Università degli Studi di Ferrara

DOTTORATO DI RICERCA IN
SCIENZE DELL'INGEGNERIA

CICLO *XXVIII*

COORDINATORE Prof. Stefano Trillo

Occurrence of pharmaceuticals and personal care products in hospital effluent, urban wastewater and combined sewage overflow. Options for their management and treatment.

Settore Scientifico Disciplinare ICAR/03

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Ph.D. IN
"SCIENCE OF ENGINEERING

XXVIII CYCLE

COORDINATOR Prof. Stefano Trillo

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Disciplinary Scientific Sector ICAR/03

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Acknowledgements

Even though writing the Acknowledgments should be the easiest part of the thesis (because there are only to put together emotions, feelings, memories and no one formula appears), for me doing this has not been so easy. It would be easier just say thanks to a list of people, but I have to force myself because there are things that worth to be written mostly because this has been a period when most of the things that I knew in my life changed. It is clear that I did not do it alone: there were people in my life that directly or indirectly put me in the conditions to arrive here. Now I will try to include them all in here.

First of all, I would like to express my sincere gratitude to my supervisors/tutor Prof.
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I well remember the first time I met her. She met me when I had to choose which university attempt, she was pregnant with her second child. When I finished all my exams she offered me my thesis dealing with the industrial wastewater. After that I became a PhD student and I started to manage a large amount of data mainly about the pharmaceutical compounds. In these years, her office door was always open for helping me in my activities as well as for "lessons of life" but also cooking, children, etc.

I must express my gratitude to my colleague and friend Dr. Mustafa Al Aukidy for his advices and patience in supporting all my long monologues. Almost in all the place where you gone for your doctoral studies I went to meet you and now I want to find you again.

I appreciate the opportunities to keep in touch with my big friend Eng. Chiozzi, that come in my office to tear me a smile, telling me his amazing adventures. He supported me in several occasions when I needed some wise advices. For all this reason and the great regard I have about him, I have chosen him as my wedding witness.
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But also all my friend belonging to the Italian Red Cross. Thank you. Thanks to all the people that for carelessness I do not mentioned here, thank you all for being part of my life!

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Sometimes doubts overcame my beliefs and it was hard to keep going. In these moments, having all these people by my side has been very important.

Elena

In Ferrara, on a sunny February afternoon

“Nessuna quantità di esperimenti potrà dimostrare che ho ragione.
Un unico esperimento potrà dimostrare che ho sbagliato.”

Albert Einstein, lettera Max Born
del 4 dicembre 1926

***Abbreviations**:*

AC, Activated carbon

AeD, aerobic digestion;

AnD, anaerobic digestion;

AOP, advanced oxidation process;

AOXs, adsorbable organic compounds;

ARB, antibiotic resistant bacteria;

ARG, antibiotic resistant genes;

AS, activated sludge;

ATC, Anatomical Therapeutic Chemical classification system

BAF, biological aerated filter;

BAT, best available technology;

BNR, biological nutrient reactor;

CAS, conventional activated sludge;

CEC, cation exchange capacity;

CFR, Code of Federal Regulations;

CFU, colony-forming units;

Chlorin, chlorination;

Coag, coagulation;

CPCs cancerogenic platinum compounds

CSO, combined sewage overflow;

CWs constructed wetlands

D617, Ndealkylverapamil;

DM, dry matter

DNA, deoxyribonucleic acid

DO, dissolved oxygen;

DOC, dissolved organic carbon;

D_{ow} octanol water distribution coefficient

D_{ow}, octanol water partition coefficient;

E1, estrone;

E2, estradiol;

E3, estriol;

EC_{50}, Effective Concentration producing an adverse effect in 50% of a test species;

EE2, ethinylestradiol;

EF, extrapolation factor;

EPA, United States Environmental Protection Agency;

EQS, environmental quality standard;

FDA, United States Food and Drug Administration;

FIB, faecal indicator bacteria;

FL, flocculation;

FLO, flotation;

foc, fraction of organic carbon,

GAC, granular activated carbon

HDPE, high density polyethylene;

HRT, hydraulic retention time;

H-SSF, horizontal subsurfaceflow;

HWW, hospital wastewater

ICM, iodinated contrast media;

K_a, dissociation constant;

k_{biol}, biological degradation rate;

K_d, solid liquid partition coefficient;

K_{ow}, octanol water partition coefficient;

LAS, linear alkyl sulfonates;

LC_{50}, Lethal Concentration producing 50% mortality in test species;

LP low pressure

MAnD, mesophilic anaerobic digestion

MBBR,moving bed biofilm reactor;

MBR, membrane biological reactor;

MCWO, molecular weight cut off

MEC, measured environmental concentration;

MP, medium pressure;

MPN, Most probable number

NF, nanofiltration;

NP, nonylphenol;

NPnEO, nonylphenol (n) ethoxylates;

NSAID, Nonsteroidal anti-inflammatory drug

O&M, maintenance and operation;

OM, organic matter;

PAC, powdered activated carbon;

PAH, polycyclic aromatic hydrocarbons;

PBT, Persistence, Bioaccumulation, Toxicity;

PCB, polychlorinated biphenyls;

PCP, personal care product;

PEC, Predicted Environmental Concentration;

PhCs, pharmaceutical compounds;

pK_a, acid dissociation constant;

PNEC, Predicted No Effect Concentration, highest concentration of the substance that does not have a harmful effect in the environment;

PPCP, pharmaceutical and personal care product;

RO, reverse osmosis;

RQ, risk quotient;

RT, sludge retention time;

SF, surface flow;

SRT, sludge retention time;

SSD, Sewage Sludge Directive;

SSF, subsurface flow;

STP, Sewage Treatment Plant.

T, temperature;

TAnD, thermophilic anaerobic digestion;

TDS, total dissolved solids;

TOC, total organic carbon;

TSS, total suspended solids;

UASB, upflow anaerobic sludge blanket

UF, ultrafiltration

UV, ultraviolet;

UWW urban wastewater

VF, vertical flow

vf, filtration velocity;

V-SSF vertical subsurface flow

WWTP wastewater treatment plant

i. *Introduction*

i.1 Introduction

The activities I did, coordinated by Prof. Verlicchi, concern the analysis of the presence of pharmaceutical compounds (PhCs) and personal care products (PCPs) in wastewater and the study and optimization of different technologies and strategies in order to reduce the release of these compounds into the environment.

More than 15,000 prescription pharmaceutical compounds (PhCs) and over the counter (OTC) drugs are registered and approved for use today, corresponding about 1300 active ingredients (FDA, 2012). Data on their consumption in terms of annual quantity administered in a specific area (country, region, etc.) or for particular users (households, hospitals, healthcare structures, etc.) are difficult to obtain, in particular for OTC drugs.
Furthermore, consumption patterns vary among study areas (local, regional, and countrywide), making prediction of PhC consumption extremely difficult.
Consumption patterns may vary due to the local economic situation, national and local healthcare system organization, drug prescription guidelines and behaviour (recommended average dose and treatment duration), as well as geographic prevalence of certain diseases at particular times. A rough estimation of the global consumption of human PhCs showed that about 100,000 tons of PhCs is used each year, which corresponds to a worldwide average consumption of 15 g/(year per capita) (Kummerer, 2004). Although more detailed analyses of PhC consumption of specific therapeutic classes by area and by country are available in terms of sales (WHO, 2004), these data do not aid evaluation of the mass flow of PhCs consumed in a specific area over a specific period of time.
Once administered, PhCs are metabolized to varying degrees, and their excreted metabolites and unaltered parent compounds can also undergo further modification due to biological, chemical and physical processes in both sewage treatment facilities and receiving water bodies (Verlicchi et al., 2013c).
Also personal care products (PCPs) are used in a large amount and their residues are discharged in wastewater.
Although many investigations have pointed out the environmental risks correlated to the occurrence of pharmaceuticals and personal care products (PCPs) in aquatic environments (surface and groundwaters) (Fick et al., 2009) and that the main source is due to wastewater treatment plant (WWTP) discharges (Daughton and Ruhoy, 2009), up to now, legal limits regarding PCPs have not thus far been set, and no technical guidelines or suggestions as to most suitable treatments for reducing their concentrations in final effluent are yet available (Verlicchi et al., 2012d). However, recent studies evidenced that hospital effluents can be considered hot-spot sources and the search for appropriate management and treatment of this kind of effluent is an extremely pressing issue (Verlicchi et al., 2010a,b, 2012a).
Conventional activated sludge (CAS) processes have been employed extensively in WWTPs all over the world, predominantly because they produce a secondary effluent that complies with global and national quality standards for discharge into surface water bodies, and they entail reasonable construction, operating, and maintenance costs. WWTPs were built and upgraded with the principal aim of removing easily or moderately biodegradable carbon, nitrogen, and phosphorus compounds and microbiological organisms, which regularly joint the treatment plant in concentrations of the order of mg/L and at least 10^6 MPN/100 mL, respectively. In raw domestic wastewaters, PCPs generally range considerably from 10^{-3} to 10^{-6} mg/L (Verlicchi et al., 2012b), and their chemical and physical properties, namely solubility, volatility, adsorbability, absorbability, biodegradability, polarity and stability, also vary greatly a lot (Le Minh et al., 2010; Ziylan and Ince, 2011), with obvious repercussions on their behavior during the treatments and consequently their removal efficiencies (Cairns, 2012). Among many factors governing the complex interactions in wastewaters and treatment systems, trace lipophilic pollutants are likely to be associated with colloids, due to their organic coating (Stumm and Morgan, 1996), on which some PhCs can be sorbed. In addition, positive charged molecules can become associated to these colloids by means of low strength Van der Waals bonds (Verlicchi et al., 2013c).

By this way, sludge originates during biological and chemical processes in WWTPs and contain a wide spectrum of PCPs as well as microorganisms and viruses which are separated from the liquid phase during treatments.

The removal of PCPs by conventional wastewater treatments (mainly activated sludge systems AS, membrane bioreactors MBR and advanced oxidation processes AOPs), in particular, have been the object of a great number of studies (among them: Verlicchi et al., 2012b; Chelliapan and Sallis, 2013),while the PCPs removal efficiencies by constructed wetlands (CWs) have only recently come under scrutiny. Constructed wetlands (CWs) are systems where oxic-anoxic-anaerobic environments may coexist, especially in subsurface flow beds or in sequence of different kinds of CW types. In surface flow systems, solar radiation may also contribute to the removal of micropollutants.

Recent studies have remarked that due to the wide spectrum of characteristics of emerging contaminants, including PCPs, it is quite difficult to find a treatment able to remove most of them at a high percentage.
Recent studies (Verlicchi et al., 2013c; Verlicchi and Zambello, 2014) pointed out that different groups of micropollutants can be removed at a medium-high extent only in those treatment trains where different removal mechanisms can occur. Multi-barrier treatment systems are necessary.
If not properly removed these may fall within the cycle via the effluent treatment plant that is discharged into a water body or can be found in sewage sludge that is applied in agriculture as fertilizer. These compounds also cannot get treatment and be discharged directly into the body of water through spillways during rainfall events of particular entities.

To get an overview of the current situation, a literature search was performed, and the findings are reported in the graph of Fig.i.1. They were obtained by searching Scopus with the following variables: document type, all; data range,1997–2014 (included); subject areas, all; and search for "pharmaceutical activated sludge" or "personal care products activated sludge" or "drug municipal wastewater treatment" or "pharmaceutical sewage" or "personal care products constructed wetland" or "pharmaceuticals constructed wetland".

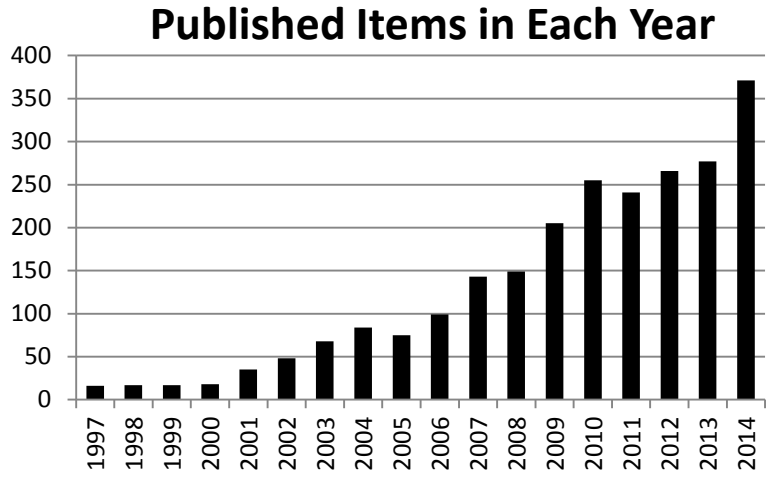

Fig. i.1: SCOPUS search for relevant publications, reported by year

It is quite evident that in the last 6 years, the number of studies dealing with occurrence of PhCs in wastewater and removal by CAS systems have greatly increased. To refine the search, these studies were screened for the terms: “pharmaceutical mass load,” “environmental risk assessment,” and “pharmaceutical prediction concentration” (Fig. i.2) and for “activated sludge modeling pharmaceutical compound,” “pharmaceutical concentration secondary sludge,” “removal mechanism pharmaceuticals activated sludge” and "combined sewage overflow" (Fig. i.3).

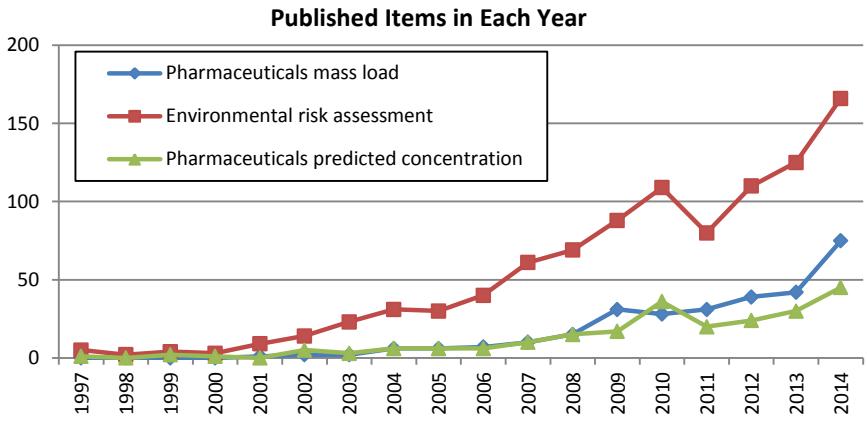

Fig. i.2: Refined search within the results for the three terms reported in the legend

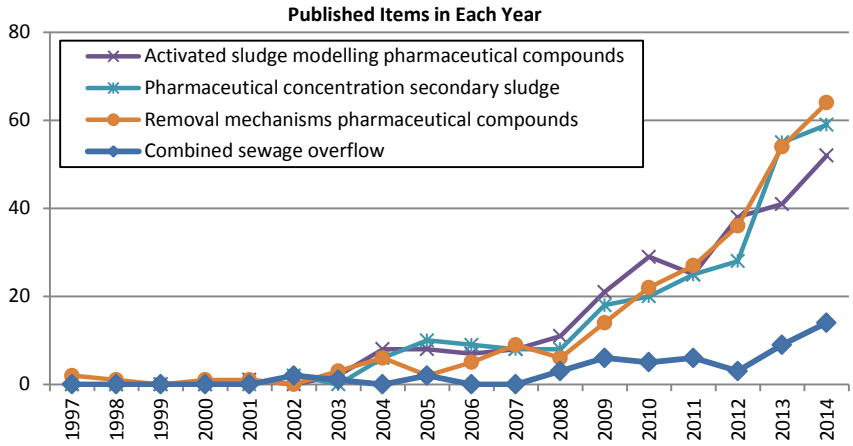

Fig. i.3: Refined search within the results for the four terms reported in the legend

These graphs show that in recent years, the main focus of such studies has been environmental risk assessment, followed by removal mechanisms, pharmaceutical concentrations in secondary sludge.
In the last place combined sewage overflow.

The following sections will show and discuss the major findings on these topics for selected PPCPs belonging to a wide spectrum of therapeutic classes.

As PPCs may show toxic effects in the long run it is very important to investigate the occurrence of these compounds in urban and hospital WW and to analyze different strategies in order to reduce their discharge into the environment. My PhD thesis has precisely this purpose, as main focus.

This thesis considered 250 pharmaceuticals belonging to 28 therapeutic classes listed in the following lines together with the number of selected compounds.

Analgesics/Anti-inflammatories (27); Anesthetics (6); Antianginal (1); Antiarrhythmics (1); Antibiotics (59); Anticoagulant (1); Antidiabetics (3); Anti diarrhea (1); Antiemetic (1); Antifungals (7); Antihypertensives (13); Antineoplastic (5); Anti-parkinson (2); Antiplatelets (3); Antiprotozoal (1); Anti spasmodic (1); Antiviral (4); Beta-agonists (4); Barbiturates (2); Beta-blokers (13); Contrast media (8); Hormones (7); Lipid regulators (10); Psychiatric drugs (54); Receptor antagonists (1); Stimulants (3); Vasodilator (1); Topical Products (1).

Moreover, 36 PCPs belonging to 11 classes are investigated. Particular attention was posed to two classes of surfactants (anionic and non ionic).

i.2 Structure of the thesis

Within the urban water cycle, reported in Fig. i.4, the activities mainly concern the treatment of wastewater coming from the drainage system for the reduction of the discharge polluting load and environmental risk.

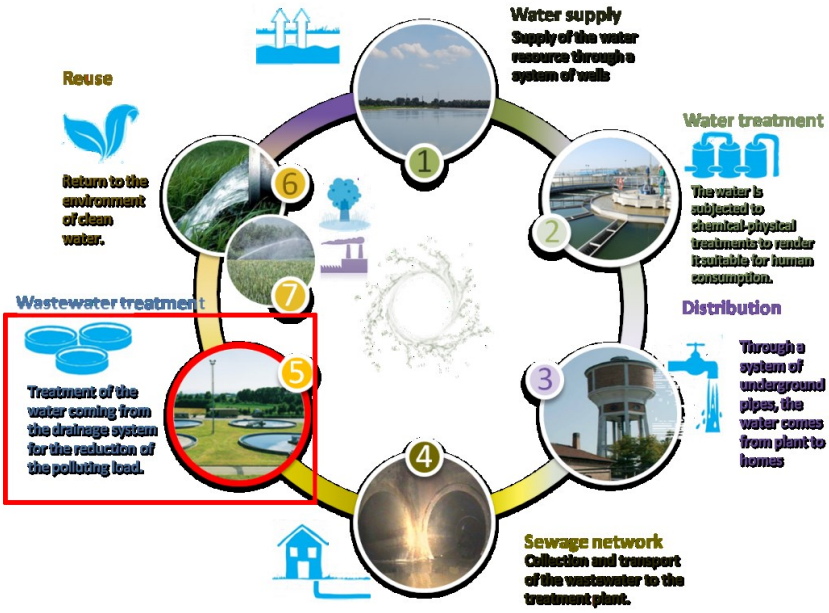

Fig. i.4: Urban water cycle

The activities developed during this Ph.D. have been showed in 3 parts, divided in chapters, that collect and analyse the available data about:

PART A) Urban Wastewater: occurrence, treatment and removal.
-Chapter 1: PhCs removal by conventional WWTPs
-Chapter 2: PPCPs occurrence in untreated and treated sewage sludge
-Chapter 3: Removal of PCPs in CWs
-Chapter 4: Removal of PhCs by CWs
-Chapter 5: Removal of PhCs by CAS followed by CW analysis of their respective contributions

PART B) Hospital Wastewater: Occurrence, Treatment and Management.
- Chapter 6: Management and treatment of hospital wastewater
- Chapter 7: Predicted and measured concentrations for selected PhCs in hospital effluent. Limits and advantages of the two approaches through the analysis of a case study.

PART C) Combined sewage overflow: Occurrence and Management
- Chapter 8: Contribution of combined sewage overflow to the load of E.Coli and PhCs. The case study of Comacchio.

After the chapters four appendixes are presented:

- Appendex A collects two tables that list the selected PPCPs combined with their physical-chemical properties;
- Appendix B collects the first page of the papers published about the issues developed in this thesis.
- Appendex C collects the summary of the supplementary data of each published paper
- Appendix D is a table that reports the excretion rate of some selected PhCs.

In the end of the topic, there is a complete Bibliography with more than 500 citations.
The following figure shows the analysed steps according to the water flow.

Fig. i.5: Various aspects considered in this thesis [1. Domestic wastewater, 2. Hospital wastewater, 3. Conventional WWTP (CAS), 4. Natural WWTP (CW), 5. Overflow, 6. WWTP effluent, 7. Sludge application to land]

Data have been collected from **literature studies** in order to describe:

-the occurrence in raw wastewater (point 1), in WWTP effluent (point 7) and in soil amended sludge (point 8), their discharged load and the corresponding environmental risk;
-the removal in conventional (point 3) and natural treatment (point 4)

Two **case study** were considered:

- Comparison between PhC measured and predicted concentration in hospital effluent in an hospital located in the North-Est of Italy (point 2).
- Estimation of PhC load discharged by overflows referring to the area of Comacchio (Ferrara) by using data of flow rate provided by the water management authority CADF spa. CADF provided us also data of the concentrations of Eschericchia Coli (point 6).

Data provided in this thesis have been presented during international conferences, in scientific papers and book chapters listed in the following lines together with the number of the corresponding chapter.

- Verlicchi P, Zambello E, Al Aukidy M. Removal of pharmaceuticals by conventional wastewater treatment plants. In: Petrovic M, Suarez S, Barcelò D, editors. Analysis, removal, effects, risks of pharmaceuticals in the water cycle: occurrence and transformation in the environment. Series works: Comprehensive Analytical Chemistry Elsevier; Vol. 62. Barcelò D. (Elsevier), Amsterdam: The Netherlands 2013c pp.231- 86. **Chapter 1**
- Verlicchi P, Zambello E. Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil – A critical review. Sci Tot Environ 2015;538:750-767. **Chapter 2**
- Verlicchi P., Zambello E., Al Aukidy M., Removal of personal Care Products in Constructed Wetlands. Chapter in "Personal Care Products in the Aquatic Environment" edited by Dr. Silvia Díaz Cruz and Prof. Dr. Damià Barceló, 2014. **Chapter 3**
- Verlicchi P, Zambello E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. Sci Tot Enviro 2014;470-471;1281-1306. **Chapter 4**
- Verlicchi P, Galletti A, PetrovicM, Barceló D, Al Aukidy M, Zambello E. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed—analysis of their respective contributions. Sci Total Environ 2013b;454–455:411–25. **Chapter 5**
- Verlicchi P, Al Aukidy M, Zambello E. What have we learned from worldwide experiences on the management and treatment of hospital effluent? — An overview and a discussion on perspectives. Sci Total Environ. 2015;514:467-491. **Chapter 6**

i.3 Analysed compounds

i.3.1 Pharmaceutical compounds

Medicaments are composed by one or more active pharmaceutical ingredients (APIs) also called parent compounds, designed to provoke the desired effect on human health: cure and prevent diseases. These molecules are generally complex, possessing different functional groups and biological physicochemical properties. They are quite often polar and have a molecular weight ranging from 200 to 500/1,000 Da. APIs are generally grouped according to their therapeutic class according to their physiological activity (i.e. analgesics/anti-inflammatories, antibiotics, anti-diabetics, anti-hypertensives, barbiturates, beta-agonists, beta-blockers, diuretics, lipid regulators, psychiatric drugs, receptor antagonists anti-neoplastics and X-ray ICMs), rather than their chemical structure, as even minute changes in the latter may cause significant difference in polarity, solubility and/or other important properties that influence and define their environmental fate (Verlicchi et al., 2013).
After administration, the active substances of medicines are metabolized, but only to a certain extent. The unmetabolized active substances (varying between 10% and 95%) are excreted, largely through the renal system (urine) and partially through the biliary system (faeces), depending from both the nature of the compound and the individual in question. As a consequence, API residues join wastewater and enter the water cycle through the sewage system as unchanged substances (parent compounds), a mixture of metabolites or conjugated with an inactivating compound attached to the molecule, whose fate in the environment will be governed by their characteristics (mainly solubility, volatility, adsorbability, absorbability, biodegradability, hydrophilicity, lipophilicity, polarity and stability) as well as environmental conditions (temperature, pH, aerobic/anaerobic/anoxic conditions) (Verlicchi et al., 2012).
Recent investigations document that PhC production and administration may vary both among countries and over time (Goossens et al., 2007, Kümmerer, 2009a), fluctuating not only on an annual basis, but also from one year to the next (Alexy et al., 2006). In addition, constant ageing population and improving quality of life worldwide mean that their consumption is set to increase in next years (Van der Aa et al., 2011).

In recent years, pharmaceutical compounds (PhCs) have provoked increasing concern, particularly as no legal requirements have been set for discharge into surface water bodies of these ubiquitous, persistent and biologically active substances (Furhacker, 2008; Salgot et al., 2006; Ternes et al., 2007). For these reasons, most PhCs belong to the group of so-called *emerging contaminants*, that is, pollutants that are quite often unregulated as yet but may be candidates for future regulation, depending on the results of research monitoring their occurrence and assessing their potential effects on health (Verlicchi et al., 2013).
The compounds considered in this thesis are those analyzed from 2013 to 2015 during the different activities. They were generally chosen according to the following criteria: high consumption, widespread occurrence in urban and hospital wastewater as well as in treated effluent, available analytical methods. Many compounds have high toxicity towards aquatic environment.
The selected PhCs are listed according with their therapeutic class in Appendix A together with the corresponding properties.

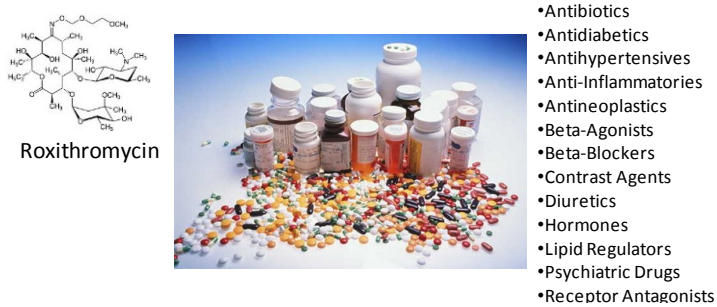

Fig. i.6:PhCs and some selected therapeutic classes

i.3.2 Personal care products

Fig. i.7: PCPs and some selected PCP groups

Every day we use products for our personal care and hygiene, in particular cosmetics (skin care products, hair sprays, and sunscreens), toiletries (bath additives, soaps, hair tonics, shampoos, oral hygiene products) and fragrances (perfumes, aftershaves). These products, commonly called personal care products (PCPs) contain synthetic organic chemicals with a specific function, the *ingredients*. They may be antimicrobial disinfectants (triclosan, triclocarban), preservatives (methylparaben, ethynilparaben, butylparaben) or sunscreen agents (oxybenzone, avobenzone). In addition, some of them may contain synthetic surfactants (generally anionic and nonionic compounds). These are substances widely used in the formulation of many commercial PCPs not only for their wetting, cleaning, foaming and emollient properties, but also as they can create dispersed systems (suspension or emulsion), modify the cosmetic rheological properties, prolong durability of the product and control the release of active ingredients (Somasundaran et al., 2006) which greatly improves the quality of the substance.
PCPs are used in the range of several thousand tons per year: parabens are used in more than 22,000 cosmetic products (Andersen, 2009), approximately 350 tons of triclosan are produced annually in Europe (Singer et al., 2002), and in 1998, 1,473 tons of galaxolide, 343 tons of tonalide and 18 tons of celestolide were consumed in Europe [Alder et al., 2007].
These products are disposed of or discharged into the environment on a continuous basis via municipal/industrial sewage facilities and also directly by untreated discharges (Ternes et al., 2003; Kunz and Fent, 2006; Bester, 2007). This means that their exposure potential may reach critical level for the environment, even for those compounds that might have a low persistence.
In recent years, increasing attention has been paid to the occurrence of some of them in aquatic environments, also due to the finding that some PCPs can induce known or suspected undesirable effects on humans and ecosystems (included endocrine disruptions) (Stuart et al., 2012).

Among these compounds, triclosan, present everywhere in soaps, shampoos, detergents, has been already reported for its endocrine function and for other aspects harmful to human health.

The compounds considered in this thesis are listed in Appendix A together with their physical-chemical properties.

i.4 Properties

In order to identify a large number of PPCs in WW and in sludge, appropriate analytical methods have to be developed for both the water phase and for the solid phase, respectively (Johnson et al., 2008). To date, the conventional chemistry analytical methods for organic micro-contaminants are mainly based on liquid or gas chromatography (LC or GC) with detection by mass spectroscopy (MS) after appropriate extraction, clean up, and derivatization procedures. For most of the organic micro-contaminants these detection instruments enable limits of quantification (LOQ) down to 1-10 ng/L for the aqueous phase and down to 1-10 ng/g for solid matrices. Large sample volumes help to lower LOQs.

Physico-chemical characteristics of PPCs - such as sorption affinity to sediments (K_d), octanol-water partitioning $\log K_{ow}$ and pH dependency for (de) protonated forms (pK_a) - are used in pharmacokinetic studies in clinical settings but their use has been transplanted in predicting their behaviour in environmental assessment by the Food and Drug Administration (FDA) (Jjemba, 2006). These properties help to identify whether the target compounds are predominantly dissolved in the water phase, sorbed onto suspended matter, or if have both compartments.

This properties may be also related to toxic potential as stated by Lienert et al., (2007). They found that in general drugs with high lipophilicity had a higher toxic potential in faces than urine. Diclofenac, erythromycon and fenofibrate are some examples. On the contrary, Norfloxacin and clofibrate.

The toxicity in this thesis was related to PNEC (predicted no effect concentration). When available this value is reported in Appendix A for the selected compounds. Further investigation are needed for determine PNEC for mixture of compounds. Moreover, current approaches in assessing the risks from PPCPs in the environment lay emphasis on acute toxicity but because these compounds typically occur at very low concentrations over a long time an ecotoxicity potential assessment that takes into account varying biological activity is proposed (Jjemba, 2006).

An in depth analysis of various properties is reported in Chapter 1.

i.5 Regulations

Attention is currently paid to the "origin" of PhCs, as set in the regulations issued by the US FDA (US FDA, 1998) and the European Community (Directive 2004/27/EC), which contains a Community code relating to medicinal products for human use, and Regulation 726/2004, which lays down Community procedures for the authorization and supervision of medicinal products for human and veterinary use), and an environmental assessment of each *new* compound is mandatory before its launch onto the market and use. Additionally, in June 2007 the European Community Regulation REACH (Registration, Evaluation, Authorization and Restriction of Chemicals) came into force, with the aim of safeguarding human health and the environment through a better and a earlier identification of the intrinsic properties of chemical substances. As a result, information about the composition of administered PhCs is readily available.

Despite PhCs in the environment is an emerging issue, the discharges of their residues in surface water are not yet covered by specific regulations. Until now, the current legislation does not impose mandatory limits under which it must maintain concentrations of these compounds.

However, European directive on water quality (DIR 2013/39/EU 12/08/3013) has never included in the list of substances to be monitored even some of these compounds, as they are subjected of great attention because of their possible negative effects even in the long term, on humans and aquatic environment (Fent et al, 2006). The EU decision 2015/495 of 20 March 2015 have established a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council. In this control list ethinylestradiol, estradiol, estrone, diclofenac, Erythromycin, Clarithromycin and Azithromycin are included. For these substances data have to be collected in order to assess effectively their risk and to revise the list.

Some recent assessments of environmental risk showed that the concentration of drugs in aquatic environment may exceed the threshold concentration below which there are no negative effects (predicted no-effect Concentrations PNEC) (Kim et al. 2007, Quinn et al. 2008).

There are some studies that propose priority list in order to identify the PhCs that should be the first to be considered in any study on pharmaceuticals regarding water management. Among them Voogt et al. (2009) propose a list of 10 high priority compounds that was extracted from the literature review work. In the European Union, USA and other countries a debate is open regarding the compilation of lists including *priority compounds* requiring monitoring in the aquatic environment [2013/39/EU, Richardson and Ternes, 2011, Bottoni et al., 2010, Lapworth et al., 2012). However, due to the lack of information on toxicity and environmental impacts, a large number of contaminants, especially organic compounds, are not included in these lists. The number of compounds which could become priorities is therefore likely to grow.

Referring to PCPS limits of concentrations have been set for surfactants with regard to wastewater treatment plant discharges into surface water bodies or for the direct reuse of treated effluents. However, limits do not exist for many other PCPs occurring in wastewaters.

i.5.1 Legislation constraints for sludge disposal

With regard to European countries, sewage sludge management and its final disposal have to respect specific directives related to wastewater and also waste management since sewage sludge, generated in WWTPs, is often transported elsewhere, either to a specific treatment platform or to final disposal and thus it becomes a waste. European Directives aim to improve aquatic environment protection, by encouraging a progressive reduction of contaminants released into the aquatic environment (Directive 2000/60/EC, CEC (Council of the European Communities) Council Directive 91/271/EEC) and thus promote an upgrade of the existing WWTPs, sometimes resulting in a higher production of sludge. Moreover, they encourage the reuse of sewage sludge (CEC (Council of the European Communities) Council Directive 91/271/EEC) and, for this objective, they set qualitative and quantitative limits as in the Sewage Sludge Directive (SSD) 86/278/EEC. Moreover, they want to reduce the amount of waste to landfill, in particular

biodegradable waste (that is waste capable of undergoing anaerobic or aerobic decomposition) (CEC (Council of the European Communities) Council Directive 99/31/EC). It is possible to use sludge on agricultural land — in accordance with the SSD, Member States have established national legislations and in particular have set conditions allowing land application of sewage sludge. The SSD sets the maximum concentrations for heavy metals (Zn, Cu, Ni, Cd, Pb, Hg) in sludge, but many Member States set more stringent limits for heavy metals, organic micropollutants (including PCBs, LAS, and PAH) and pathogens (in particular Salmonella, enteric viruses and helminth eggs) (Inglezakis et al., 2014; Kelessidis and Stasinakis, 2012). No limits have been set for organic compounds and in particular for PhCs.

The SSD favors the agricultural use of sludge subjected to a “biological, chemical or heat treatment, long-term storage or any other appropriate process” in which “fermentability and health risks resulting from its use” have been significantly reduced.

At EU level, revision of the SSD is ongoing, addressing different issues concerning: (i) the intention to reduce the chemical content in sludge promoted by REACH (that is a regulation regarding the Registration, Evaluation, Authorization and Restriction of Chemicals), (ii) the possibility of increasing the treatment of biological wastes to produce compost, characterized by a lower content of hazardous substances with respect to sewage sludge, and to favour its spreading on soil and (iii) the interest in encouraging the use of sludge for biogas production and other forms of energy recovery. Bearing this in mind, in the coming years limits will be revised for the regulated substances and set for organic pollutants (absorbable organically bound halogens (AOX), surfactants, PCB, PCDD, etc.) and pathogens, as discussed in Inglezakis et al. (2014). In order to avoid the risk of pathogen spread into the environment in Sweden, a new regulation is under discussion which would require a sanitation step including chemical and thermal treatments for all those sludges allocated to agriculture purposes (Malmborg and Magnér, 2015). A comparison of the efficacy of the different sludge treatment in removing the typical pathogens contained in sludge is described in the study by Arthurson (2008). This new regulation will come into force in January 2019 (Malmborg and Magnér, 2015).

An in-depth discussion and comparison of the legislation adopted in EU-27 is reported in Kelessidis and Stasinakis (2012). With regard to USA regulations (USA Code Part 503), Standards for the use or Disposal of Sewage Sludge (generally called biosolids) are found Part 503 of Section 40 of the Code of Federal Regulations (40 CFR 503, hereafter simply “Part 503”). US limits for heavy metals are less severe than those set by the SSD. Part 503 distinguishes between two types of biosolids (Class A and Class B) on the basis of the level of treatment the sludge is subjected to. The distinction is briefly reported in Chapter 2, while an in-depth discussion is reported in Jones-Lepp and Stevens (2007) and in McClellan and Halden (2010).

Part A

Urban Wastewater

Occurrence, Treatment and Removal

Chapter 1:

1 PhCs removal by conventional WWTPs

1.1 Introduction

The first part of the Chapter presents a brief description of the CAS process, focusing on the most common treatment trains for both wastewater and sludge (Paragraph 1.2). The historical development of the activated sludge process is then discussed, in order to identify the most common reactor configurations, which will then be considered as the Chapter progresses. The selection criteria for compounds to include in this study are outlined in Paragraph 1.3, which also reports the list of selected PhCs grouped according to their therapeutic class.

The occurrence of the selected PhCs in domestic raw influent and CAS effluent is reported in Paragraph 0, while their occurrence in the primary, excess and treated sludge is detailed in Paragraph 1.4. Aqueous and overall pharmaceutical removal efficiencies are discussed in Paragraph 1.5, as well as their percentage partitions (where data available) among effluent, sludge and removed fraction during secondary biological treatment. How PhC removal efficiencies can be affected by the main chemical and physical properties of selected compounds and operational parameters within the biological reactors is discussed, respectively, in Paragraphs 1.5.3 and 1.5.4.

The average mass load rankings, based on the collected data pertaining to the secondary effluent and the corresponding average flow rate, are reported and discussed in Paragraph 1.6. Paragraph 1.7 outlines an environmental risk assessment of secondary effluent as well as treated sludge, and in particular reports results in terms of risk quotient both for the two kinds of CAS outlets. The PhCs are then ranked according to their presence in secondary effluent and sludge, highlighting those with the highest risk and enabling identification of the most critical compounds in terms of load and environmental risk. The aim is to contribute to the debate by raising issues to consider further to reducing the impact of PhCs in secondary effluent and treated sludge, which are generally directly discharged into surface water bodies or applied to the land, respectively. Some indications about the available tools for modelling the behaviour of PhCs in CAS are also reported (Paragraph 1.10).

The chapter concludes with a focus on a special kind of wastewater that contains a great amount of PhCs: the effluent from pharmaceutical manufacturing facilities (Paragraph 1.11).

All reported concentration data are measured rather than predicted, but it is important to note that they (PhC occurrence in water and sludge, removal efficiency, mass load) were reported in a host of previous investigations carried out in different countries and at different times. Hence, the findings are unavoidably affected by uncertainty. For instance, measured PhC concentrations will depend on protocols used for sampling, preparation, conservation and chemical analysis. Furthermore, removal efficiency is strictly correlated to measured influent and effluent concentrations, while mass load will depend on assumed (average) flow rate and (average) concentration, and the risk quotients are calculated using assumed measured concentrations and predicted no-effect concentrations, and so on (Liebig et al., 2006; Ort et al., 2006, 2010b,c). Hence, for in-depth analysis of the reported data, the specific cited studies should be consulted. Nevertheless, the data reported and analysed in this study should provide a snapshot of the current state of affairs, and provide a springboard for further debate on this crucial issue.

1.2 Conventional Wastewater Treatments

Domestic (also known as urban) wastewaters are generally subjected to a treatment sequence including preliminary treatments (screening, grit removal, and oil and grease removal), a primary gravity settling (sometimes this step is absent), secondary biological treatment (by activated sludge, fixed-film reactors, lagoon systems, and/or sedimentation), and finally tertiary steps, sometimes including advanced treatments (chemical coagulation, flocculation, sedimentation, activated carbon filtration, disinfection, and chemical oxidation).

Fig. 1.1 reports the sequences generally adopted for raw wastewater and the resulting sludge.
For the secondary step, activated sludge treatment is that most extensively employed all over the world for processing both urban wastewaters from small and large communities and industrial effluents. This type of treatment was developed by two English researchers, Ardern and Lockett, in 1914, and since then, it has been implemented on a global scale. Activated sludge treatment consists mainly of flocculating microorganisms held in suspension and contact with wastewater in a mixed aerated tank. The so-called CAS system consists of a biological reactor (where activated sludge may develop and grow) followed by a secondary clarifier: The simplest diagram of this process is that shown in Fig. 1.2, and subsequent configurations developed over the years are shown in Fig. 1.3.

The biological reactor may consist of one (Fig. 1.2 and Fig. 1.3A) or more compartments (Fig. 1.3B–F). Multiple compartments provide different operational conditions, namely, aerobic, anoxic, and anaerobic, and enable C, N, and P removal. Adsorption, absorption, flocculation, oxidation–reduction reactions, and sedimentation are the main physical and biochemical processes occurring within the activated sludge process. Biochemical reactions (anabolic, catabolic, and cometabolic reactions) take place within the biological reactor and bring about the degradation of the organic compounds in the influent wastewater. The reactions are performed by the microorganisms suspended in the liquid, namely, bacteria, protozoa, rotifers, and fungi, which together form the biomass (see image on the left in Fig. 1.2), which develops and grows as these reactions take place. Organic compounds subject to biodegradation include not only lipids, proteins, and carbohydrates, which occur at the order of mg/L, but also micropollutants (i.e., pharmaceuticals and personal care products), occurring at concentrations of ng/L or μg/L.

After enough time for the appropriate biochemical reactions, the mixed liquor is transferred to a settling tank (secondary clarifier) to allow gravity separation of the suspended solids (in form of floc particles) from the treated effluent. Some of the settled solids are returned to the biological reactor return activated sludge) in order to maintain the desired biomass concentration inside (about 3–4 g/L). The remainder is considered waste (the so-called excess sludge) and is subjected to thickening, by removing a portion of the liquid fraction in order to increase its solid content. Through the processes of stabilization, dewatering, drying, and combustion, both the water and organic fractions are considerably reduced, and the processed solids (treated or digested sludge) are suitable for reuse or disposal. Over the years, different configurations of the activated sludge process were developed to promote nitrification, denitrification, and phosphorus removal.

More recent evolutions in CAS include membrane bioreactors (MBRs, Fig. 1.3E) and moving bed biological reactors (MBBRs, Fig. 1.3F). MBRs were developed with the primary aim not only to improve effluent quality but also to upgrade existing WWTPs by replacing the previous secondary settler with a membrane compartment able to better separate the solid from the liquid phase.

They generally operate at higher biomass concentrations and higher sludge ages with respect to CAS. MMBRs were designed to enhance biological processes by promoting the growth of both suspended and attached (on the surface of carriers present in the biological reactor) biomass, thereby increasing the biomass concentration in the aeration tank. One of the main advantages of the two new configurations is that they are able to treat a higher pollutant load in the “original” reactor volume (Metcalf & Eddy, 2004). Although these two treatments are becoming more diffuse, CAS is still by far the most common in operation (and most studied).

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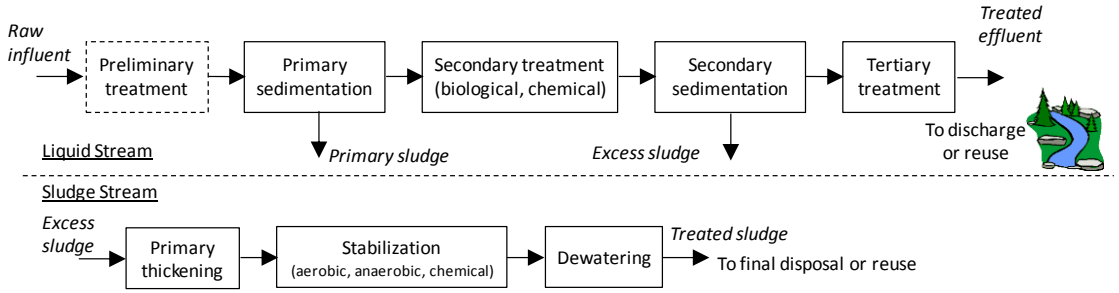


Fig. 1.1: Common treatment sequences adopted for domestic effluent and sludge produced during their treatment.

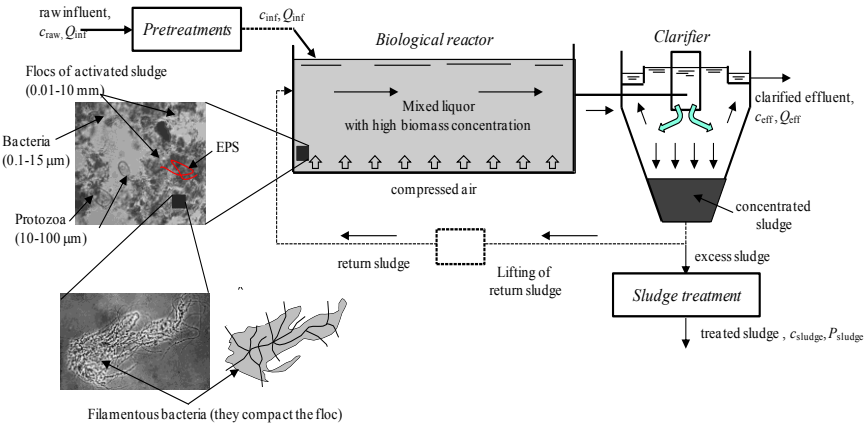
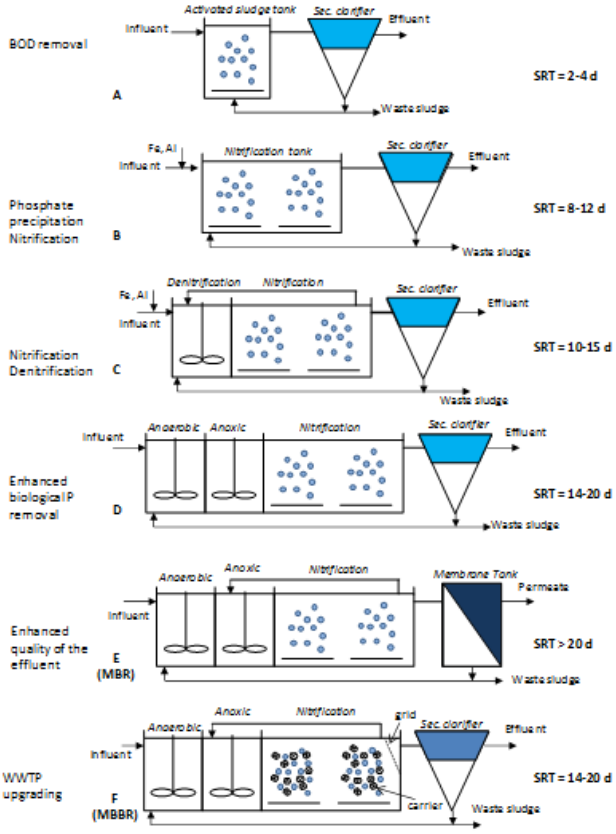


Fig. 1.2: Simplified layout of the activated sludge process. On the left, an image obtained by optical microscopy of activated sludge in the presence of protozoa; bottom left, an image and a schematic of a sludge floc containing filamentous bacteria, which make it more robust. (EPS= extracellular polymeric substance which acts as a bond between flocs).

Fig. 1.3: Historical development of the activated sludge process: from CAS for BOD removal to MBR and MBBR for enhancing the quality of the final effluent and upgrading the existing CAS while maintaining or reducing the existing footprint.



1.3 Pharmaceutical compounds included in the study

PhCs include a wide spectrum of highly active substances designed to interact with receptors in humans and animals. They are generally grouped into therapeutic classes according to their physiological activity. However, it is worth noting that these compounds, even if they belong to the same therapeutic class, may have very different chemical structures and chemical–physical properties, resulting in very different behaviors during wastewater treatment.
To narrow the field somewhat, a group of PhCs was selected according to the following criteria: high consumption, widespread occurrence in urban wastewater and treated effluent all over the world, as documented by the recent studies (see Verlicchi et al., 2012a,c; Al Aukidy et al., 2012; Jelic et al., 2011, Radjienovic et al., 2009b; Martin et al., 2012a,b), and available analytical methods. By these means, 74 PhCs were selected, spanning the following 15 therapeutic classes: analgesics and anti-inflammatories (A), antibiotics (B), antidiabetics (C), anti-hypertensives (D), beta-blockers (E), diuretics (F), lipid regulators (G), psychiatric drugs (H), receptor antagonists (I), hormones (J), beta-agonists (K), anti-neoplastics (L), topical products (M), antiseptics (N), and contrast agents (O). Among these compounds, data pertaining to 64 in water and 54 in sludge were considered, as shown in Table 1.1. Physico-chemical properties of these compounds are reported in Appendix A.

Table 1.1: Selected compounds included in this study. Compounds whose data are only available for water phase are in italics, and compounds whose data are only available in sludge phase are underlined

Therapeutic class	Compounds	Water	Sludge
Analgesics/anti-inflammatories (**A**)	Acetaminophen, *acetylsalicylic acid*, codeine, diclofenac, *fenoprofen*, ibuprofen, *indomethacin*, ketoprofen, mefenamic acid, naproxen, *phenazone*, *propyphenazone*, salicylic acid, *tramadol*	14	8
Antibiotics (**B**)	Azithromycin, *cefalexin*, *chloramphenicol*, chlortetracycline, ciprofloxacin, clarithromycin, doxycycline, enrofloxacin, erythromycin, fleroxacin, gatifloxacin, lomefloxacin, *metronidazole*, micocylcine, moxifloxacin, norfloxacin, ofloxacin, oxytetracycline, roxithromycin, sarafloxacin, sparfloxacin, *sulfachloropyridazine*, *sulfadimethoxine*, sulfamethoxazole, sulfanilamide, *sulfapyridine*, *sulfasalazine*, *sulfathiazole*, tetracycline, trimethoprim	20	22
Anti-diabetics (**C**)	Glibenclamide, metformin	1	2
Anti-hypertensives (**D**)	Diltiazem, hydrochlorothiazide	2	2
Beta-blockers (**E**)	Atenolol, *bisoprolol*, *celiprolol, metoprolol*, propranolol, sotalol	6	3
Diuretics (**F**)	Furosemide	1	1
Lipid regulators (**G**)	Bezafibrate, clofibric acid, *fenofibric acid*, gemfibrozil, *pravastatin*	5	3
Psychiatric drugs (**H**)	Carbamazepine, diazepam, fluoxetine, *gabapentin*, paroxetine	4	4
Receptor antagonists (**I**)	Cimetidine, famotidine, loratadine, ranitidine	2	4
Hormones (**J**)	Estradiol E2, estriol E3, estrone E1, ethinylestradiol EE2	4	4
Beta-agonists (**K**)	*Salbutamol*	1	0
Anti-neoplastics (**L**)	*Ifosfamide*	1	0
Topical products (**M**)	*Crotamitron*	1	0
Antiseptics (**N**)	Triclosan	1	1
Contrast media (**O**)	Iopromide	1	0

1.3.1 PhCs occurrence in the influent and in the effluent

Fig. 1.4 shows the occurrence of the selected PhCs, grouped according to their therapeutic class, reported for raw municipal WWTP influent (on the left) and CAS effluent (on the right). These graphs are plotted from data collated in the review by Verlicchi et al. (2012c) of 244 full-scale CAS systems of different nominal capacities operating in various global locations. The bars of the graph show the variability range observed for each PhC and the corresponding average values measured in the raw influent and secondary effluent. As discussed in Verlicchi et al. (2012c), measured concentrations generally refer to 24-h composite, flow-proportional, or time-proportional water samples. As reported and discussed in Ort et al. (2006, 2010a, 2010b), the sampling mode may greatly influence the reliability of experimental data.

Referring to the influent, six compounds had an average concentration >10 μg/L, 21 PhCs were detected in the range 1–10 μg/L, and the remaining 37 had a mean concentration below 1 mg/L. The highest average values were found for the analgesics/anti-inflammatories acetaminophen, ibuprofen, and tramadol (all about 30 μg/L), followed by the psychiatric drugs diazepam and gabapentin (on average, respectively, 21 and 13 μg/L) and then the analgesic salicylic acid (17 μg/L). The antibiotics cefalexin, ciprofloxacin, clarithromycin, erythromycin, and sulfapyridine were, on average, detected at concentrations higher than 1 μg/L. The widest variability ranges were observed for the analgesic/anti-inflammatory, antibiotic, and lipid regulator classes. As discussed in Verlicchi et al. (2012c), and elsewhere, influent concentrations may vary over the course of the day (Plosz et al., 2010), the week (Salgado et al., 2011) and the year (Martin et al., 2012a), depending on many factors, including differences in the nature and consumption patterns of the PhCs in question, as well as CAS influent flow rate.

In general, CAS effluent contains smaller average concentrations than its influent, but they are, nonetheless, far from negligible. Indeed, for 2 compounds, the mean concentrations were still >10 μg/L (tramadol and enofibric acid); for 9, they were between 1 and 10 μg/L; and only for the remaining 63 substances were detected effluent levels below 1 μg/L. The highest average values were found for tramadol (20 μg/L as reported by Kasprzyk-Hordern et al., 2009 and Wick et al., 2009), fenofibric acid (10 μg/L), diazepam (6.5 μg/L), ibuprofen (3.90 μg/L), atenolol (3.74 μg/L), and cimetidine (3.47 μg/L). Differences in the values observed in the CAS effluent are due not only to different influent concentrations values and the characteristics of the compounds but also to the design and operational characteristics of the WWTP, as will be discussed later.

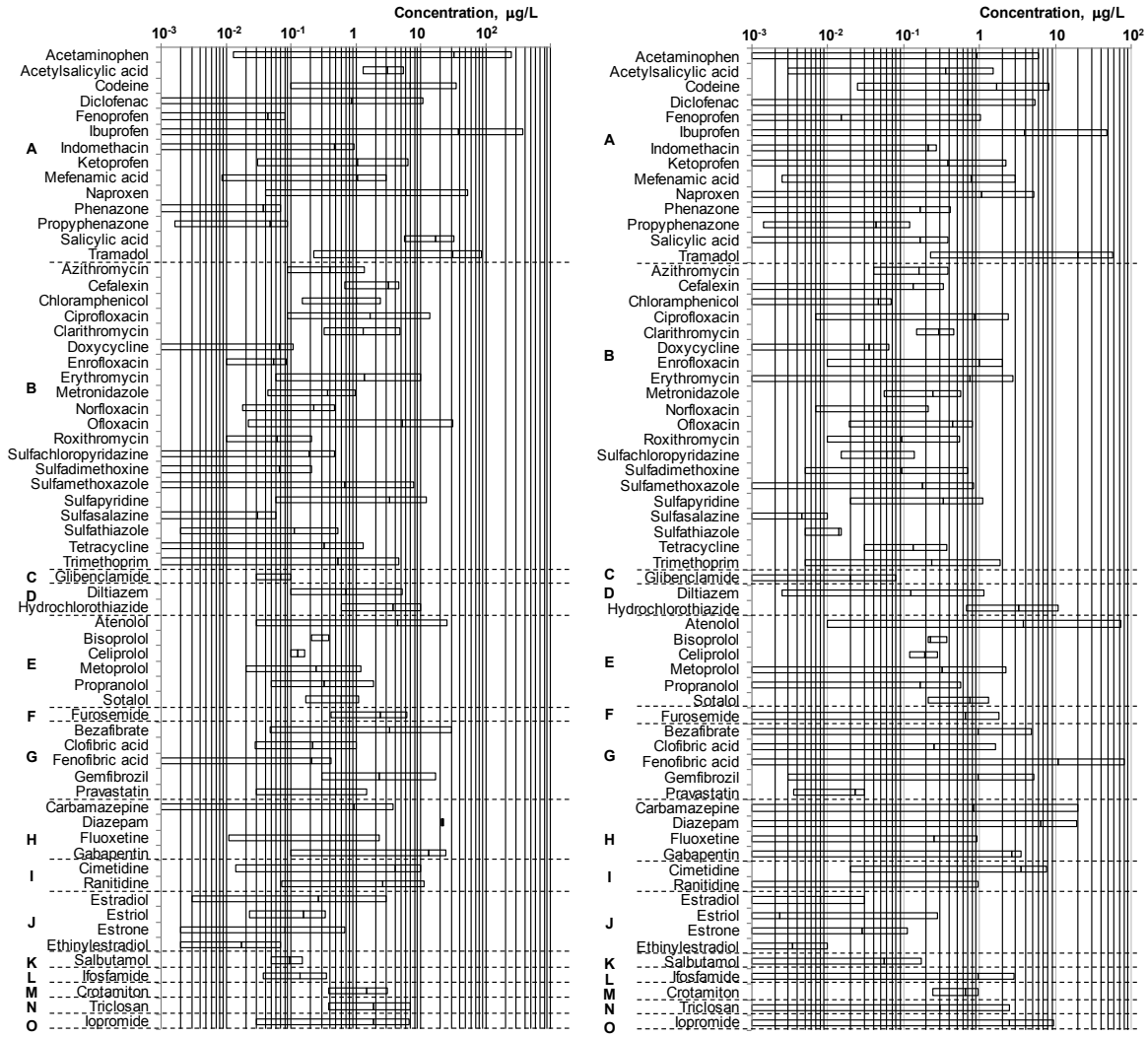


Fig. 1.4: Occurrence of the 64 selected PhCs from 15 therapeutic classes in the influent (left side) and effluent (right side) of a conventional activated sludge system.

1.4 Occurrence in sewage sludge

Investigations on the occurrence of selected PhCs in sewage sludges from different stages of their treatment have been carried out less often than wastewater investigations. As a result, data pertain to a smaller number of compounds and a limited number of full-scale treatment plants. The analysis reported here includes 54 common PhCs that were investigated in the major studies on the issue (among them Joss et al., 2005, Jelic et al., 2011, Suarez et al., 2010, Gobel et al., 2007, Jia et al., 2012, Lindberg et al., 2006, and Martin et al., 2012a,b). Collected data refer to (generally grab-) samples of primary (diverting from the primary clarifier), excess (secondary) and treated (thickened) sludges. The main results, in terms of concentration variability and means of the selected PhCs (grouped according to their therapeutic class), are reported in Fig. 1.5. The number in brackets after the name in the X-axis corresponds to the logarithm of solid liquid/distribution coefficient of the compound Log K_d, (with K_d in L/kg$_{ss}$). As discussed in Paragraph 1.5.3, in an initial analysis, the affinity of a compound for the solid phase is expressed by K_d, which is experimentally determined as the ratio between the concentration of compound sorbed to solid and the concentration of compound in the liquid phase at equilibrium. For most PhCs, removal by sorption is negligible in comparison with the total mass balance, as evidenced by the relatively low K_d values (K_d < 500 L/kg$_{ss}$), corresponding to Log K_d < 2.6 (Ternes et al., 2004b).

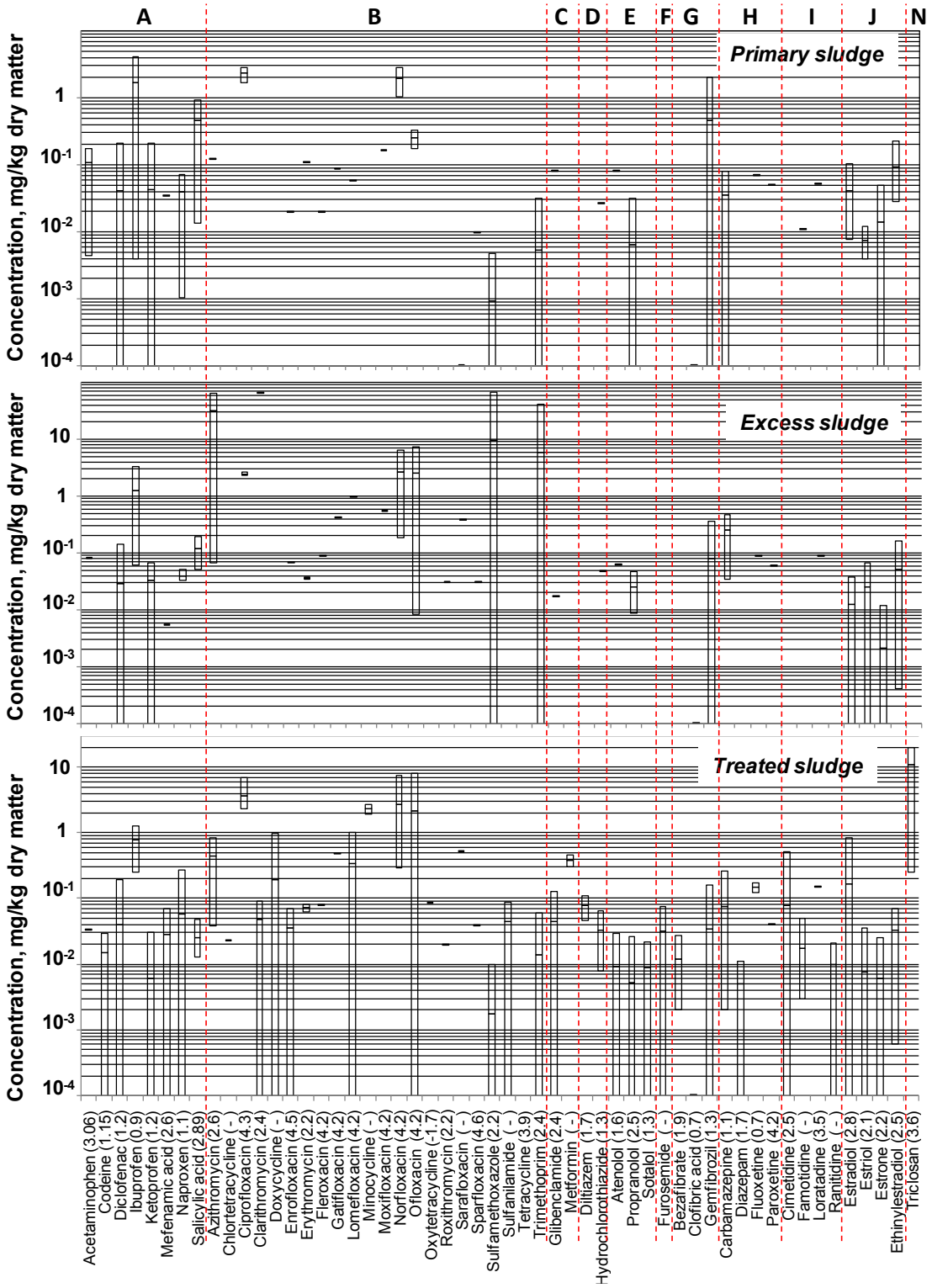


Fig. 1.5: Occurrence of selected compounds in primary (top), excess (middle) and treated sludge (bottom). Number in brackets after the name of the compound corresponds to Log K_d value reported in the literature (K_d is in L/kg$_{ss}$). [K_d data from: Pomiès et al., 2013; Roepke et al., 2005; Hutchinson et al., 1999 Sludge concentration data from: Andersen et al., 2003; Braga et al., 2005; Esperanza et al., 2007; Göbel et al., 2005; Golet et al., 2003; Jelic et al., 2011; Jia et al., 2012; Khan and Ongerth, 2002; Lindberg et al., 2005; Lindberg et al., 2006; Martín et al., 2012 (distib); Martín et al., 2012 (Occurrence); Martin-Ruel et al., 2010; McClellan K and Halden RU, 2010; Miao et al., 2005; Radjenović et al., 2009 analytical; Xu et al., 2007].

In general, data on the presence of PhCs in sludges is few and far between. Antibiotics have been the most analysed and found to be the most abundant. Other classes investigated in sludges are: analgesics and anti-inflammatories, hormones, lipid regulators, psychiatric drugs, and receptor antagonists.

An interesting study recently published by Martin et al. (2012a) details the evolution of the concentration levels of 16 common PhCs (analgesics and anti-inflammatories, antibiotics, lipid regulators, psychiatric drugs, hormones, beta-blockers) in the sludge treatment sequence over the course of the year. These authors found that the time of year may influence the concentration of PhCs in sludge, mainly due to different seasonal consumption (as for ibuprofen and salicylic acid or some antibiotics) and, to a lesser extent, the changes in degradation rates at the elevated temperatures during the summer season. This was found to apply to PhCs such as carbamazepine and ethinylestradiol, whose consumption is not influenced by the season. Gao et al. (2012a) found similar results regarding the concentrations of three types of antibiotics: fluoroquinolones, sulfonamides and macrolides, whose concentrations were slightly higher in winter than in spring and autumn, due to both a greater consumption and a decline in water use in winter. They concluded that the antibiotics in raw sewage are more prone to transfer from the aqueous to the solid phase in winter, causing an increase in the amount of antibiotics in the sludge.

Martin et al. (2012a) found that the concentrations of most of the selected compounds increased between primary and secondary sludges, with the exception of diclofenac, ibuprofen and salicylic acid. They ascribed this behaviour to the different physical-chemical properties of the investigated compounds (namely chemical structures, pK_a, and K_{ow} values) and the different chemical compositions of primary and secondary sludges, which resulted in different absorption/adsorption patterns. The highest concentration of PhCs found in secondary sludge could be explained by the hydrolysis of conjugates or by the higher organic matter content of secondary sludge, which is mainly composed of biomass, considering that the retention of PhCs occurs mainly in the organic fraction of sewage sludge (Andersen et al., 2003; Urase and Kikuta, 2005). The higher concentration of diclofenac, ibuprofen and salicylic acid found in primary sludge could be due to a retention mechanism based on electrostatic interactions (Diaz-Cruz et al., 2003). Despite their hydrophilic potential (negative Log K_{ow}), the fluoroquinolones ciprofloxacin and norfloxacin have a high tendency for sorption due to their zwitterionic character (p$K_{a,COOH}$ = 5.9-6.4; p$K_{a,NH2}$ = 7.7-10.2) (Golet et al., 2003).

Martin et al. (2012a) also noted that the concentrations of most of the investigated PhCs (ibuprofen, naproxen, ketoprofen, salicylic acid, sulfamethoxazole, carbamazepine, propranolol, ethinylestradiol, estriol) decrease in an anaerobically treated sludge, contrasting with data reported by Radjenovic et al., 2009b, who detected an increase in ibuprofen, diclofenac, gemfibrozil, loratadine and glibenclamide. In any case, biodegradation of pharmaceutically active compounds is influenced by desorption of pharmaceuticals from the sludge matrix and microbial activity, and the final outcome will depend on the balance between these two processes in each particular case (Radjenovic et al., 2009a). An increase in the concentrations of compounds such as ibuprofen, diclofenac, gemfibrozil, loratadine and glibenclamide could be explained by lower biodegradation potential of the sludge. Triclosan is present at high concentrations in digested sludge; it has a Log K_{ow} of 4.8 and a pK_a of 7.9, and under wastewater conditions (pH about 7) can be considered a hydrophobic compound prone to sorption onto sludge. Gao et al., 2012b found that tetracyclines manifest strong sorption to sludge via complexation with metals associated with the sludge and cation exchange reactions. Their sorption removal is affected by the temperature, pH, and Ca^{2+} and Mg^{2+} concentrations of the sludge, as well as its organic matter content.

As for the psychiatric drugs, paroxetine and fluoxetine were the antidepressants most retained on sludge (they have a high sorption potential as shown by their Log K_d > 4), whereas carbamazepine showed a wide variability, but in general its partition to solids remained quite low.

1.5 PhCs removal by conventional WWTPs

Over the last decade, most studies have dedicated more attention to the liquid than the solid phase, assessing its impact on the environment following discharge of the effluent from the treatment plant. For this reason, authors have predominantly evaluated the efficiency of selected PhC removal from the *liquid phase*, considering the raw influent and the treated liquid effluent, but not the sludge produced during either primary or secondary treatment. This removal efficiency can therefore legitimately be termed the "apparent removal" or "aqueous phase removal," to distinguish it from the overall removal efficiency, which also takes into account the sludge phase.

According to many authors (Ternes and Joss, 2006; Yasojima et al., 2006; Watkinson et al., 2007; Zorita et al., 2009, Verlicchi et al., 2010b, Gabet-Giraud et al., 2010, Leung et al., 2012), preliminary treatments and primary settling are generally fairly inefficient at removing PhCs (almost always less than 10 %) from wastewaters. Removal depends mostly on sorption potential to suspended solids deposited during primary sedimentation. In some cases, compounds may even be released during this process, presumably due to the simultaneous presence of deconjugable substances, i.e., human metabolites, of these compounds in the raw influent (Carballa et al., 2004, Göbel et al., 2005).

A relatively high removal efficiency has been found for norfloxacin, reported at 28 % (Golet et al., 2003) and even as high as 40 % (Leung et al., 2012). This latter study also reported high efficiency of removal of tetracycline, 40 %, and oxytetracycline, 35 %. As regards tetracycline, this has been tentatively ascribed to a strong tendency of the compound to form complexes with iron (III) ions, which may enhance removal by coagulation and flocculation during sedimentation (Gu and Karthikeyan, 2005).

Leung et al., (2012) found that mechanical coarse screening (> 6 mm) combined with a very short HRT (< 0.5 h) should not be expected to remove micro-pollutants. Chemically enhanced sedimentation moderately increased the removal of norfloxacin (47 %) and tetracycline (41 %) alone.

No significant reduction was found for ibuprofen, ketoprofen naproxen, mefenamic acid or gemfibrozil (Carballa et al, 2004, Behera et al., 2011). This can be correlated to their acidic structures (negative charge of the molecule at pH 7), accompanied by a very low solid–liquid partition coefficient K_d, which results in their presence mainly in the aqueous phase. For the hormone estrone, a higher concentration was observed at the end of primary sedimentation with respect to the influent (Carballa et al., 2004), very likely due to the oxidation of the estradiol present, which would explain the high negative removal efficiencies seen for estrone and the positive reduction of estradiol.

Whatever the configuration of the biological reactor, the main removal mechanisms invariably include: biological degradation, adsorption, absorption, flocculation and sedimentation. Chemical transformations may also occur within the biological reactor, and generally consist of deconjugation of certain micropollutants, that is conversion back to their original compounds, but this is not a particularly influential occurrence (Omil et al., 2010).

The different mechanisms that occur within the biological reactor may be favoured by different operational conditions (namely: redox, pH, temperature, sludge retention time SRT, and hydraulic retention time HRT) and different reactor configurations (plug flow or complete-mix reactors, single tank or reactors in series with alternate anoxic-oxic-anaerobic compartments), as discussed in Paragraph 1.5.4.

CAS processes are not able to efficiently remove all the different kinds of PhCs (Monteiro and Boxall, 2010) for various reasons. In particular, PhCs are designed to be biologically stable, and their sorption tendency depends on the types and properties of both the suspended solids (sludge) and the PhC molecule, not to mention the conditions inside the bioreactor, mainly pH, redox potential and temperature.

As preliminary and primary treatments are fairly inefficient at removing PhCs, raw influent can be considered to possess the same pollutant load as the influent to the biological tank (hence, c_{raw}= c_{inf} in Fig. 1.2). Moreover, Q_{inf} can be assumed as equal to Q_{eff}. As a consequence, removal from the liquid phase $\eta_{aqueous}$ can be evaluated by applying eq. 1.1:

$$\eta_{aqueous} = \frac{Q_{inf}\ c_{inf} - Q_{eff}\ c_{eff}}{Q_{inf}\ c_{inf}} \text{ x } 100 = \frac{c_{inf} - c_{eff}}{c_{inf}} \text{ x } 100 \qquad (1.1)$$

where Q is the average influent (subscript inf) or effluent (subscript eff) flow rate expressed in terms of L/d, c is the average concentration in the influent (subscript inf) or in the effluent (subscript eff), µg/L, as shown in Fig. 1.2.

A limited number of investigations have thus far considered the WWTP as a whole: a black box with only *one* inlet (influent water) and *two* outlets (namely effluent water and treated sludge). Accordingly, the overall removal efficiency can be evaluated by means of 1.2:

$$\eta_{overall} = \frac{Q_{inf}\ c_{inf} - \left(Q_{eff}\ c_{eff} + P_{sludge}\ c_{sludge}\right)}{Q_{inf}\ c_{inf}} \text{ x } 100 \qquad (1.2)$$

where P_{sludge} is the sludge production rate (tons/d) and c_{sludge} is the concentration of PhC in the treated sludge (ng/g dry matter).

The difference between overall and aqueous removal is the fraction that is sorbed to sludge matter; as a consequence, $\eta_{aqueous}$ is expected to be higher than $\eta_{overall}$.

Fig. 1.6 shows the variability ranges and the mean value of the removal efficiencies $\eta_{aqueous}$ for the 64 selected PhCs (listed in Table 1.1) based on data presented in the review by Verlicchi et al. (2012c). The graph only reports PhC removal, and does not show any release that may occur. An in-depth analysis of this is reported in the cited review, whereas in this Chapter only a few cases will be discussed.

Out of the 64 compounds, data are not available for 4 PhCs: the antibiotic sulfasalazine, the beta-blockers bisoprolol and celiprolol and the anti-neoplastic ifosfamide. The best average removal efficiencies (> 75 %) were found for 15 PhCs, with the highest values (> 95 %) for salicylic acid, estriol and chloramphenicol. 23 compounds showed good removal, in the range 50-75 %, whereas for 17 compounds the removal was modest (25-50 %) and quite lowfor the remaining compounds , as in the case of metoprolol, fenofibric acid, tramadol, carbamazepine and diazepam.

As mentioned earlier, the extent to which a compound can be removed in a CAS system depends on many factors: the chemical and physical properties of the compound, wastewater composition, operational conditions and reactor configurations. Hence, high variations in reported removal were observed for most compounds (e.g., diclofenac, ketoprofen, clarithromycin, atenolol, propranolol, salbutamol, etc., as shown in Fig. 1.6), and no clear and definitive conclusions can be drawn on their removal, and even less can be stated about the fate of a particular therapeutic group.

Among the influential operating parameters (HRT, SRT, T, redox and recirculation ratio), SRT seems to be the most critical for activated sludge design, as it affects the treatment process performance, aeration tank volume, sludge production and oxygen requirements. It has been proven that longer SRT improves the removal of most of the PhCs during sewage treatments (Gobel et al., 2007, Suarez et al., 2012). Indeed, WWTPs with high SRTs allow the enrichment of slowly growing bacteria, and consequently the establishment of a more diverse biocoenosis with broader physiological capabilities (e.g., nitrification or the capacity for certain pathways) than WWTPs with low SRTs (Clara et al., 2005a). All of these parameters will be taken into consideration in the following discussion of the behaviour of specific compounds under particular conditions.

Acetaminophen, ibuprofen, acetylsalicylic acid, salicylic acid, estrone, estriol and estradiol were efficiently removed by CAS systems. Biodegradation of both acetaminophen (Gomez et al., 2007) and ibuprofen (Clara et al., 2005b) are known to be rapid. Diclofenac, on the other hand, was one of the selected PhCs that showed a modest removal efficiency (< 29 %). This may be due to the combination of degradation in wastewater and the liberation of additional diclofenac molecules by

deconjugation of glucuronidated or sulphated diclofenac and/or its desorption from particles (Zorita et al., 2009). According to Cirja et al., 2008 compounds with chlorine groups within the molecule may more readily persist during biological treatment. This could explain the poor average removal efficiencies reported for diclofenac and clofibric acid (on average < 40 %).

For fluoroquinolones (namely norfloxacin, ciprofloxacin, enrofloxacin and ofloxacin), adsorption is a potentially major elimination process. Although these compounds are very hydrophilic and zwitterionic (Xu et al., 2007), their higher concentrations in sludge (Fig. 1.5) and their percentage partition onto sludge (Fig. 1.7) support this conclusion.

As regards sulfamethoxazole, Gobel et al., (2007) observed that in some cases a release occurred due to the presence of metabolites in the influent that can subsequently be transformed into their parent compounds during biological processes.

Macrolides, namely erythromycin, clarithromycin and roxithromycin, were removed to a lesser extent in CAS systems. One possible reason is that sometimes particles larger than 0.45 μm are not included in the analysis. This may lead to an underestimation of the concentrations of these compounds in the influent (Gao et al., 2012a). Gobel et al., (2007) also proposed a gradual release of the macrolides from faecal particles during biological treatment as an explanation for the possible negative removal efficiencies sometimes observed. According to Xu et al., (2007) the conjugated metabolites in raw influent samples can be deconjugated during the treatment. They also propose that analyte behaviour, such as adsorption to particles, may be altered by changing physical-chemical parameters during the treatment process, thus influencing the removal efficiency.

Modest to good removal efficiencies were found for the lipid regulators, which, however, displayed quite wide variability ranges, in particular for bezafibrate, gemfibrozil and clofibric acid. Modestly average removals were observed for the beta-blockers, in particular for metoprolol (< 20 %). It is possible that microbial clearance of conjugates could be responsible for an underestimation of its removal efficiency, as this is well known to influence the balance in WWTPs (Andersen et al., 2003).

Carbamazepine is quite a stable compound, and may even be considered an anthropogenic marker (Clara et al., 2004a). Due to its hydrophilic nature, it is removed from wastewater by sorption onto sludge. It has quite often been detected at a higher concentration in the CAS effluent. This may be due to conversion of carbamazepine glucuronides and other conjugated metabolites to the parent compounds by enzymatic processes in the CAS (Monteiro, 2010).

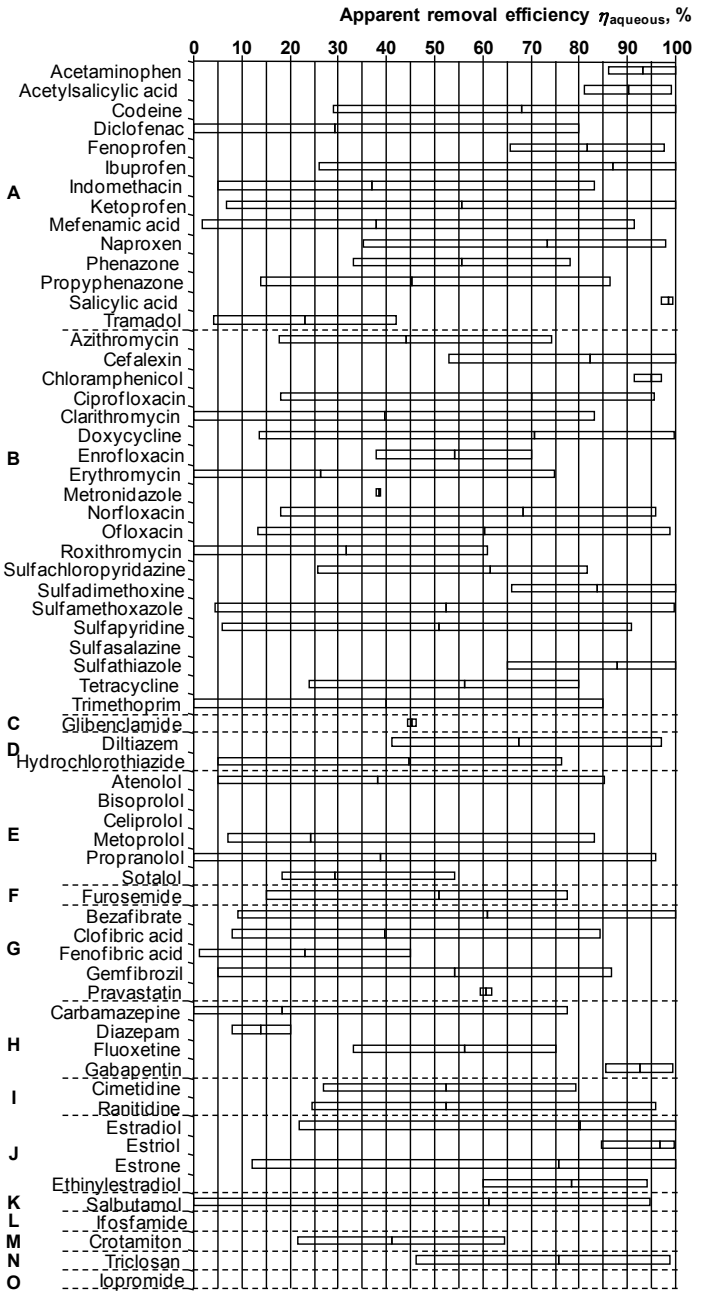


Fig. 1.6: Average removal efficiencies from the liquid phase for the selected compounds

It is important to observe that the term *removal* in CAS quite often implies conversion of the original PhC (parent compound) to other different compounds (metabolites) rather than complete mineralization (*elimination*). Moreover, it is important to note that low removal efficiencies could also be due to the fact that contaminants are present at *very low* concentrations in the influent, and unavoidable instrumental errors may affect their "observed" removal values (Clara et al., 2005b; Verlicchi et al., 2012c). At the other extreme, high removal efficiencies, greater than 99 %, which corresponds to a reduction of the influent concentration of two orders of magnitude, may nevertheless not be enough to consistently reduce the PhC concentrations to a low level of risk to aquatic life. For instance, if ibuprofen presents an influent concentration of 350 μg/L, even if 99 % is removed, its final concentration would still amount to 3.5 μg/L, i.e., a considerable mass load when discharged by the WWTP, as discussed below.

1.5.1 Solid–liquid partition and pharmaceutical "loss" through biodegradation

As reported above, sludge tends to concentrate poorly degradable micropollutants. These are quite often hydrophobic substances with a high sorption potential. High aqueous removal efficiencies for some PhCs would seem to indicate very efficient removal during the treatments. However, only a certain fraction of the total mass is really lost (degraded); for some compounds a considerable portion of the influent mass load could accumulate onto the sludge. Thus, determining the mass balance at a particular WWTP requires evaluation of the percentage mass loads of the selected PhCs discharged with the effluent, sorbed onto to sludge and removed during biological treatment, with respect to the influent mass load. Table 1.2 reports the corresponding fractions reported by different investigations that performed both liquid (raw influent and CAS effluent) and solid phase (sludge) analysis. Where available, the SRT of the investigated plant is reported. This parameter seems to be one of the factors that can influence the behaviour of micropollutants in biological reactors, as will be discussed later (Paragraph 1.5.4).

Table 1.2: Fractions of selected PhCs removed via sorbtion to sludge and discharge with secondary effluent during biological treatment, with respect to the influent mass load.

Class	Compound	SRT [d]	Biodegraded %	Sorbed %	in Effluent %	References
	Acetaminophen	--	> 99	< 0.01	<0.2	Gao et al., 2012b
Analgesics and anti-inflammatories **A**	Diclofenac	4-60 6 16 <20 >50	5-45 25 10 5 10-30	<5 <5 5 0 0	55-95 70-75 85 95 70-90	Joss et al., 2005 Jelic et al., 2011 Jelic et al., 2011 Suarez et al., 2010 Suarez et al., 2010
	Ibuprofen	4-60 2 <20 >50 >20	90-100 <5 35-40 95 96	<5 <5 0 0 0	0-10 95-100 60-65 5 4	Joss et al., 2005 Clara et al., 2005b Suarez et al., 2010 Suarez et al., 2010 Fernandez-Fontaina et al., 2012
	Indomethacin	6 16	27 40	0 <5	73 58-60	Jelic et al., 2011
	Ketoprofen	6 16	70 <95	0	30 5-10	Jelic et al., 2011
	Mefenamic acid	6 16	65 55-58	7 <30	28 <20	Jelic et al., 2011
	Naproxen	10-30 6 16 <20 >50 >20	55-85 77 95-98 5 85-90 91	<5 0 0 0 0 0	15-45 23 <5 95 10-15 9	Joss et al., 2005 Jelic et al. 2011 Jelic et al., 2011 Suarez et al., 2010 Suarez et al., 2010 Fernandez-Fontaina et al., 2012
Antibiotics **B**	Azithromycin	10-30	< 40	< 10	60-90	Gobel et al., 2007
	Chloramphenicol	6	0	0	100	Jelic et al., 2011
	Chlortetracycline	--	100			Gao et al., 2012b
	Ciprofloxacin	10-12 20	< 10 < 10	70-80 77	≤30 <4	Golet et al., 2003 Lindberg et al., 2006
	Clarithromycin	< 20 >50 <20 6 16	< 10 90 <10 0 0	< 5 <5 ≤10 18 <45	75-90 10 >90 82 55-60	Gobel et al., 2007 Gobel et al., 2007 Gobel et al., 2007 Jelic et al., 2011 Jelic et al., 2011
	Doxycycline	---	47	3	50	Gao et al., 2012b
	Enrofloxacin	20-25	19	65	17	Jia et al., 2012
	Erythromycin	<20 >20	20 93	0 0	80 7	Suarez et al., 2010 Fernandez-Fontaina et al., 2012
	Lomefloxacin	20-25		60	40	Jia et al., 2010
	Metronidazole	6 16	 15-18		100 82-85	Jelic et al., 2011
	Norfloxacin	10-12 20	< 10 < 10	80-90 72	≤ 20 < 4	Golet et al., 2003 Lindberg et al., 2006
	Ofloxacin	20-25		60	40	Jia et al., 2012
	Oxytetracycline	---	37	2.2	61	Gao et al., 2012b
	Roxithromycin	4-30 <20 >20	< 60 18 93	< 5 2 0	>35 80 7	Gobel et al., 2007 Suarez et al., 2010 Fernandez-Fontaina et al., 2012
	Sulfamethoxazole	--	>89	< 0.1	11	Gao et al., 2012b

Class	Compound	SRT [d]	Biodegraded %	Sorbed %	in Effluent %	References
		4-12 <20	50-90 20	< 5 0	10-50 80	Gobel et al., 2007 Suarez et al., 2010
	Sulfapyridine	10-30	≤ 70	< 10	≥30	Gobel et al., 2007
	Tetracycline	---	93	7.1		Gao et al., 2012b
	Trimethoprim	<50 <20 6 16 <20 >20	~90 <10 40 38-40 18 78	≤5 ≤5 < 5 5-10 0 0	~10 >90 < 60 50-55 72 22	Gobel et al., 2007 Gobel et al., 2007 Jelic et al., 2011 Jelic et al., 2011 Suarez et al., 2010 Fernandez-Fontaina et al., 2012
Antidiabetics **C**	Glibenclamide	6 16 15	 73	<10 60 7	90-95 40 20	Jelic et al., 2011 Jelic et al., 2011 Jelic et al. 2011
Antihypertensives **E**	Hydrochlorothiazide	6 16		100 100		Jelic et al., 2011
Beta-blockers **G**	Atenolol	6	< 70	< 5	< 35	Jelic et al., 2011
	Metoprolol	6 16	~35 0	0 0	~65 100	Jelic et al., 2011
	Sotalol	6 16	10 <50	< 5 <5	< 90 50	Jelic et al., 2011
Diuretics **H**	Furosemide	6 16	35-40 75-80	<5 2-5	60-65 20	Jelic et al., 2011
Lipid regulators **I**	Bezafibrate	6 16 2	12 <80 45-50	2 <5 <5	86 20-25 50	Jelic et al., 2011 Jelic et al., 2011 Clara et al., 2005b
	Gemfibrozil	6 16	0 90	3 <5	97 5-10	Jelic et al., 2011 Jelic et al., 2011
	Pravastatin	6 16	45 62	0 2	55 <40	Jelic et al., 2011
Psychiatric drugs **J**	Carbamazepine	--- 4-60 6 16	-41 <40 22 0	0.6 <5 3 5	141 >60 75 95	Gao et al., 2012b Joss et al., 2006 Jelic et al., 2011 Jelic et al., 2011
	Diazepam	6 16	0	42 65	58 35	Jelic et al., 2011
	Fluoxetine	<20 >50 >20	80 90 78	0 0 2	20 10 20	Suarez et al., 2010 Suarez et al., 2010 Fernandez-Fontaina et al., 2012
Receptor antagonists **K**	Cimetidine	6 16	42 60	4 5-8	54 32-35	Jelic et al., 2011
	Famotidine	6 16	< 10 80	10 20	85 0	Jelic et al., 2011
	Ranitidine	6 16	< 20 75	< 5 <5	80 20-25	Jelic et al., 2011
Hormones **L**	Estradiol, E2	10-30 5-15	85-99 93	<5 0	<15 7	Joss et al., 2004 Zhou et al., 2012
	Estrone, E1	10-30 5-15	35-97 95	≤5 0	5-60 5	Joss et al., 2004 Zhou et al., 2012
	Ethinylestradiol, EE2	10-30 <20 >50 5-15	45-95 25 80-90 25	≤5 5 0 63	5-50 70 10-20 12	Joss et al., 2004 Suarez et al., 2010 Suarez et al., 2010 Zhou et al., 2012
	Estriol, E3	5-15	100			Zhou et al., 2012
Beta-agonists **M**	Salbutamol	6 16	<60 40-42	<5 2	<45 55-60	Jelic et al., 2011
Contrast agents **Q**	Iopromide	10-30	20-95	<5	5-80	Joss et al., 2005

1.5.2 Considerations about biological degradation and sorption removal mechanisms

Biodegradation of PhCs may occur through (*i*) metabolic reactions in which the pollutant is used as a source of primary carbon or nutrients for microorganism growth (anabolic reactions) and/or as an energy source (catabolic reactions), or (*ii*) co-metabolic reactions in which the pollutants are transformed by the action of extracellular polymeric enzymes (called EPS in Fig. 1.2) produced by the cells, but without any benefit for the microorganisms. It is less probable that the biological compartment contains specific microorganisms able to metabolize micropollutants exclusively. For instance Forrez et al. 2008 found that the enzyme ammonium mono-oxygenase, which is involved

in the nitrification processes, was responsible for the degradation of the hormone ethinylestradiol. In any case, CAS systems operating at high SRTs could promote a higher and more specific enzymatic activity through increased cell lysis (Omil et al., 2010). The enzymatic mechanism responsible for the degradation of certain PhCs may be not activated as long as there are more readily degradable carbon or nutrient sources available, as may be the case in conventional municipal WWTPs. In this context, Drillia et al., 2005a found that the antibiotic sulfamethoxazole can serve as a source of both carbon and nitrogen for enriched consortia, but is only biodegraded whenever there is a depletion of carbon and nitrogen or both in the medium. In the presence of acetate and ammonium nitrogen, however, the antibiotic was not degraded and remained unaltered. For this reason, sulfamethoxazole is expected to be detected in many municipal WWTP effluents, only in extended aeration systems will a depletion of carbon and nitrogen source occur, making sulfamethoxazole degradation more likely.

Few studies have investigated the long-term effects of PhCs on the performance of biological reactors, namely removal of COD, nitrogen and phosphorus compounds, and bacteria. Schmidt et al. 2012, investigated the influence of a mixture of ciprofloxacin, gentamicin, sulfamethoxazole, trimethoprim and vancomycin, up to a final concentration up to 30-40 mg/L, on the removal of COD, ammonia and bacteria by activated sludge processes in lab-scale WWTPs. These concentrations are unlikely to be found in urban and hospital wastewater (Verlicchi et al., 2010b), but they may be a feature of pharmaceutical industry wastewaters, as will be discussed in Paragraph 13. Schmidt and colleagues observed that at 30 mg/L of the total antibiotic concentration, the nitrification ended at nitrite, while no nitrification at all occurred at 40 mg/L antibiotic concentration. They also determined that the nitrifiers were more sensitive to antibiotics than heterotrophic bacteria. COD removal in antibiotic-stressed lab plants was not influenced by ≤ 20 mg/L antibiotics, and antibiotics were not found to negatively affect the total viable count of bacteria. Furthermore, removal of antibiotics varied during the observation period, and these fluctuations were not strictly influenced by the total antibiotic concentrations.

Gao et al., 2012a investigated the potential effect of fluoroquinolones on microorganisms in CAS, and concluded that these compounds are unlikely to have adverse effects as their concentrations did not generally exceed the threshold of 8 μg/L at which genotoxic effects may occur. Discussion of the behaviour of some other common PhCs is reported in Paragraph 1.5.3.

Sorption mechanisms are quite difficult to assess and to predict (Stevens-Garmon et al., 2011). As discussed in Paragraph 8.6, these will depend not only on the sorbate in question, but also on the sorbent, i.e., the composition of the solid phase, in particular its organic carbon fraction (f_{oc}) and cation exchange capacity (CEC) (Hyland et al., 2012). Indeed, compounds may absorb into/adsorb onto bacterial lipid structures and the fat fraction of sewage sludge through hydrophobic interactions (this is the case of aliphatic and aromatic groups); adsorb onto polysaccharide structures, which often feature a negative charge, on the outside of bacterial cells through electrostatic interactions (this is the case of amino groups); and/or they can bind chemically to bacterial proteins and nucleic acids. The partitioning between the aqueous and the solid phase is described by the solid–water distribution coefficient $K_{d,}$ i.e., the ratio of the equilibrium concentration of the chemical on the solids to the corresponding equilibrium concentration in the aqueous fraction which analyses different case studies and specific PhCs.

1.5.3 Properties predicting removal in CAS

The behavior of a PhC in conventional WWTPs will depend upon many factors, including the chemical and physical properties of the compound and the configuration and operational conditions of the biological reactor and the settling tank. The properties of a particular compound will influence whether it will remain in the aqueous phase (like many acidic, neutral, and basic compounds), degrade (such as ibuprofen and acetaminophen), or interact with solid particles (such as certain antibiotics, which have a higher potential for adsorption onto sewage sludges). In this context, the chemical structure, volatility, acidity, lipophilicity, biodegradability, and sorption potential of PhCs are the main properties investigated up to now by different research teams and are therefore those that are reported in the succeeding text, with particular focus on their significance, values, and reliability as predictors, based on knowledge about their behavior in a CAS. The popular rules of thumb defining threshold values of each of these properties are also reported, alongside the limitations plaguing their application.

Chemical structure

Poor removal efficiencies in CAS systems have been documented for compounds with complex molecular structures, like those featuring aromatic rings (as in naproxen and ketoprofen), and for small PhC molecules containing halogens groups (like clofibric acid and diclofenac) Kimura et al., 2005. Very small differences in chemical structure can result in very different behavior in the CAS. Take, for example, the hormones estradiol and ethinyl estradiol. Although they have basically the same chemical structure, the latter features an ethinyl group, which results in a great difference in biodegradability. Indeed, microorganisms in biological reactors are able to degrade estradiol quite easily,while ethinyl estradiol is more persistent.

Volatility

Volatility is the tendency of a compound to volatilize that is, to evaporate from the liquid phase into the gaseous phase. This property is strictly correlated to the Henry coefficient H of a compound, defined as the ratio between the concentration of this compound in solution and its concentration in the gas above the solution, at the equilibrium. In fact, Ternes and Joss (2006) found that a significant amount of compound will be stripped in a bioreactor with fine bubble aeration if $H>10^{-3}$. However, most PhCs are characterized by H values $<10^{-5}$ (often $<10^{-10}$), since they are designed to take effect in an aqueous environment (for instance blood) and are therefore rather hydrophilic. As a consequence, the amount of PhCs stripped in the aeration tank of a CAS system is very low (Table 1.3).

Table 1.3: Henry coefficient rule of thumb (Ternes and Joss, 2006)

Parameter	Conditions	Rule of thumb
H	$< 10^{-3}$	Low volatility
H	$> 10^{-3}$	High volatility

Acidity

Acidity indicates whether or not a specific ionic interaction is relevant for the sorption potential of a given PhC. It is measured through the dissociation constant pKa of the compound. pKa can be used to determine the fraction of the dissolved chemical that exists in a neutral, non-ionized state at the system pH. Since pKa is the negative logarithm of Ka ($pKa = - \log Ka = pH - \log([A^-]/[AH])$), it follows that the lower the value of pKa, the stronger the acid, and that a difference in the unit in pKa on a log scale reflects a tenfold difference in acid strength. On the other side, the higher the value of pKa, the stronger the base. Common acidic drugs are ketoprofen (pKa =3.88) and acetaminophen (pKa = 9.5), which are mainly present in anionic form at pH = 7, and common basic drugs are diazepam (pKa = 3.3) and nadolol (pKa = 9.76), which are mainly present in their cationic form. Verlicchi et al., 2012c provides values of pKa for most common PhCs.
The complex molecule of a PhC often contains heteroatoms and multifunctional groups, and can be polar and ionizable. These properties are arguably closely linked to and influenced by the pH of

the mixture. Moreover, many compounds have more than one ionizable functional group (for instance ciprofloxacin, see Fig. 1.7), which will generate several equilibrium constants that have to be considered separately. The degree of ioniziation is correlated to the pH of the solution containing the compound, and as ionized and no-ionized species typically behave differently, this is a crucial factor. For instance, an ionized molecule will generally be more water soluble and less likely to partition to lipid-like substances than its non-ionized form.
Naturally, the potential of a molecule to participate in the environmental ion-exchange processes ubiquitous in soil and sludge systems will also be affected by whether the charge is positive or negative [84] (Cunningham, 2008). At the pH of wastewater, compounds tend to be classified as either non-ionized (neutral) or ionized (basic or acidic). Acidic compounds may carry a negative charge, while basic compounds may carry a positive charge. As reported in detail in the supplementary data of the review by Verlicchi et al., 2012c, at pH = 7, some of the selected PhCs may have a positive charge overall, some a negative charge, and some will be neutral.
Table 1.4 reports the rules of thumb usually adopted for pKa.

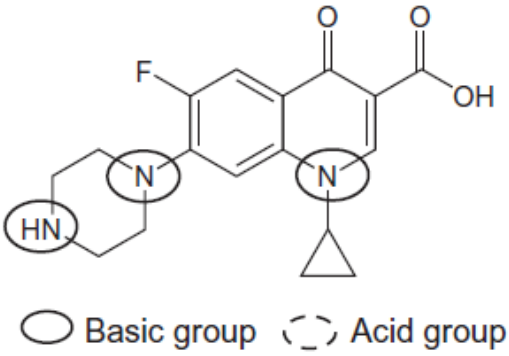


Fig. 1.7: Chemical structure of ciprofloxacin containing both acid and basic

Table 1.4: pKa rule of thumb

Parameter	Conditions	Rule of thumb
pK_a	2-12	Low acidity
pK_a	< 2	High acidity

Hydrophobicity/Lipophilicity

Hydrophobicity is the physical property of a compound that allows it to be repelled by a mass of water. Different coefficients have been used to evaluate the tendency of a substance to stay in the aqueous phase, and the most common parameters are the octanol-water *partition* coefficient (K_{ow}) and the octanol-water *distribution* coefficient (D_{ow}). In the past, K_{ow} was generally used for evaluating and predicting PhC behaviour in the aquatic compartment by considering high K_{ow} values as characteristics of hydrophobic substances, poor water solubility, and in some cases a high potential to sorb on organic material of sludge (Rogers, 1996, as reported in Table 1.5).

Nonetheless, PhCs are complex multifunctional organic compounds, which, in some cases, are ionized in the aquatic environment. Thus, one PhC may generate non-ionized species, which will predominate in partition into octanol from water, and ionized species, which will generally remain in the aqueous compartment. Hence, the pH at which measurements are made for evaluating K_{ow} is a crucial parameter, prompting Cunningham (2008) to recently state that K_{ow} does not properly describe environmental partitioning or dynamic interactions in the environment of polar and ionizable compounds such as PhCs. He suggested that for these compounds the coefficient D_{ow} is more suitable, as it is pK_a dependent at environmental pH. D_{ow} is defined by eq. 1.4, and, according to Schwarzenbach et al. (2003), evaluated through eq. 1.4 and eq. 1.5:

$$D_{ow} \equiv \frac{\text{concentration in } n-\text{octanol}}{\text{concentration in water}} \qquad \text{(eq. 1.3)}$$

$$log_{10}\, D_{ow} = Log\, K_{ow} + Log \frac{1}{1 + 10^{pH - pK_a}} \quad \text{(acidic compound)} \qquad \text{(eq. 1.4)}$$

$$log_{10}\, D_{ow} = Log\, K_{ow} + Log \frac{1}{1 + 10^{pK_a - pH}} \quad \text{(basic compound)} \qquad \text{(eq. 1.5)}$$

In the case of neutral moieties, the two previous correlations result in eq. 1.6:

$$\mathrm{Log} D_{ow} = Log K_{ow} \,. \qquad \text{(eq. 1.6)}$$

According to Wells (2006), since most water treatments are conducted at a pH between 7 and 8, and as D_{OW} simultaneously embodies the concepts of hydrophobicity and ionogenicity, D_{OW} at pH 7–8 is an appropriate physicochemical parameter for understanding and regulating PhC water treatment. Table 1.5 reports the rule of thumb when using lipophilicity to predict PhC behaviour in aquatic compartments.

Table 1.5: Lipophilicity - Rule of thumb

Parameter	Conditions	Rule of thumb	Reference
Log K_{ow}	< 2.5	Low sorption	Rogers 1996
Log K_{ow}	> 4	High sorption	Rogers 1996
Log D_{ow}	< 1	Low sorption	Cunningham 2008
Log D_{ow}	>3	High sorption	Cunningham 2008

However, the parameter Log D_{ow} assumes that any charged species is completely water soluble, and that only the neutral fraction of an acidic or basic trace organic contaminant can partition to the solid phase. In fact, charged species can participate in interactions that are not necessarily electrostatic, hence sorption of those analytes carrying a charge is likely to be a function of both the electrostatic properties of sorbent and sorbate (Suarez et al., 2010) and the van der Waals interactions between them.

Biodegradability

The biodegradability of a compound is measured using the experimentally determined kinetic constant k_{biol}, (Joss et al., 2006). The constant k_{biol} is influenced by many factors: the biochemical versatility of the sludge (correlated to SRT), the bioavailability and chemical structure of the substance to degrade (i.e., the potential of microorganisms to interact with them, which is correlated to its concentration in the aqueous phase, generally very low), the availability of a co-substrate, the fraction of inert matter contained in the sludge (influenced by influent composition and sludge age) (Ternes and Joss, 2006). The degradation rate may also be influenced by temperature, biological reactor configuration and sludge floc dimension and characteristics. Values may vary in a wide range, for instance 0.002 L/(g_{ss} d) for roxithromycin and 350 L/(g_{ss} d) for estradiol (Pomiès et al., 2013).

Temperature can be accounted for by the known model based on Arrhenius equation 1.7.

$$k_{biol,\,T} = k_{biol,T_0} e^{\theta(T - T_0)} \qquad \text{(eq. 1.7)}$$

where $k_{biol,T}$ is the constant (L/g_{ss} d) at the desired temperature T (°C), $k_{biol,T0}$ is the constant at the reference temperature T_0 (°C), and is the temperature coefficient (0.03-0.09).

Biomass is usually approximated by the amount of total or volatile suspended solids (respectively TSS and VSS), which can easily be determined by routine measurements. However a major drawback of utilizing TSS is that only a fraction of them can be considered as viable biomass, while an inert fraction is also present (Cronje et al., 2002). Although this has been successfully overcome, for instance for COD and ammonia transformation, by classifying activated sludge bacteria into heterotrophic and autotrophic fractions, the issue of identifying bacteria responsible for PhC degradation still remains to be addressed (Majewsky et al., 2011).

The sludge characteristics that may influenced the values of k_{biol} are:

- Floc size: the CAS floc has a smaller dimension than that found in MBRs. Cicek et al., (1999), found that the average diameter of particles in the MBR was about 3.5 μm, with 97% of the particles being smaller than 10 μm. Most of the surface area was made up of particles in the size range of 3 to 5 μm in diameter. In a CAS system, only 88% of the particles were smaller than 10 μm, and a large number of particles ranging from 20 to 120 μm were detected. In this case, the main contribution to the total surface area was provided by particles in the size range of 80 to 120 μm. Their analysis showed that the CAS sludge contains large size flocs, while the MBR sludge is primarily composed of single bacteria and small flocs. Ternes and Joss (2006) found that diffusion limits transformation of the compound, which occurs only in the outer floc layers, not contributing to the biological activity. As a result, for many PhCs, the k_{biol} in a CAS is smaller than the corresponding k_{biol} determined for an MBR (Ternes and Joss, 2006, Joss et al., 2006);
- Diversity of the activity of the biomass due to either differences in microbial population or the enzyme activity expressed (i.e., sludge age, as reported by Clara et al., 2005a)
- The fraction of active biomass within the total suspended solids (Joss et al., 2006).

Furthermore, a complex structure and the presence of toxic groups in the compound will make breaking down the molecule more difficult (Omil et al., 2010). Table 1.6 reports the rule of thumb for evaluating biodegradability of a PhC.

Table 1.6: Biodegradability: rule of thumb

Parameter	Conditions	Rule of thumb (Joss et al., 2006)
k_{biol}	< 0.01 L/g_{ss} d	No removal by biodegradation. (< 20 % for strongly sorbing compounds with K_d > 1 L/g_{ss}, due to transfer to sludge)
k_{biol}	0.1-10 L/g_{ss} d	Partial removal (20-90 %)
k_{biol}	> 10 L/g_{ss} d	Removal greater than 90 %. Degradation strongly depends on reactor configuration.

To give a few examples, high values of have been found for ibuprofen (9-35 l/g_{ss} d), paracetamol (58-80 L/g_{ss} d) estradiol (350 L/g_{ss} d) and estrone (600 L/g_{ss} d), while very low k_{biol} levels have been reported for the recalcitrant carbamazepine (0.08 L/g_{ss} d), iopamidol (< 0.36 L/g_{ss} d) and tetracycline (0.44 L/g_{ss} d). Values of k_{biol} for many common PhCs are listed in the review by Pomiès et al., (2013) along with the corresponding references.

Sorption potential

Sorption of an organic contaminant mainly occurs by *absorption*, which involves hydrophobic interactions between the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and the fat fractions of the sludge, and by *adsorption*, where positively charged groups on the PhC (e.g., amino groups) electrostatically interact with the negatively charged surfaces of the microorganisms. These positively charged groups can also bind chemically to bacterial proteins and nucleic acids. As a result, sorption depends on the characteristics not only of the compound (presence of amino groups, COOH groups, etc., in the molecule), but also of the sludge, namely the organic compound fraction (f_{oc}), cation exchange capacity (CEC), suspended solid size and SRT.

While primary sludge contains few microorganisms and a large fat fraction, microorganisms make up the greatest portion of suspended solids in the secondary sludge. Interestingly, Hyland et al., 2012, found that f_{oc} appears to be fairly similar in different activated sludge solids (43-47 %, on average 44 %), appearing relatively unaffected by the location and operational conditions of the treatment plants investigated. Likewise, the CEC of the sludge solids is consistent across sludges (CEC= 54-75 meq/100 g). These authors also confirmed that SRT has no significant impact on the sorption potential of a compound. Instead, sorption potential is often correlated to the solid–water distribution coefficient K_d (=X/S), which describes the ratio between the concentration sorbed onto

sludge and the dissolved concentration S at equilibrium. The pertinent rule of thumb for predicting PhC behaviour is reported in Table 1.7.

The coefficient K_d of various PhCs has been experimentally evaluated for different primary, activated and digested sludges, as well as for soils and sediments (Urase and Kikuta, 2005; Carballa et al., 2008a; Radjenovic et al., 2009, Wick et al., 2009; Martin et al., 2012b). Among these, activated sludges have been investigated the most, and a recent review by Pomiès et al., 2013, reports K_d data for a great number of compounds. Some authors found that for some compounds K_d values are greater in secondary sludge than in primary (Ternes and Joss, 2006, Golet et al., 2003), for example ciprofloxacin, whose K_d was found to be equal to 2000 L/kg_{ss} in primary sludge and 2 10^4 L/kg_{ss} in activated sludge. Despite being an extremely polar compound, it sorbs readily onto the suspended solids in the sewage sludge (Golet et al., 2003). At a neutral pH, this sorption is likely to rely mainly on electrostatic interactions between the positively charged amino group (Fig. 1.7) and the negatively charged surfaces of the microorganisms.

As microorganisms in the secondary sludge make up the greatest proportion of the suspended solids, a relatively high sorption constant of $K_d \approx 20$ L/g of suspended solids and a relatively high sorbed fraction were observed. In contrast, primary sludge contains few microorganisms and has a large fat fraction, so the K_d of ciprofloxacin in the primary sludge is only ≈2 L/gSS. This means that ~20% of the ciprofloxacin is sorbed onto the primary sludge, whereas more than double this load partitions onto the secondary sludge (Ternes et al., 2004b).

When employing literature values for K_d, great care must be taken to choose the right ones. This is because in evaluating K_d, some studies have used PhC concentration in the range μg/l to mg/L (Urase and Kikuta, 2005; Wick et al., 2009), which are higher orders of magnitude than those usually observed in raw municipal wastewaters for many compounds. Moreover, as reported by Stevens-Garmon et al. (2011), some studies have relied on single point calculation rather than sorption isotherms, which may not be suitable at other PhC concentration ranges.

Hyland et al. (2012) suggest that for hydrophobic, non-ionized compounds, partition to organic matter in activated sludges can be estimated using K_d derived from K_{ow} values. The assumption is that the chemical will partition solely into the organic fraction of the solid. However, in general, the sorption of polar compounds and/or compounds with charged functional groups may be governed by a combination of different mechanisms, including electrostatic interactions, van der Waals forces, cation exchange, cation bridging, surface complexation and hydrogen bonding (Cunningham 2008). The extent of sorption does not correlate with their hydrophobicity (hence K_{ow}) as can be seen for neutral compounds. This implies that some electrostatic interactions or others may be driving the specific sorption of these species, but no conclusions can yet be drawn as to the specific nature of these mechanisms and how they may differ between analytes.

Specific sorption coefficients generally decrease with increasing temperature, and the measured effect of temperature on sorption isotherms is ascribable to a combination of the temperature-dependence of both sorption coefficient and solubility (Lajeunesse et al., 2012). K_d may also be influenced by pH (Horsing et al., 2011). For instance many psychiatric drugs (fluoxetine, carbamazepine) present basic properties with their amine moieties (pK_a around 9). Having a higher pH value close to 8 would result in a higher ratio of un-dissociated, and hence more hydrophobic, molecules in the sludge and consequently higher K_d values.

Table 1.7: Sorption potential – Rule of thumb

Parameter	Conditions	Rule of thumb	Reference
K_d Log K_d	> 500 L/kg > 2.67	High sorption	Ternes and Joss 2006
K_d Log K_d	< 500 L/kg < 2.67	Low sorption	Ternes and Joss 2006

As reported in Table 1.2, norfloxacin is mainly removed by sorption onto sludge. It has a high sorption potential (Log K_d ~4) and a high hydrophobic potential (Log D_{ow} =1-3) and, being a positively charged compound, it partly sorbs to solid sludge surfaces by electrostatic interactions. This behaviour can be explained by the fact that microorganisms have a negative charged surface

acting as a cation exchanger, meaning a stronger association will occur between this surface and a positively-charged species than with a neutral one (Schwarzenbach et al. 2003). That being said, atenolol, (K_d ~ 30 L/kg_{ss}, Log K_d ~ 1.4) another positively charged molecule at pH = 7, was observed to possess a noticeably lower potential to sorb onto sludge solids.

However, the compound is less hydrophobic than norfloxacin (Log D_{ow} = - 2.14), suggesting that hydrophobic sorption interactions are still important for positively charged compounds (Stevens-Garmon et al. 2011).

The neutral hormones ethinylestradiol, estradiol and estrone have high Log K_d (2.6-3.2) and high Log K_{ow} (3.7-4.3), but they are not removed by sorption, as they have very high k_{biol} (ethinylestradiol ~10 L/(g_{ss} d) and one order of magnitude higher the other two hormones). The negatively charged compounds atorvastatin and gemfibrozil have Log K_d values in the range 1.5-1.7 and 2-2.3, and Log D_{ow} values of 1.9 and 2.8 respectively. Other negatively charged substances, namely ibuprofen, diclofenac, naproxen, sulphamethoxazole and enalapril, have very low Log K_d (< 1.4) and Log D_{ow} < 1.7. For neutral and negatively charged compounds, increasing Log D_{ow} is indicative of increasing sorption potential. For non-ionic compounds, sorption is assumed to be governed by partitioning to the organic phase in the activated sludge (Stevens-Garmon et al., 2011).

1.5.4 Operational factors affecting PhC removal

There are a number of operational factors likely to influence the biological removal of PhCs in CAS. These include carbon load, hydraulic retention time (HRT), solid retention time (SRT), food-microorganism ratio (F/M) mixed liquor-suspended solids (MLSS), pH, temperature, redox potential and reactor configuration. The following paragraph discusses these factors through interesting case studies found in the literature.

Initial organic carbon concentration and applied organic load – Urase and Kikuta (2005) found higher degradation rates of selected PhCs (hormones, analgesics, lipid regulator, psychiatric drugs) with lower initial organic carbon concentrations. Their investigations, carried out in batch experiments in lab reactors fed with synthetic wastewaters, showed that microorganisms in the activated sludge degrade the target compounds more rapidly in the absence of easily biodegradable substances such as glucose and peptones. The lower TOC operational condition was found to be preferable for the removal of target substances in the batch experiment, as under these conditions, microorganisms are forced to utilize micropollutants as sources of C and N.
Gabet Giraud et al. (2010) found that in low-loaded activated sludge with an applied F/M ratio below 0.1 kg BOD_5 (kg MMLVSS d)$^{-1}$, higher removal was achieved for the ten selected beta-blockers and the investigated estrogens (estrone, estradiol, estriol, and ethinylestradiol) than in medium-loaded activated sludge processes (0.5 kg BOD_5 (kg MMLVSS d)$^{-1}$).

HRT – This parameter determines the mean residence time of soluble compounds within the biological compartment. In this time, PhCs may biodegrade to a greater or lesser extent, depending on their biological degradation kinetics. Based on literature data pertaining to PhC removal collected in their database, Miège et al., (2009) revealed that higher PhC removal occurs at higher HRT. Unsurprisingly, therefore, Yang et al., (2011) found that the contact time required for activated sludge to degrade sulfamethoxazole and sulfadimethoxime is longer than the HRT of 4–6 h usually provided by CAS processes in urban WWTPs.
Gros et al., (2010) and Garcia-Galan et al. (2011) found that those compounds with a half-life, $t_{1/2}$, less than WWTP HRT generally exhibited high removal efficiencies, concluding that $t_{1/2}$ can give us an idea of the time the compounds need to remain in the biological reactor to ensure their efficient removal. In particular they found that three different situations applied: (*a*) for compounds with high removal efficiency and high degradation rate (low $t_{1/2}$), like ibuprofen, naproxen, salicylic acid, acetaminophen and enalapril, and (*b*) for compounds with poor or no elimination and low degradation (high $t_{1/2}$), like carbamazepine, clofibric acid and diclofenac, HRT does not influence compound removal; (c) for compounds with medium removal and moderate degradation rate (including famotidine, ranitidine and pravastatin), HRT seems to play a role, as their removal

efficiencies were higher at increased HRT. Gros et al. (2010) concluded that substances that are biodegradable (high k_{biol} or low $t_{1/2}$) and have low Log K_d (low sludge–water distribution coefficient, corresponding to low tendency to adsorb on sewage sludge) are more influenced by HRT, while compounds with high Log K_d and low k_{biol} are more influenced by SRT. However, there are other PhCs like ibuprofen with high k_{biol} and low Log K_d that are efficiently removed, irrespective of HRT and SRT.
Based on experimental findings on Canadian WWTPs (SRT from 2 to 10 d), Metcalfe et al. (2003) proposed the following correlation for naproxen and ibuprofen, between HRT and percentage PhC removal η:

$$\eta = 1.735\ e^{0.886\ HRT} \qquad \text{(eq. 1.8)}$$

They concluded that due to the high half-lives observed for most of the investigated compounds in WWTP effluents, higher HRTs should be required to enhance compound degradation.

SRT – Many authors (among them Weiss and Reemtsma, 2008) have found that a long SRT promotes the adaptation of different kinds of microorganisms, as well as the presence of slower growing species that could have a greater capacity for removing xenobiotics while simultaneously greatly improving suspended solid separation. This is the case for ibuprofen and diclofenac, as reported by Suarez et al. (2012), who found removal only after the growth of specific bacteria.
For compounds with a significant sorption potential, such as estrogens and sulfamethoxazole, SRT is known to exert a significant effect only on the degree of their transformation (Clara et al. 2005a, Suarez et al. 2012), while no clear correlation was found between SRT and the removal of beta-blockers, carbamazepine, ciprofloxacin, ofloxacin and norfloxacin (Vieno et al., 2005).
For lipophilic substances, in general, the retention time inside the reactor may be more strongly influenced by the SRT rather than the HRT of the plant, which could explain how compounds with relatively slow kinetics can be biologically transformed during secondary treatment steps operating at high SRTs. Varying SRT in a secondary biological treatment system may influence the biological activity of the activated sludge, as well as potentially affecting the nature of the organic matter (Hyland et al., 2012). SRT may potentially be indicative of the degree of oxidation of the organic matter present, or it might influence the composition and activity of the biomass or even of the active fraction of the biomass (Joss et al., 2006).
A minimum SRT of 10–15 days has been suggested as necessary to ensure the development of a diverse biocoenosis, comprising nitrification, denitrification and phosphorus removal (Clara et al., 2005a).
An increase in SRT may also cause differences in sludge characteristics and performance. Indeed, Massé et al., (2006) observed a deterioration of sludge settleability and CAS effluent quality in the presence of filamentous bacteria, and therefore an increase in protein and polysaccharide release.
Clara et al. (2005a) found that if a specific substance is degraded in an SRT-dependent fashion, a critical value for the sludge age can be determined. In WWTPs operating SRTs below this critical value, effluent concentrations in the range of influent concentrations or a distribution according to the adsorption equilibrium must be expected, whereas degradation will occur in WWTPs operating at SRTs higher than the critical value. Generally speaking, high removal efficiencies and low effluent concentrations are achieved at SRTs higher than 10 days at an environmental temperature of 10 °C. This corresponds to the requirements for WWTPs situated in sensitive areas, according to the urban wastewater directive of the European Community 91/271/EEC (CEC, 1991) in moderate climatic zones.

Sludge characteristics *(floc size, biomass concentration, acclimation)* – Few studies have thus been carried out on this issue. Nonetheless, microscopic analysis carried out by Cicek et al., (1999) showed that with respect to the sludge of a MBR, CAS sludge is composed of larger flocs, fewer free-swimming bacteria, greater amounts of filamentous organisms inside the flocs (see Fig. 5, left bottom), and higher concentrations of nematodes and crawling or free-swimming ciliates. Biomass in CAS has a lower viable fraction than in the MBR. Moreover, metabolic activity and specific enzymatic activity tests showed that overall activity is lower in the CAS than in the MBR

sludge. The CAS contains fewer enzymes in the soluble phase than found in the MBR, and CAS cultures are capable of degrading a narrower spectrum of carbon substrates than MBR cultures.

Microbial communities evolve according to the prevailing environmental conditions, and therefore largely depend on the composition of the incoming wastewater, including its organic loading rate. Kraigher et al. 2008 showed that a significant structural shift in the bacterial community caused by permanent PhC presence occurred only at a concentrations > 50 μg/L, which are unlikely to occur in municipal WWTPs receiving urban effluents. However, interesting considerations are raised by the long-term study conducted by Suarez et al., 2012 on a CAS pilot plant fed by a synthetic mixture containing selected PhCs. They revealed that the removal efficiency observed for naproxen was directly proportional to the concentration of the mixed liquor volatile suspended solids (MLVSS) in the bioreactor. The removal efficiency increased from 27 % to 99 % during the first 300 d of investigation, when the VSS increased from 1 to 4 g/L, and remained stable during the following 300 days. This initial enhancement could be attributable to a possible acclimation of bacteria to this compound. Similarly, in an aerobic pilot reactor, diclofenac removal increased from 0 to 25 % during the first 170 days, which coincides with the death and wash of heterotrophic bacteria and the development of strictly nitrifying biomass. Removal of ibuprofen in an anoxic reactor increased gradually with time from below 16 % (up to day 200) to 75 % (on day 340) (Omil et al., 2010). These examples confirm that the type of bacteria flourishing in biological systems can influence the behaviour of micropollutants to a very significant extent. According to Ternes et al. (2004b) existing microorganisms could acclimate to the presence of PhCs by broadening their enzyme spectrum in response to the lower sludge loading with bulk organics when working at higher SRT. Suarez et al. (2012) confirmed that biological transformation of PhCs follows pseudo-first-order kinetics, the transformation rate being directly proportional to the soluble substance concentration, as well as to the sludge concentration, although the effect of the latter will only be significant for compounds with moderate biological degradation constants. Hence, an increase in SRT will cause an increase in the relative amount of inert mass in the activated sludge (Joss et al., 2006). Majeswsky et al. (2011) found that active heterotophic bacteria, known to govern COD removal, could be considered a determining factor for biological PhC removal.

Internal recirculation ratio – Suarez et al. (2012) found that the effect of an increase in the internal recirculation ratio from 3 to 4 (from the aerobic to the anoxic compartment of the pilot reactor) was relevant for substances with moderate biological degradation constants, such as the psychiatric drug citalopram (0.41 L/g_{ss} d), whose removal efficiency increased from 25 % to 50 %. A slighter improvement (about 10 %) was found in the removal efficiency of compounds with higher $k_{biol,}$ including ibuprofen (k_{biol} =3.7 L/g d), naproxen (k_{biol} =3.3 L/g_{ss} d) and fluoxetine (k_{biol} = 1.6 L/g d). Nonetheless, these three compounds were already transformed to a high extent (70-80 %) at a recirculation ratio equal to 3.

Temperature – The effect of temperature on the efficiency of PhC removal has been investigated by many authors. Among them Vieno et al. (2005) reported that at low winter temperatures nitrification did not occur in the investigated activated sludge plants in Finland, and far lower removal efficiencies were observed for analgesics (naproxen, ibuprofen, ketoprofen and diclofenac) and lipid regulators (bezafibrate). Likewise, Valder et al., (2000) found that removal of ethinylestradiol in activated sludges ceased when the sludge lost its nitrification capacity due to falling temperatures. Suarez et al. (2012) concluded that the influence of temperature is inversely proportional to the biological degradation rate constants of PhCs. As a consequence, temperature is a significant factor for substances with moderate to low k_{biol} that undergo transformation through mechanisms involving microbial activity.

pH value – pH may influence the removal of micropollutants from wastewater by influencing both the physiology of microorganisms (optimal pH for microbial enzyme activities) and the solubility of the micropollutants present in wastewater. Depending on their pK_a values, PhCs can exist in various protonation states as a consequence of pH variation in the aquatic compartments. At pH 6–7, tetracyclines are neutral molecules, and for them adsorption becomes the most incisive removal mechanism. Moreover, Horsing et al. (2011) found that pH can be an important factor for the partition coefficient K_d.

Redox – Suarez et al. (2012) found that anoxic conditions favour the removal of fluoxetine, trimethoprim and erythromycin, while aerobic conditions are better for the removal of naproxen, ibuprofen, hormones, citalopram, sulfamethoxazole and roxithromycin. Their investigations confirmed that operating at different redox conditions could result in an increased microbial diversity and a broader enzyme spectrum inside the biological reactor.

Reactor configuration – Joss et al. (2006) found that where sorption levels are high (K_d > 100 L/kg_{ss}), the impact of dividing the reactor volume into cascades becomes less significant (i.e., the removal of the plug flow, the configuration becomes increasingly similar to a single completely mixed tank, even for compounds with high degradation constant k_{biol}). This is due to the fact that with increasing K_d, the soluble concentration is increasingly controlled by sorption/desorption, while the influent load has limited impact.
Clara et al. (2005a) and McAdam et al. (2010) found that high removal efficiencies and low effluent concentrations of ibuprofen and bezafibrate are achieved at the design criteria for nitrogen removal. Relatively high removal efficiencies for estrogens may be observed in the absence of nitrogen removal, implying that effective biodegradation can proceed in heterotrophically dominated microbial consortia. Vieno et al. (2007) found that atenolol and sotalol were slightly more efficiently eliminated in the WWTPs where nitrogen removal was greater than 60 %, compared with those that removed less than 30 % nitrogen. Similarly, Lajeunesse et al. (2012) found that biological nutrient reactors (BNR), including anoxic-oxic-anaerobic tanks operating at different redox conditions, and microbial environments may contribute to the decomposition of more persistent compounds such as the antidepressants carbamazepine and fluoxetine.
That being the case, it is still not entirely clear how the type of technology affects micro-pollutant removal, as in many cases, discussion is based on data referring to activated sludge reactors, which differ in their configurations, operational conditions and concentration of the influent wastewater. Nonetheless, Behera et al. (2011) found that carbamazepine, metoprolol and triclosan were more efficiently removed in a modified CAS called Daewoo Nutrient Removal (DNR) treatment, comprised of a sludge denitrification tank, anaerobic, anoxic and aerobic zones, which help in the simultaneous removal of nitrogen and phosphorus. The same authors found improved removal efficiencies for clofibric acid, gemfibrozil, atenolol, estriol and estradiol in WWTPs adopting a Symbio treatment, wherein both aerobic and anoxic conditions coexist in a single stage, within a single tank. They ascribed the increase in the removal of those PhCs with the development of a dual zone within the sludge floc, brought about by a controlled air supply to the aeration tank maintaining dissolved oxygen at the desired low level. In this scenario, the outer region of the floc has access to the dissolved oxygen and promotes nitrification, while the inner part is oxygen-depleted and maintained under anoxic (denitrifying) condition, resulting in simultaneous nitrification and denitrification in a single tank.
Suarez et al. (2010) divided PhCs into three groups according to their potential to be removed in a biological reactor. In this system, ibuprofen, fluoxetine and natural estrogens were classed as highly biodegradable compounds under aerobic and anoxic conditions; diclofenac, naproxen, ethinylestradiol, roxithromycin and erythromycin as highly biodegradable compounds under aerobic conditions but persistent in anoxic conditions; and finally sulfamethoxazole, trimethoprim, carbamazepine and diazepam were classed as resistant to biological transformation.

1.6 PhCs mass load discharged by CAS systems

Up to now, attention has been paid to the behaviour of PhCs during their passage through a CAS system and how chemical and physical properties as well as operational and design conditions influence the removal of selected compounds in order to improve it. The amount of compounds not degraded during the treatment still remains in the treated effluent or in the sludge. An attempt to quantify the mass load for selected PhCs discharged by means of municipal CAS effluent has been made in order to define the most critical compounds, according to the amount discharged into the environment.
Mass loads L_i were evaluated for selected PhCs *i* on the basis of the data (PhC mass load and average flow rate and PhC concentrations in many WWTPs) collected in the review by Verlicchi et al. 2012c. These data are reported in the graph in Fig. 1.8 in terms of variability range and average

value. L_i was evaluated via eq. 1.9, using the effluent concentration $c_{i,j,h}$ (h = min, max, average observed value) from the WWTP j, the average treated flow rate Q_j and the population served by the WWTP j. Each mass load is expressed in mg/1000 inhabitants/day.

$$L_{i,j,h} = \frac{c_{i,j,h} Q_j}{\text{served population}} \text{ x } 1000 \qquad \text{(eq. 1.9)}$$

The graph in Fig 1.8 reports, in descending order, the range of variability of mass loads L_i .
As discussed in Verlicchi et al., 2012c, these findings may be affected by different sources of uncertainty, as pointed out in Ort and Gujer (2006), for this reason they have to be considered with caution. That being said, the highest average mass loads (greater than 200 mg/1000 inh/d) were found for the antihypertensive hydrochlorothiazide (368 mg/1000 inh/day), the psychiatric drug carbamazepine (364 mg/1000 inh/day), the receptor antagonist cimetidine (332 mg/1000 inh/day) and the beta-blocker atenolol (316 mg/1000 inh/day), followed by the analgesics/anti-inflammatories: naproxen (295), ibuprofen (273), diclofenac (241), ketoprofen (217) and mefenamic acid (211). The antibiotics clarithromycin (140), trimethoprim (124), ofloxacin (123) and erythromycin (100), exhibited lower average daily mass loads.
It was not possible to correlate the mass load to the sludge production due to lack of data for each WWTP. However, this is a pressing issue as an increase of sewage sludge production has taken place in Europe in recent years. The amount of sludge generated in European countries in 2006 was estimated to be more than 8 million tons, of which 50 % were land applied. Estimates of sewage sludge annual production are of 11.6 million for 2012 (42 % land applied) and more than 13 million for 2020 (44 % land applied) (Milieu et al., 2008). Although land disposal is regulated by European Directives and national laws, none of these regulations take into account the problem of PhCs, which can be transferred to soil after land application of biosolids. This gives them the potential to enter surface water, leach groundwater or to accumulate in vegetation or other living microorganisms. For this reasons, further research is necessary to complete the mass balance and to identify the most urgent mitigation measures required to reduce the impact of this widespread practice on the environment.

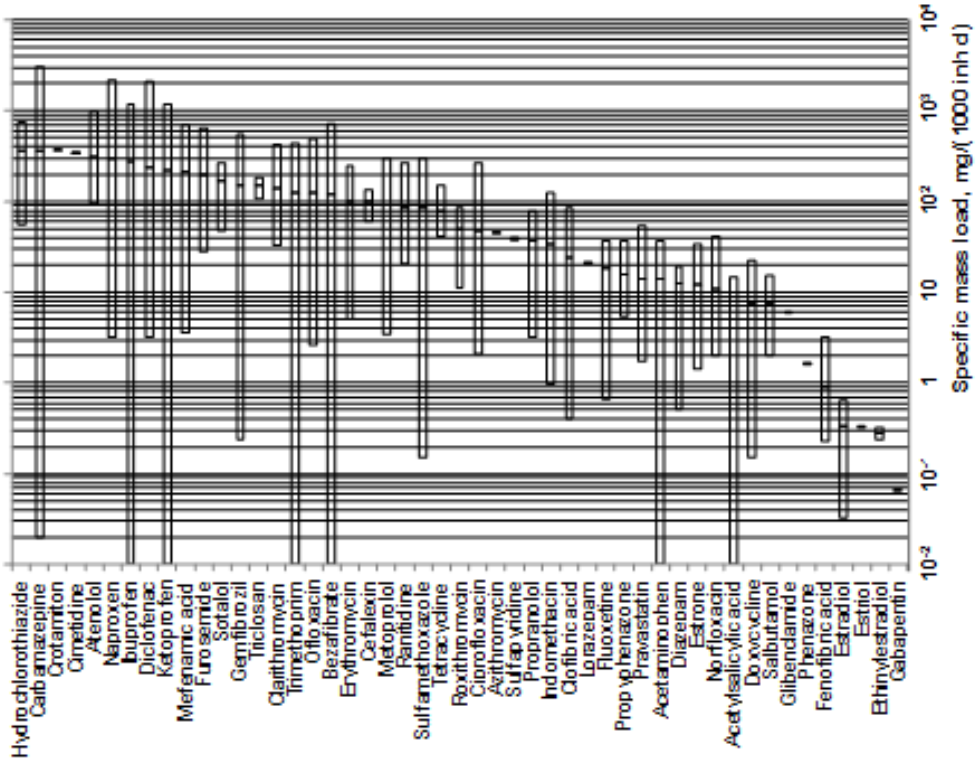


Fig. 1.8: Mass load in CAS effluent discharged into the environment mg/(1000 inh d)

1.7 Environmental risk of residual PhCs in treated effluent and sludge

Ecotoxicological risk assessment was performed for PhCs in secondary effluents and treated sludge by means of risk quotient *RQ*, which is evaluated by means of eq. 1.10:

$$RQ = \frac{MEC_i}{PNEC_i} \qquad i=1 \text{ (water)}, 2 \text{ (digested sludge)} \qquad \text{(eq. 1.10)}$$

where MEC_i is the measured environmental concentration of the PhC in the secondary effluent (i=1) or digested sludge (i = 2) and $PNEC_i$ is the corresponding predicted no-effect concentration in water (i = 1) or sludge (i = 2). In EC (2003) and EMEA (2006), $PNEC_{water}$ values were estimated from the lowest acute or chronic toxicity data reported in literature from toxicological studies using bacteria, algae or fish species as target organisms and applying an assessment factor of 1000, which takes into account inter-species variations in sensitivity, intra-species variability, and laboratory data to field impact extrapolation, as already discussed and reported in Verlicchi et al., 2012c. A different approach was adopted for estimating $PNEC_{sludge}$. As to date little toxicological data regarding PhCs in terrestrial organisms has been reported in the literature, $PNEC_{sludge}$ values were estimated from $PNEC_{water}$ values by applying the equilibrium partition approach, as suggested by the European Commission (EC, 2003) and according to Gonzalez et al., 2010 and Martin et al., 2012b as follows:

$$PNEC_{sludge} = PNEC_{water} \times K_d \times 1000 \qquad \text{(eq. 1.11)}$$

where K_d, the solid–water partition coefficient referred to the sludge is in L/kg_{ss} and $PNEC_{sludge}$ in μg/L.
Common criteria for interpreting RQ values in risk assessment studies establish different risk levels: low risk (RQ< 0.1) medium risk (0.1<RQ< 1) high risk (RQ> 1) (Hernando et al., 2006).

1.7.1 RQ in treated effluent

Fig. 1.9 reports the RQ ranges and the corresponding average values of some of the selected compounds found the secondary effluent, taken from graph on the right in Fig. 1.9. PhCs are listed in descending order of risk on the Y-axis, alongside their $PNEC_{water}$ (μg/L). PNEC values are those used in Verlicchi et al., 2012c, Roepke et al., 2005 and Hutchinson et al., 1999.

As shown in Fig. 1.9, out of the 50 selected PhCs, average effluent concentration data yields high environmental risk figure for 12 compounds (from erythromycin to azithromycin), while a moderate risk is posed by 14 substances (from estradiol to metronidazole), and a low risk by the remaining 24 compounds.

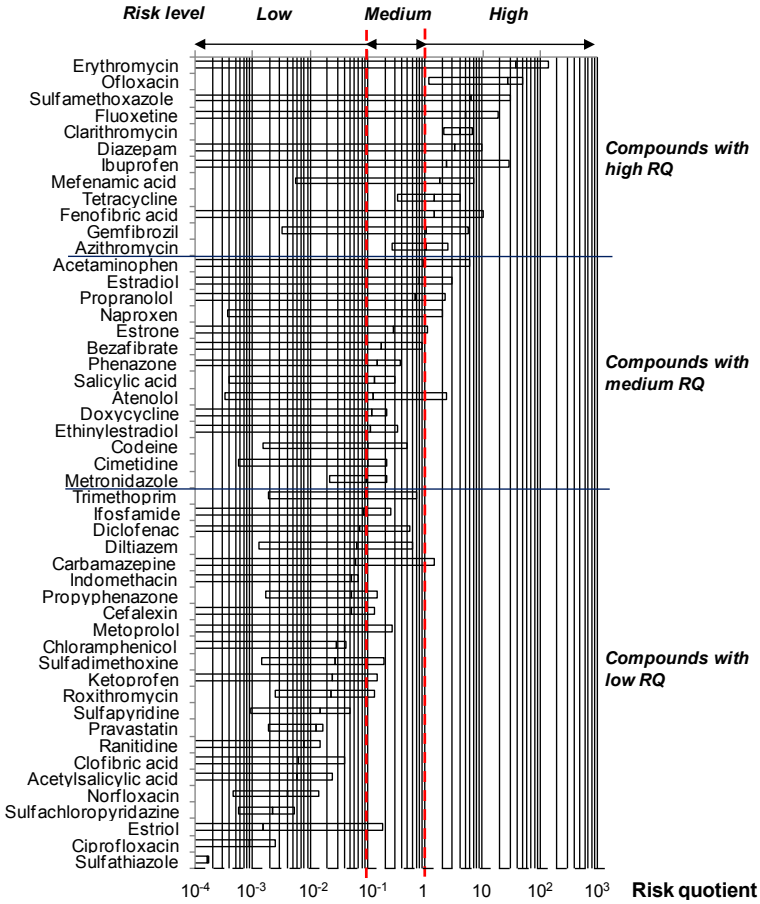


Fig. 1.9: Mass load in CAS effluent discharged into the environment mg/(1000 inh d).

The most critical compounds are antibiotics (6 pose a high risk and 2 a moderate one), psychiatric drugs (fluoxetine and diazepam present a high risk), analgesics and anti-inflammatories (for two compounds RQ > 1 and for 5 compounds RQ is between 0.1 and 1) and the lipid regulators gemfibrozil and fenofibric acid).

Once the effluent is discharged into the surface water body, dilution occurs, and its extent will depend on the receiving body flow rate. This will result in some decrease in the concentration of the pharmaceutical compounds. If a dilution factor equal to 100 can be assumed, the risk quotient in surface water for all the compounds decreases by two orders of magnitude. According to data reported in Fig. 1.10, only two compounds (erythromycin and ofloxacin) still have RQ > 0.1 (medium risk), on the basis of the average PhC concentration, the remaining compounds having an RQ < 0.1. However, if the environmental risk assessment is based, more prudently, on the maximum PhC concentration measured, the risk level is still high for erythromycin, and medium for ofloxacin, sulfamethoxazole, fluoxetine, diazepam, ibuprofen, and fenofibric acid).

The dilution effect is vital for mitigating the adverse effects posed by the presence of micropollutants in receiving water bodies. In this context, Al Aukidy et al. 2012 show the importance of the hydrodynamic characteristics of the receiving water body (mainly flow rate) and the risk related to effluent dominant rivers for which the dilution effect is quite modest (about 1 or less), resulting therefore in an equally modest mitigation of the risk. In any case, it is important to remember, as remarked by Martin et al. (2012b) that even if acute toxic effects in the aquatic environment may seem unlikely, chronic environmental exposure to toxic chemicals may still harm aquatic species with a long-life cycle.

1.7.2 RQ in treated sludge

Fig. 1.10 reports the RQ ranges for treated sludge, based on the concentration data reported in Fig. 1.5 and available $PNEC_{water}$ data. K_d values are those reported in brackets, after the name of each substance, on the X-axis in Fig. 1.5. The compounds responsible for the highest environmental risks in digested sludges (based on average concentrations detected in digested sludge sample) are the six antibiotics: oxytetracycline, erythromycin, azithromycin, ofloxacin, tetracycline and clarithromycin; the two analgesics/anti-inflammatories ibuprofen and naproxen; the two hormones estradiol and ethinylestradiol; the lipid regulator gemfibrozil; and the psychiatric drug fluoxetine.

The risk posed by the presence of PhCs in digested sludge applied to land can be evaluated according to European Commission Technical Guidance on Risk Assessment EUR 20418 EN/2 [EC, 2003] as the ratio between their predicted environmental concentration in soil (PEC_{soil}) and the corresponding $PNEC_{soil}$. This document recommends evaluating PEC_{soil} one year after one sludge-dose application by means of eq. 1.12:

$$\mathrm{PEC_{soil}} = \frac{c_{\mathrm{sludge}}\ x\ APP_{\mathrm{sludge}}}{DEPTH_{\mathrm{soil}}\ x\ RHO_{\mathrm{soil}}} \qquad \text{(eq. 1.12)}$$

where c_{sludge} is the measured concentration in digested sludge (μg/kg dry matter), APP_{sludge} is the application rate of dry sludge onto soil (0.5 kg/m^2 for agricultural soils), $DEPTH_{soil}$ is the mixing depth (0.20 m for agricultural soils) and RHO_{soil} is the bulk density of wet soil (1700 kg/m^3 for agricultural soils).

$PNEC_{soil}$ is evaluated by means of an equation formally similar to eq. 1.11, using K_d values for soil.

Very few values for soil are available in the literature, and as remarked in Paragraph 8.6 a considerable difference has been found between K_d in sludges and soils in some cases, as reported by Martin et al. (2012b). In that study they found a drastic decrease of RQ values after sludge application onto soil. The only toxic effect expected is the one caused by estradiol, since its RQ has been calculated as 2.7. This means that an ecotoxic risk is still present to terrestrial ecosystem in spite of the significant decrease in the concentration of estradiol from digested to amended digested sludge. Additionally, Yang et al. (2011) found that sorption onto sludge of sulfonamide antibiotics like sulfamethoxazole and sulfadimitidine is reversible. This implies that they can be released from the sludge upon their release into the natural environment, highlighting the fact that these compounds pose a potential risk for the environment if there are no suitable processes to eliminate them from the sludge.

Recent studies investigated the occurrence and distribution of PhCs in soil irrigated with reclaimed water (Ternes et al., 2007) and soil that received biosolids from urban sewage treatment plants (Gielen et al., 2009). They confirmed that conventional WWTPs, currently adopted all over the world, are not efficient enough to remove these micropollutants from wastewaters and sludge, and as a result they found their way into the environment. Once in the environment, pharmaceutically active compounds can produce subtle effects on aquatic and terrestrial organisms, especially on the former since they are exposed to long-term continuous influx of WW effluents as remarked in Verlicchi et al. (2012c) and biosolids as pointed out in Martin et al., (2012a).

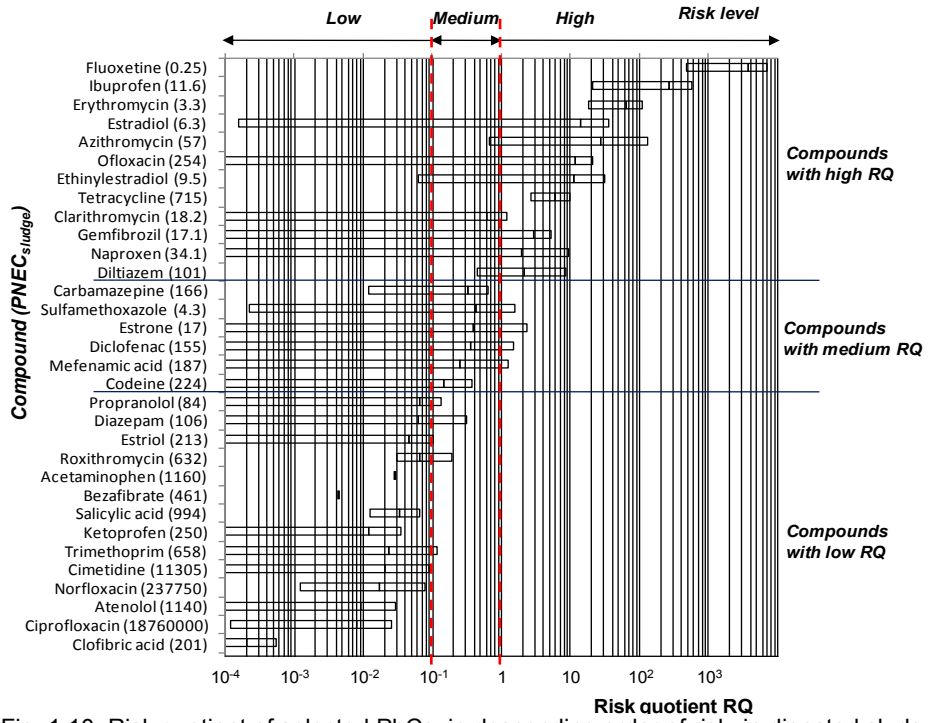


Fig. 1.10: Risk quotient of selected PhCs, in descending order of risk, in digested sludge

1.8 The most critical compounds

The current study highlights the fact that the most critical PhCs, namely those posing a high risk to the environment, will depend on the matrix investigated: secondary effluent or treated sludge. If we compare these groups of compounds with those with the highest mass load discharged into the environment reported in Fig. 1.9, we find that the two groups do not overlap, as shown in Fig. 14. In fact, this graph shows the RQ of the selected compounds in both sludge and water (the two series of histograms previously shown in Fig. 1.9 Fig. 1.10) together with their corresponding mass load (the black line, data from Fig. 1.11). Compounds are reported from the highest to the lowest mass load. Using these criteria, the most critical compounds are found to be: ibuprofen (high RQwater, high RQsludge and high load), fluoxetine, ofloxacin, erythromycin, tetracycline, azithromycin (high RQwater, high RQsludge), and gemfibrozil, estradiol and ethinylestradiol (high RQwater, medium RQsludge).

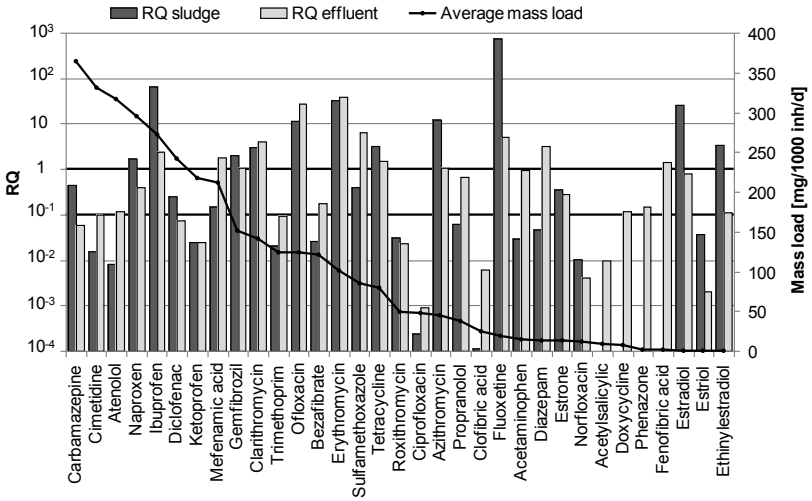


Fig. 1.11: Comparison of average specific mass load discharged by CAS effluent and RQs for secondary effluent and sludge.

1.9 Risk of antibiotic-resistant bacteria and genes

In addition to the environmental risk based on PNEC values for effluents as well as for sludge, there is another source of risk linked to the occurrence of the class of antibiotics both in the effluent and sludges: the development and release of antibiotic-resistant bacteria (ARB) and genes (ARG). ARG and ARB have been found to be several orders of magnitude higher in raw WWTP influents than in treated effluents, but, due to their high bacterial content, digested sludges also represent a significant environmental contamination route (Munir et al., 2011). It has been reported in the literature that the percentage antibiotic resistance in a treated wastewater effluent was generally higher than the percentages in the river water, but these were observed to increase downstream of a wastewater treatment plant (Iwane et al., 2001). WWTPs play a vital role in the elimination or spread of ARB and ARG, as the treatment systems and their operational conditions are likely to influence their fate. While it is likely that treated effluents with trace amount of ARGs and ARB from the treatment plants discharged into rivers or streams can add to the contamination of the environment, comparison of release loads of ARGs and ARB, Munir et al., (2011) showed that land application of biosolids from WWTPs seems to be the main source of entry of ARGs and ARB into the natural environment. Further research is necessary to determine how best to reduce the spread of such bacteria.

1.10 Modelling

Various attempts have been made to create and propose a model able to simulate the fate and behaviour of selected pharmaceuticals in a CAS in support of their design process. In this context, Plosz et al., (2012) recently suggest using mechanistic models, i.e., ASM-X, in regional risk assessment. Pomiés et al. (2013) reviewed 18 different literature models describing micropollutant removal in CAS, and remarked that an explanation for the validity of proposed models is often lacking, and for this reason future developments are necessary to improve modelling of micropollutant removal in WWTP. Indeed, in their current form, they are not ready to be used in process design.

1.11 CAS: treatment of PhC wastewaters

Wastewaters generated by pharmaceutical manufacturers contain a variety of organic and inorganic constituents including spent solvents, catalysts, additives, reagents and small amounts of intermediates, byproducts, raw materials and active pharmaceutical ingredients, which makes them particularly difficult to treat (Sreekanth et al., 2009). In addition, concentrations of COD, BOD, SS, nitrates are generally very high, of the order of tens–hundreds mg/L. The ratio BOD/COD is about 0.45-0.60, and pH may vary in the range 5–8. For instance, pharmaceutical wastewater investigated by Sreekanth et al. (2009) contained: 8500–9000 mg/L total dissolved solids, 2800–3000 mg/L TSS, 13,000–15,000 mg/L COD, 7000–7500 mg/L BOD, 600–750 volatile fatty acids, 2500–3000 mg/L alkalis, such as $CaCO_3$, 200–250 mg/L chlorides, 120–170 mg/L nitrates, 300–450 mg/L sulphates, and 100–120 mg/L phosphates, and the pH of the bulk drug in pharmaceutical wastewater was 7.0–7.5. In this effluent, the target PhC was carbamazepine, which was detected at levels of 10–15 mg/L. In some areas, PhC concentration may be even higher: Sirtori et al. (2009) reported a concentration of 45 mg/L of nalixid acid (a fluoroquinolone-type antibiotic was found) in an industrial effluent, and Chelliapan et al. (2006) found tylosin concentrations of up to 20–200 mg/L in pharmaceutical effluent they investigated. Indeed, it is estimated that approximately half of the pharmaceutical wastewaters produced worldwide are discharged without specific treatment (Enick and Moore, 2007). When treated, they are generally subjected to physicochemical processes(Kulik et al., 2008) and then to aerobic biological steps (Suman Raj and Anjaneyulu, 2005).

The operational parameters most influential in the removal of pollutants from pharmaceutical effluent are: HRT, temperature, pH, dissolved oxygen, organic load, microbial community, presence of toxic and persistent compounds, and batch operation of pharmaceutical production facilities (Suman Raj and Anjaneyulu, 2005). Hence activated sludge processes for the pharmaceutical industry effluent are generally designed with long HRT (Oz et al., 2004), operational temperature not greater than 30 °C (between 30–60 °C the number of bacterial species

decline with temperature, and activated sludge process fail at temperatures above 60 °C, LaPara et al., 2001). In fact, cooling of pharmaceutical effluent may even be necessary. Suman Raj and Anjaneyulu (2005) found that pharmaceutical wastewater can be biologically treated using mixed consortia by integrating chemical coagulation as a pretreatment. They found that a chemical coagulation with lime followed by aerobic oxidation with activated sludge increased the biodegradability through reduction in sulphate concentration (down to 44-48 %). They also found that the best results in the biological step were achieved at a mixed liquor concentration of about 4000 mg/L, confirming earlier results by Suman Ray et al. (2004).

Unfortunately, the impact of high concentrations of PhCs in activated sludges, as seen in pharmaceutical wastewaters, has not been yet investigated, and the worry is that their concentrations may inhibit biological processes. In any case, biological treatments are not able to complete removal of PhCs and other pollutants, and so complementary treatments should be used in conjunction with the traditional methods. These additional treatments include membrane filtration, reverse osmosis, activated carbon. In this context, Larsson et al. (2007) monitored the effluent of a WWTP situated in Patancheru, near Hyderabad, in India. This plant receives about 1500 m^3/d of wastewaters, mainly from 90 bulk drug facilities (BOD_5 = 1300 mg/L; COD = 6000 mg/L; SS = 500 mg/L, dissolved solids = 9000 mg/L) and the treatment sequence consists of an equalization tank (HRT = 2 d), a chemically assisted SS removal tank, a biological reactor (HRT = 4 d) in which 20 % of domestic wastewaters is added to improve the removal efficiency, and a secondary clarifier. Excess sludge is subjected to centrifugation. The final effluent (BOD = 270 mg/L; COD = 1300 mg/L; SS = 300 mg/L and dissolved solids = 5000 mg/L) is discharged into surface water bodies, and the treated sludge is disposed of in landfill. An investigation on the occurrence of some PhCs in the final effluent of this plant showed the following concentration ranges: 28–31 mg/L for ciprofloxacin; 0.8–0.95 mg/L for metoprolol; 0.7–0.9 for enrofloxacin; 0.39–0.42 for norfloxacin, 0.15–0.30 for enoxacin, 0.15-0.16 for ofloxacin and 0.09–0.16 for ranitidine.

Deegan et al. (2011) review many common treatments (traditional as well as advanced) and conclude that the problem of pharmaceuticals in wastewater cannot be solved merely by adopting end-of-pipe treatments, but source measures such as replacement of critical chemicals and reduction in raw material consumption also need to be adopted.

1.12 Conclusions

Most of the municipal WWTPs consist of preliminary, primary and secondary treatments, mainly activated sludge systems with the final effluent being discharged into a surface water body and often indirectly reused for irrigation purposes or recreational activities and the treated sludge often land applied. Many PhCs are usually present in raw influent at concentrations in the range 10-3-102 μg/L and even more, and common WWTPs are not able to efficiently remove all of them from liquid effluent as well as sludge. Observed removal efficiencies vary in a wide range for the different compounds, as well as for the same substance, due to the different chemical and physical characteristics of PhCs and to operational conditions.

This study highlights the fact that the occurrence of some PhCs in the secondary effluent discharged into surface water bodies may pose a medium–high (acute) risk to aquatic life. Furthermore, many other compounds, even if their environmental risk was found to be low, are discharged at high daily mass loads, which could contribute to negative effects on aquatic organisms in the long term due to chronic and mixture toxicity. For these reasons, it would be more prudent to begin monitoring the most frequently and most persistent administered PhCs, as well as those with the highest environmental risk, namely antibiotics (including erythromycin, ofloxacin, sulfamethoxazole, clarithromycin, amoxicillin, tetracycline and azithromycin), psychiatric drugs (like fluoxetine, diazepam and carbamazepine), analgesics/anti-inflammatories (ibuprofen, mefenamic acid, naproxen, diclofenac and ketoprofen) and lipid regulators (fenofibric acid, fenofibrate and gemfibrozil). Unfortunately, up to now, PhCs are not included among those compounds to be monitored, outwithstanding their occurrence have been documented since more than 20 years in many European countries. For this reason, further researches are necessary (i) to analyse the occurrence of scarcely investigated PhCs in the influent and outlets of municipal WWTPs; (ii) to evaluate the environmental impact of mixtures of different PhCs, (iii) to evaluate the best end-of-pipe measures for the existing WWTPs to guarantee better removal of the most persistent compounds, and (iv) to suggest source control options to reduce the quantity and variety of PhCs in the water cycle.

Reference

- Verlicchi P, Zambello E, Al Aukidy M. Removal of pharmaceuticals by conventional wastewater treatment plants. In: Petrovic M, Suarez S, Barcelò D, editors. Analysis, removal, effects, risks of pharmaceuticals in the water cycle: occurrence and transformation in the environment. Series works: Comprehensive Analytical ChemistryElsevier; Vol. 62. Barcelò D. (Elsevier), Amsterdam: The Netherlands 2013c pp.231- 86.

Chapter 2:

2 PPCPs occurrence in untreated and treated sewage sludge

2.1 Introduction

Sludge originates during biological and chemical processes in wastewater treatment plants (WWTPs) and may contain a wide spectrum of organic and inorganic substances as well as microorganisms and viruses which are separated from the liquid phase during treatments.

In Europe, its production is expected to increase from 11.5 M tons of dry matter (DM) (2010) to over 13 M tons of DM by 2020, chiefly due to increased sewerage and treatments in East European countries (Palfrey, 2010; Eriksson et al., 2008). The main disposal routes are incineration, landfill, land application, composting, with the specific percentages varying from country to country. For instance incineration of sludge is about 90% in Belgium, 50% in Germany and 45% in Denmark, while reuse in agriculture reaches 50% in Denmark and 25% in Sweden, where 50% is landfilled or allocated to construction work (Malmborg andMagnér, 2015; Kelessidis and Stasinakis, 2012). Recently Kelessidis and Stasinakis (2012) reported that 53% of sludge in EU-27 is reused in agriculture either directly or after composting, whereas Citulski and Farahbakhsh (2010) reported thatmore than 40% is spread on land in Canada and according to Venkatesan et al. (2014) more than 50% is spread on land in the USA.

The interest in using sewage sludge in agriculture is due to its nutrient content and soil-conditioning properties that are useful for restoring overexploited land to agricultural use or for improving the humus content and water-holding capacity of light-textured sandy soil as well as in cases where soils are depleted or subject to erosion (Clarke and Smith, 2011; Inglezakis et al., 2014).

Disposal routes of sewage sludge must fulfill specific regulations. With regard to the land application option, in the last 20 years great attention has been placed mainly on the occurrence of heavy metals in sludge and their fate once it is applied on agriculture land, and limits have been set and adopted in different countries (Stasinakis, 2012). Sometimes, additional limits have been implemented for surfactants (mainly linear alkyl sulfonates, LAS), polycyclic aromatic hydrocarbons (PAH), nonylphenol(n)ethoxylates (NPnEO), polychlorinated biphenyls (PCB), phthalates and pesticides in sludge, and studies monitoring their fate once spread on the land are ongoing (Kelessidis and Stasinakis, 2012). No limits have been set for pharmaceuticals (PhCs) and other contaminants of emerging interest, including personal care products (PCPs) in sewage sludge.

Land disposal of sewage sludge is regulated at EU level by the socalled Sewage Sludge Directive (SSD) 86/278/EEC (CEC, 1986) and in each EU country national regulations have also been set in accordance with the SSD. Generally, they set the maximum allowable concentrations of potentially toxic elements in soil after the application of sewage sludge, and maximum annual rates of application. They do not set concentration limits for organic compounds for either sludge or soil. A draft of a working document on sludge and biowaste is under discussion within the EU, where cut-off values are set for other groups of organic compounds (EC, European Commission, 2010).

There is ongoing debate within the scientific community in order to evaluate potential (environmental) risks in this kind of practice, due to the occurrence of toxic and persistent substances in sludge, such as aquifer contamination, the accumulation of pollutants in soil, and their transfer into the food chain. It has been estimated that loads of up to some kilograms per hectare may enter agricultural soils, and that concentrations of antibiotics similar to pesticides may be reached (Thiele-Bruhn, 2003).

The occurrence of antibiotics may cause resistance in pathogens. Moreover, antibiotic residues and resistant microorganisms can affect the natural soil microbial community and soil function and they may enter the food chain (Thiele-Bruhn, 2003).

The sorption on sludge and in soil of an organic contaminant is strongly affected by many factors, including the characteristics of the compound (molecular structure, in particular the presence of amino groups or COOH groups in the molecule, and chemical properties, including K_{ow}, pK_a, K_d) and the sludge (soil) (organic compound fraction, cation exchange capacity CEC, suspended solid size) and operating (environmental) conditions (pH, sludge retention time). As discussed in Verlicchi et al. (2012), rules of thumb have been proposed and used for a rough prediction of the

behavior of pharmaceuticals and personal care products (PPCPs), but they often lead to scenarios that are quite different fromthe observed behavior. The distribution of sewage sludge on farmland may result in an accumulation of persistent compounds in soil, representing a threat for the soil ecosystem and in particular, for soil living organisms. Once the sludge is amended to soil, PPCPs will still remain adsorbed or released, thus entering the soil water pore, it may also degrade. In the water phase they may be subjected to biodegradation and/or photodegradation or remain unchanged. They could volatize, reach groundwater, surface water bodies, or be taken up by plants, crops and grass growing on the land (Monteiro and Boxall, 2010).

In the last few years, many investigations have been carried out addressing different aspects of this complex topic. The aim of this study is therefore to provide a critical review of the main issues related to the presence of selected PPCPs in raw and treated sewage sludge and in sludge-amended soil. Collected data will be used to carry out an environmental risk analysis based on the risk quotient approach in order to identify the most potentially critical compounds in the case of sludge amended soil. This analysis will lead to a focus on the main critical aspects related to the acquired knowledge and the fields requiring future research.

2.2 Definition and types of sludge included in this chapter

Sewage sludge is defined as a mixture of the residuals from WWTPs receiving urban wastewater, or other wastewater of a similar composition. In general it is a liquid or a semi-liquid phase, with a solid percentage varying from 0.25 to 12% by weight, depending on the operations and processes used (Metcalfe and Eddy, 2004). A rough distinction is made between primary, secondary and treated sludge (mainly thickened, digested, composted, conditioned, dried and dewatered). The data collected from literature and presented in this study refers to this distinction. In addition, the term biosolids is often used for treated sewage sludge in order to underline the fact that sludge contains nutrients and other substances that can positively contribute to the improvement of soil properties and fertility (Clarke and Smith, 2011). Table 2.1 clearly presents the main characteristics of all these kinds of sludges, which will be referred to in the figures and tables discussed throughout the paper.

The data collected in this review regards the occurrence of selected PPCPs in the sludge originating from all the treatments reported in Fig. 2.1. Some studies refer to other names/types of sludge, for instance rural sludge and urban sludge in Peysson and Vulliet (2013). However, in the current manuscript we maintain its original name. In addition to the sludge compiled in Table 2.1, the biomass attached to gravel in constructed wetlands has also been included (Zhu and Chen, 2014) and defined “sediments (in CW)”.

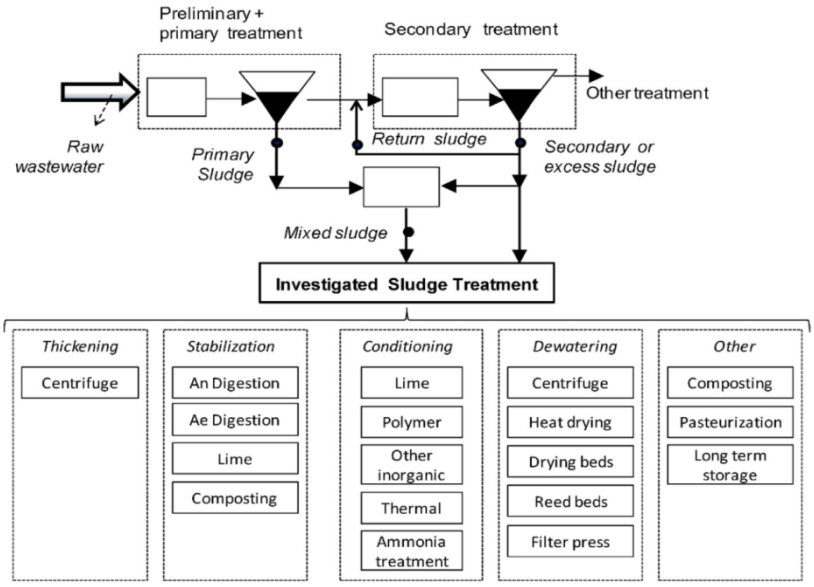


Fig. 2.1: Diagram of the types of sludge included in this review with regard to the occurrence of a wide spectrum of PhCs and PCPs.

Table 2.1: Types of untreated and treated sludge included in the review.

Sludge type	Description
Untreated sludge	
Raw	It contains suspended solids collected by filtering raw sewage (Jia et al., 2012; Lindberg et al., 2010) or in a grit chamber (Miao et al., 2005).
Primary	It derives from primary clarifiers which may also be chemically enhanced (for instance with FeCl3, Lajeunesse et al., 2012), and contains about 2–8% of total dry solids. Water content can be easily reduced by thickening or dewatering. It has a larger particle size than secondary sludge.
Secondary	It is generated in secondary biological treatments — conventional activated sludge systems (CAS), membrane biological reactors (MBR), biological nutrient reactors (BNR), or attached biological systems, such as trickling filters, and biological aerated filters (BAF). Sludge produced in UASB has also been included in this type of sludge.
Mixed	It is the mixture of primary and secondary sludges
Lagoon sludge	SF Sludge produced and settled in deep anaerobic stabilization ponds or in aerobic surface flow basins.
Treated sludge	
Digested sludge	Stabilized sludge produced in aerobic or in anaerobic digesters. The main aim of digestion is to reduce organic content and pathogens and also eliminate odors. Anaerobic digestion may occur both at a low temperature (mesophilic digestion, around 37 °C) and at a high temperature (thermophilic digestion, around 55 °C).
Composted sludge	Stabilized sludge resulting from the decomposition of organic compounds by microorganisms under aerobic conditions ensuring proper aeration by regularly turning sludge.
Biosolids	This term reflects the fact that the solids (“sludge”) are organic products that can be beneficial after treatment with processes such as biological stabilization and/or digestion of primary and secondary sludges and composting. In the USA a distinction is made between Class A and B on the basis of the treatment the sludge is subjected to. In a Class A sewage sludge treatment has greatly reduced pathogens below detectable limits and thus can be distributed as a soil amendment without any restriction. Class B sewage sludge may contain pathogens and therefore restrictions on crop harvesting, animal grazing and public access are requested after sludge application on land (Jones-Lepp and Stevens, 2007; Citulski and Farahbakhsh, 2010)
Conditioned sludge	Sludge from systems aiming to reduce its water content by chemical and physical processes (for instance by addition of FeCl3).
Dried sludge	Sludge from systems aiming to reduce its water content by thermal processes.
Thickened and Dewatered sludge	Sludge from systems aiming to reduce its water content by mechanical and physical processes.
Other types of treated sludges	Sludges obtained by disinfection (aiming to reduce the pathogen concentration), pasteurization (aiming to eliminate most pathogens by heating the sludge at 70 °C for 60 min), thermal hydrolysis (aiming to improve biodegradation of organic content by heating the sludge at 165 °C, at 6 bar for 30 min), advanced oxidation (adopted to treat or stabilize the organic material in the sludge) in particular Fenton's reaction (by adding sulfuric acid and hydrogen peroxide to the sludge), and ammonia treatment (by mixing dissolved ammonia or urea to the digested sludge) (Malmborg and Magnér, 2015; Arthurson, 2008).

2.3 Framework of the chapter

The current study is mainly based on 59 papers, published between 2002 and 2015, referring to about 450 treatment trains (full scale plants for more than 90% of the plants) operating in 24 different countries, providing data regarding sludge concentrations for 169 compounds — 152 pharmaceuticals (PhCs) and 17 personal care products (PCPs), grouped into 28 different classes (see Table 2.1: 23 for PhCs and 5 for PCPs). The papers were selected based on a research on SCOPUS, by applying the keywords: wastewater treatment plants, sludge, sludge amended soil, pharmaceuticals occurrence, environmental risk assessment. The main issues addressed in the published investigations included in the chapter are detailed in Table SD-1 of Verlicchi and Zambello, 2015, whereas in Appendix A the main chemical and physical properties (molecular structure, log K_{ow}, pK_a, and molecule charge at pH 7) were listed for all the selected compounds.

After a discussion of the legislative scenario governing the final disposal of treated sludge in European countries and the USA, the current study provides a snapshot of the occurrence of selected PPCPs in (untreated and treated) sludge and after its application on soil. Occurrence data refers to concentrations of PhCs and PCPs in primary, secondary, mixed and differently treated sludge originating in municipal WWTPs mainly fed with urban wastewater. In a few cases, factories slightly contribute to the WWTP feeding (Golet et al., 2003; Miao et al., 2005; Radjenović et al., 2009a). Moreover, one case (Jelic et al., 2012) deals with an anaerobic codigestion of the sewage sludge with the organic fraction of biowaste. In most studies, when the concentration was found to be less than the detection limit, it was assumed to take half the reported limits, according to many authors (among them von der Ohe et al., 2011). Data collected in spiking investigations was not included as, according to Eggen and Majcherczyk (1998), it does not represent reality because added compounds behave differently compared to “aged” compounds, which are more linked to a matrix and therefore require more energy to be degraded.

Most of the collected data refers to grab samples of sludge, and in just a few cases to composite samples. According to many authors, grab samples of treated sludge may be considered sufficiently representative of the treatment line (Lajeunesse et al., 2012; Jelic et al., 2012).

Concentrations of PPCPs in manure and sludge originating from livestock WWTPs are not reported. These may contain much higher concentrations not only of nutrients but also of estrogens (E1, E2, EE2 and E3), as shown in Sim et al. (2011).

In addition to measured concentrations, predicted concentrations in sludge were also reported and the most common models adopted for this prediction are critically discussed. They are generally based on the parameter K_d. For this reason, the current study also includes a reconnaissance of the different values of K_d referring to the different kinds of sludge.

The study then reports the data regarding the measured concentrations of PPCPs in sludge-amended soil and analyzes the main model used for predicting them. Finally, it carries out an environmental risk assessment posed by the occurrence of PPCPs in soil in the case of land application of sludge and compares the results obtained by different authors. The study concludes with a focus on the main issues that should be further investigated.

For the full list of Supplementary Data presented in Verlicchi and Zambello (2015) see Appendix B.

2.4 Results

2.4.1 Sorption mechanisms and attempts to predict the PPCP sorption potential

Sorption can be ascribed to two kinds of mechanisms: absorption, due to hydrophobic interactions of aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and the lipid fraction of the sludge, and adsorption, due to electrostatic interactions caused by contact between positively charged groups of chemicals and the negatively charged surfaces of the microorganisms (Ternes et al., 2004a). In the past,many attempts have been made to predict the sorption behavior of a compound on the basis of its specific properties, in particular its lipophilicity, expressed in terms of K_{ow} (octanol water distribution coefficient) and its affinity to the solid phase, expressed in terms of K_d (solid liquid partition coefficient).

Rules of thumb have been proposed in recent years (Table 2.3), but their application led to rough estimations that were quite often differed too much from the evidence. For instance, Jones et al. (2014) did not find any correlation between LogK_{ow} and concentration for 7 PhCs and a disinfectant for primary, secondary and mixed sludges collected in 28 different WWTPs in the UK. In Verlicchi et al. (2013) an in-depth discussion is reported.

Ambient pH may play a critical role for compounds containing functional groups which can be protonated and de-protonated. Further attempts to predict sorption behavior also tried to include the effect of pH and pK_a (acidic dissociation constant), leading to another rule of thumb based on the parameter D_{ow} (octanol water partition coefficient). But discrepancies between predictions and measurements still occurred for many compounds. The conclusion is that sorption mechanisms may hardly be correlated to the value of one parameter(K_{ow}, D_{ow}, K_d) as due to the complexity of the molecule, the fate of a PPCP depends on all of them (Table 2.3).

It is well-know that concentrations of (micro and macro)pollutants in sewage sludge are strictly affected by the characteristics of the influent wastewater, the sludge characteristics (pH, organic matter and cation concentration), the adopted wastewater and sludge treatments, and the operational conditions. In secondary sludge, microorganisms represent the greatest proportion of suspended solids, while primary sludge essentially contains fewer micro-organisms and has a large lipid fraction (Ternes et al., 2004a). A characterization of the different kinds of sludge is reported in Table 2.4 that can be useful in explaining the results that will be presented and discussed in the following sections.

Table 2.2: Groups of classes of PhCs and PCPs included in the review and, in brackets, their corresponding symbol and number of compounds.

Class			
Analgesics/anti-inflam. (A, 11)	Anti-histamines (I, 2)	Hormones (Q, 6)	Antiseptics (a, 2)
Antianginals (B, 1)	Anti-hypertensives (J, 6)	Hypnotics (R, 1)	Insect repellents (b, 1)
Antiarrhythmics (C, 2)	Anti neoplastics (K, 2)	Lipid regulators (S, 6)	UV filters (c, 1)
Antibiotics (D, 45)	Antiplatelets (L, 3)	Psychiatric drugs (T, 31)	Synthetic musks (d, 6)
Anticoagulants (E, 1)	Antiprotozoals (M, 1)	Contrast Media (U, 1)	Non-ionic surfactants (e, 7)
Antidiabetics (F, 2)	Beta-agonists (N, 3)	Receptor antagonists (V, 5)	
Antiemetics (G, 1)	Beta-blockers (O, 10)	Stimulants (W, 3)	
Antifungals (H, 7)	Diuretics (P, 1)		

Table 2.3: Rules of thumb in predicting the sorption behavior of a compound.

Parameter	**Conditions**	**Rule of thumb**	**Reference**
Log K_{ow}	<2.5	Low sorption	Rogers (1996)
Log K_{ow}	>4	High sorption	Rogers (1996)
	>500 L/kg	High sorption	Ternes and Joss (2006)
Log K_d	>2.67		
K_d	<500 L/kg	Low sorption	Ternes and Joss (2006)
Log K_d	<2.67		
Log Dow	<1	Low sorption	Cunningham (2008)
Log Dow	>3	High sorption	Cunningham (2008)

Table 2.4: Characterization of the main characteristics of the sludges. Data from: Butler et al., 2011; Carballa et al., 2007c, 2008; Drillia et al., 2005b; Gao et al., 2012b; Golet et al., 2003; Horsing et al., 2011; Hyland et al., 2012; Jelic et al., 2012; Li, 2014.

Property	Primary	Secondary	Mixed	AnD	AeD	Dew	SOIL
pH	6.6–7	6–8		5.8–7.5	7–7.5		4–8
OM %				31–48 (MAnd)			1–8
				24–51 (TAnD)			
C %	70	7–72		18–28 (MAnD)			0.4–7
				14–30 (TAnD)			
foc %	37.7	12.2		5.8–14 (MAnD)		36.7	
		42–47		3.2–15 (TAnD)			
N tot %		5.1–5.9		2–3 (MAnD)			
				1.4–2.5 (TAnD)			
P %		0.7–5		2.1–4.3 (MAnD)	0.26		
				0.29–4.4 (TAnD)			
TSS, g/L	50–125	10–35	30–95				
CEC, meq/100g		54–75					16–28

2.4.2 Measured concentrations in different kinds of sludge

Raw sludge

An interesting analysis carried out by Lindberg et al. (2010) on the occurrence of antifungal agents in sludges, highlights that in raw sewage articles, ketoconazole and econazole were detected at 980 and 470 ng/g DM respectively. In raw sludge the concentrations were 1300 and 240 ng/g DMrespectively. Jia et al. (2012) found that the concentrations of some antibiotics and the antiseptic pipemic acid were similar in raw sludge and primary sludge, ranging in the interval of 10 and 70 ng/g DM. The variability range was higher for norfloxacin, ofloxacin and moxifloxacin — between 170 and 1060 ng/g DM.

Lindberg et al. (2006) found higher concentrations of norfloxacin and ciprofloxacin in raw rather than primary sludge s, occurring in the ranges of 4700–5800 ng/g DM and 5700–7700 ng/g DM in raw sludge, and 1700–4200 and 2000–4000 ng/g DM in primary sludge.

Primary sludge

Fig. 2.2 and Fig. 2.3 refer to concentrations measured in primary sludge.

It emerges that the most investigated therapeutic classes are antibiotics (20 compounds), analgesics and anti-inflammatories (7 compounds), and antifungals, hormones and psychiatric drugs (4 compounds). Moreover, the most investigated compounds are ciprofloxacin and norfloxacin (10 data), ibuprofen (9), and estradiol, ethinylestradiol and caffeine (7 values). The highest concentrations were found for the fragrances galaxolide (187,000 ng/g DW) and tonalide (183,000 ng/g DM) (Ternes et al., 2004a), triclosan (14,700 ng/g DM) (McAvoy et al., 2002) and salicylic acid (13,800 ng/g DM) (Khan and Ongerth, 2002).

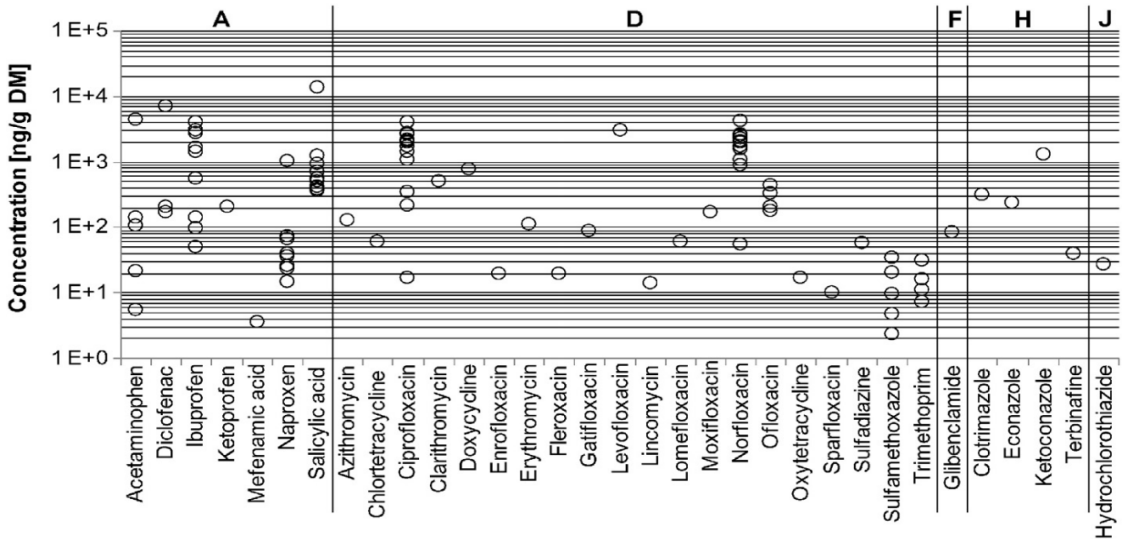


Fig. 2.2: Occurrence of compounds belonging to classes A, D, F, H and J in primary sludge. [Data from: Carballa et al., 2007b; Gao et al., 2012b; Golet et al., 2002; 2003; Jia et al., 2012; Khan and Ongerth, 2002; Lindberg et al., 2006, 2010; Martin et al., 2012a,b; Okuda et al., 2009; Radjenović, Jelić, Petrović and Barceló, 2009a; Stasinakis et al., 2013; Ternes et al., 2004a].

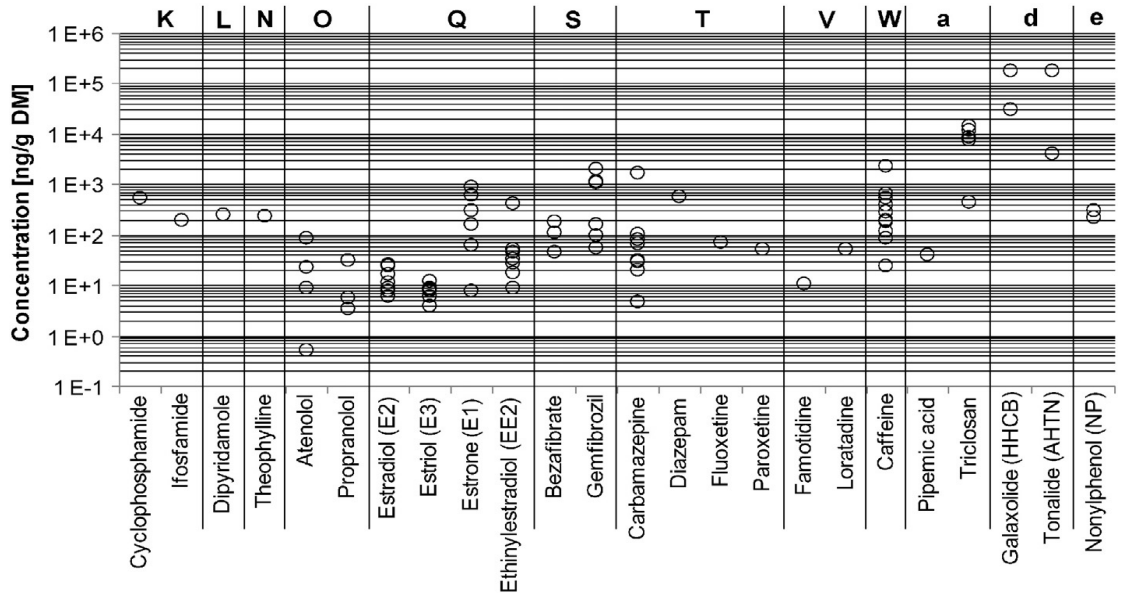


Fig. 2.3: Occurrence of compounds of classes K, L, N, O, Q, S, T, U, V, W, a, d and e in primary sludge. [Data from: Andersen et al., 2003; Carballa et al., 2007b; Gao et al., 2012b; Jia et al., 2012; Khan and Ongerth, 2002; Martin et al., 2012a, b; McAvoy et al., 2002; Muller et al., 2010; Okuda et al., 2009; Peterakis et al., 2012; Radjenovic et al., 2009a; Stasinakis et al., 2013; Ternes et al., 2004a].

Secondary sludge

Fig. 2.4 and Fig. 2.5 refer to secondary biological (excess) sludge from activated sludge processes, including conventional systems (CAS, BNR) and MBR. The most investigated classeswere antibiotics (135 data referring to 29 compounds), analgesics and anti-inflammatories (36 data regarding 7 compounds), hormones (49 data regarding 4 compounds).

The most studied compounds were ciprofloxacin (19 data), estradiol and ethinylestradiol (16), ciprofloxacin (14), ofloxacin and carbamazepine (13), sulfamethoxazole (12), and triclosan (10). Ten compounds were found at a concentration N10,000 ng/g DM: azithromycin (64,000 ng/g DM), clarithromycin (67,000 ng/g DM), ofloxacin (21,000 ng/g DM), sulfamethoxazole (68,000 ng/g DM), trimethoprim (41,000 ng/g DM), triclosan and triclocarban (17,500 and 43,200 ng/g DM respectively), galaxolide and tonalide (131,000 and 10,200 ng/g DM respectively). It emerges that the range of the observed concentrations may bewide up to 3–4 orders ofmagnitude for many compounds, namely diclofenac, azithromycin, josamycin, norfloxacin, ofloxacin, spiramycin, sulfamethoxazole, estradiol, ethinylestradiol, carbamazepine and tonalide. This can be ascribed to the adopted biological reactor configuration which may include anoxic, aerobic and anaerobic compartments, promoting C, N and P removal and different SRT values.

Jones et al. (2014) found that although the quality of the WWTP influent and of the effluent discharged may exhibit a consistent variability between different WWTPs, the sludge quality is more “homogeneous”, that is the variability range is generally narrower. This could be related to the prolonged residence time of the sludge which promotes good mixing and higher degradation processes in its bulk.

With regard to the seasonal variation of the concentration of PPCPs, Gao et al. (2012a) and Martin et al. (2012a) observed a consistent variability in the concentrations of antibiotics in sewage sludge from different municipal WWTPs. The highest concentrations were found in winter, and the lowest in autumn.

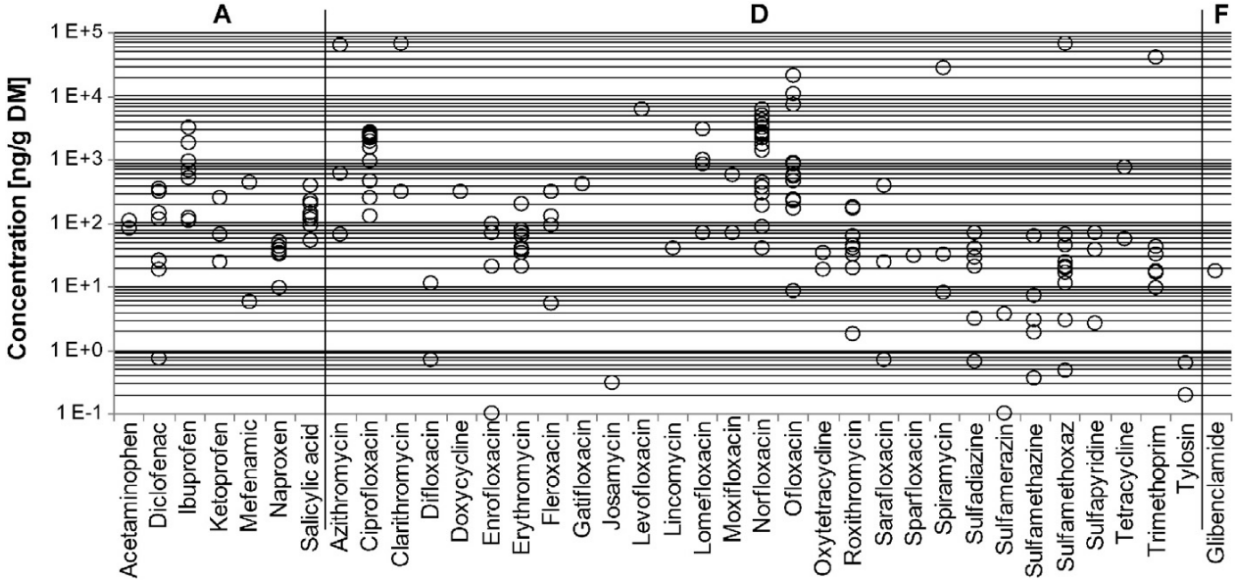


Fig. 2.4: Occurrence of compounds belonging to classes A, D and F in secondary sludges. [Data from: Carballa et al., 2007b; Gao et al., 2012a, b; Göbel, Thomsen,McArdell, Joss and Giger, 2005; Golet et al., 2003; Jia et al., 2012; Lindberg et al., 2006;Martin et al., 2012a,b, 2015; Okuda et al., 2009; Radjenović, Jelić, Petrović and Barceló, 2009a; Stasinakis et al., 2013; Ternes et al., 2004a; Xu et al., 2007; Yan et al., 2014].

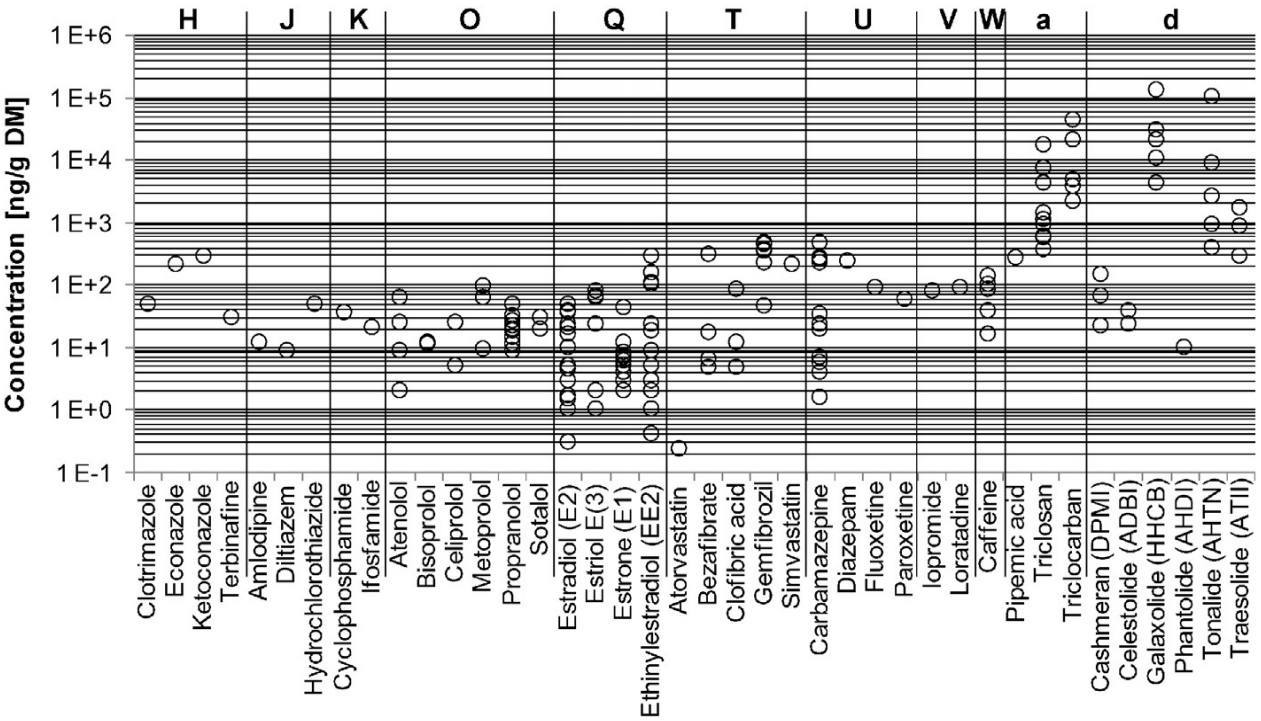


Fig. 2.5: Occurrence of compounds of classes H, J, K, O, Q, T, U, V, W, a and d in secondary sludges. [Data from: Andersen et al., 2003; Braga et al., 2005; Carballa et al., 2007b; Chu and Metcalfe, 2007; Clara et al., 2011; Gao et al., 2012b; Heidler and Halden, 2009; Jia et al., 2012; Lindberg et al., 2010; Martin et al., 2012a, b, 2015; McAvoy et al., 2002; Muller et al., 2008, 2010; Okuda et al., 2009; Radjenović, Jelić, Petrović and Barceló, 2009a; Scheurer et al., 2010; Stasinakis et al., 2013; Ternes et al., 2004a; Yan et al., 2014].

Mixed sludge

Jones et al. (2014) provided data regarding the average concentrations for 7 PhCs, triclosan and 3 NPnEO in mixed sludges concerning different WWTPs in the UK. The highest concentrations were found for NP3EO (176,000 ng/g DM), oxytetracycline (7630 ng/g DM), NPEO (5000 ng/g DM) triclosan (4900 ng/g DM), NP2EO (1100 ng/g DM), and diclofenac, ibuprofen, propranolol, erythromycin, ofloxacin and fluoxetine (60–270 ng/g DM).

Digested sludge

Fig. 2.6, Fig. 2.7 and Fig. 2.8 refer to concentrationsmeasured in aerobically or anaerobically digested sludge. The most studied classes were psychiatric drugs (19 compounds) and antibiotics (16), followed by analgesics/anti-inflammatories, antifungals, hormones and non ionic surfactants (6 compounds in each class). The most investigated compounds were carbamazepine (41 values), ibuprofen (27), estradiol (26), diclofenac (22), estrone (21), ciprofloxacin (20), caffeine (19) and norfloxacin (18). Anaerobic digestion (AnD) was more frequently investigated than aerobic (AeD) (in the cited figures, circles refer to AnD, squares to AeD and stars to an undefined digestion process). The highest concentrations were found in AnD sludge with the only exceptions of galaxolide and tonalide. Compounds that occurred at concentrations higher than 10_4 ng/g DM (=10 μg/g DM) are (in descending order) galaxolide (81,000 ng/g DM), triclocarban (63,000 ng/g DM), triclosan (46,000 ng/g DM), NP2EO (25,000 ng/g DM), estrone (22,000 ng/g DM), OP2EO (20,000 ng/g M), tresolide and tonalide (16,000 ng/g DM).

A consistent seasonal variation was also noted by Nieto et al. (2010) for acetaminophen, diclofenac and ibuprofen in AnD sludges. The authors ascribed it to higher consumption in winter than in spring–summer.

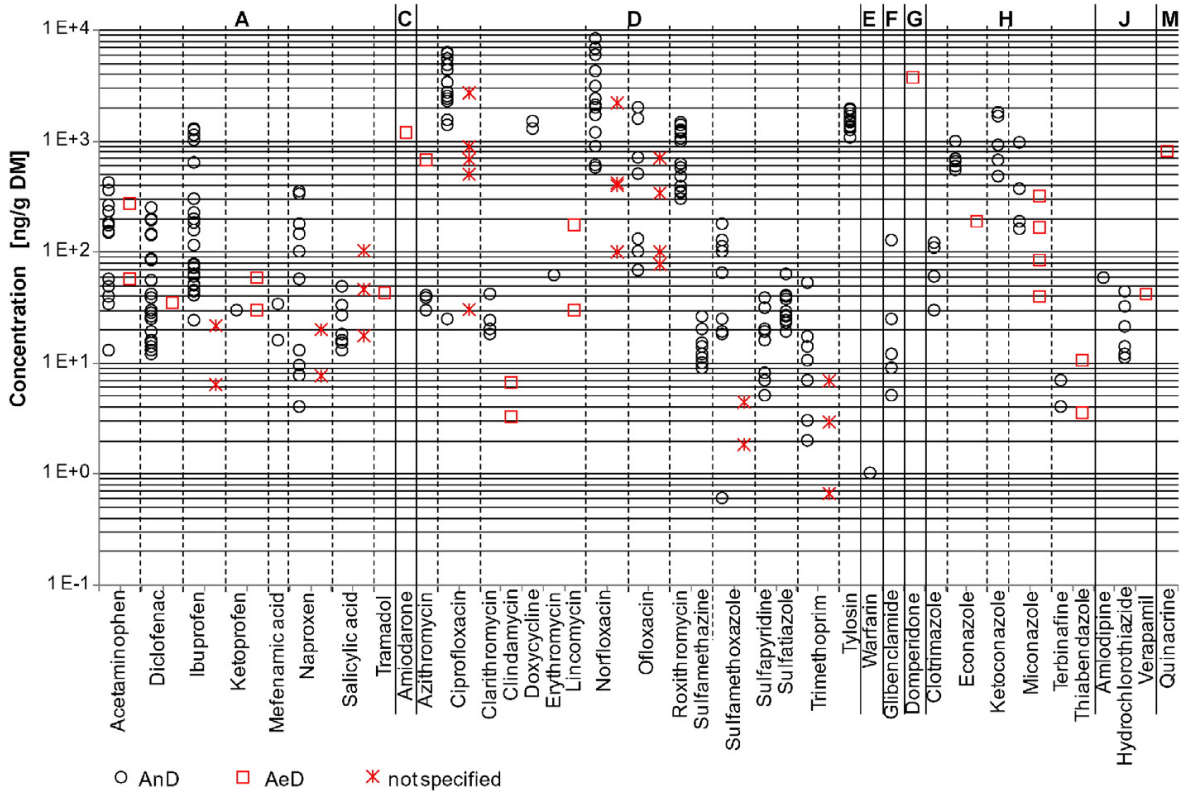


Fig. 2.6: Occurrence of compounds belonging to classes A, C, D, E, F, G, H, J and M in biologically digested sludges. Data from: Carballa et al., 2007c; Golet et al., 2002; Jelic et al., 2011, 2012; Khan and Ongerth, 2002; Lillenberg et al., 2009; Lindberg et al., 2005, 2006, 2010; Malmborg and Magnér, 2015; Martin et al., 2012a,b,2015; Nieto et al., 2010; Peysson and Vulliet, 2013; Radjenović, Jelić, Petrović and Barceló, 2009a; Stasinakis et al., 2013; Subedi et al., 2014.

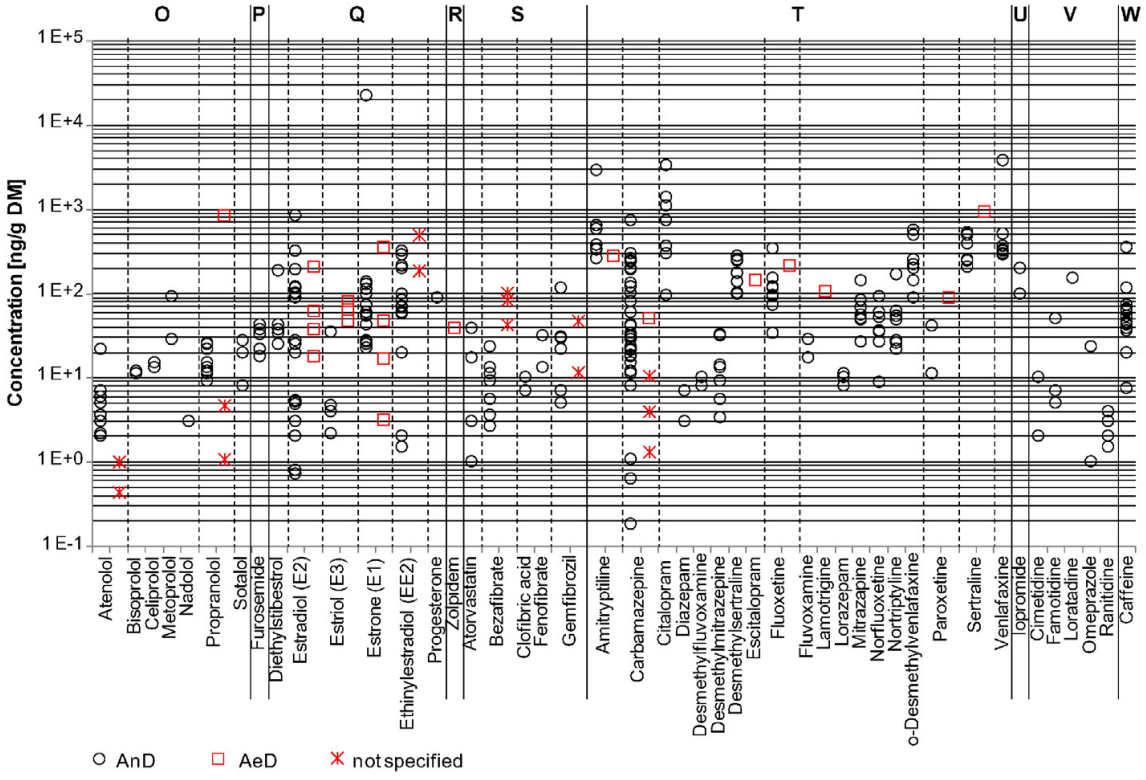


Fig. 2.7: Occurrence of compounds of classes O, P, Q, R, S, T, U, V and Z in biologically digested sludges. Data from: Andersen et al., 2003; Carballa et al., 2007c; Jelic et al., 2011, 2012; Khan and Ongerth, 2002; Lajeunesse et al., 2012; Lindberg et al., 2010;Malmborg and Magnér, 2015;Martin et al., 2012a,b,2015; Miao et al., 2005; Muller et al., 2010; Nieto et al., 2010; Peysson and Vulliet, 2013; Radjenović, Jelić, Petrović and Barceló, 2009a; Scheurer et al., 2010; Simet al., 2011; Subedi et al., 2014.

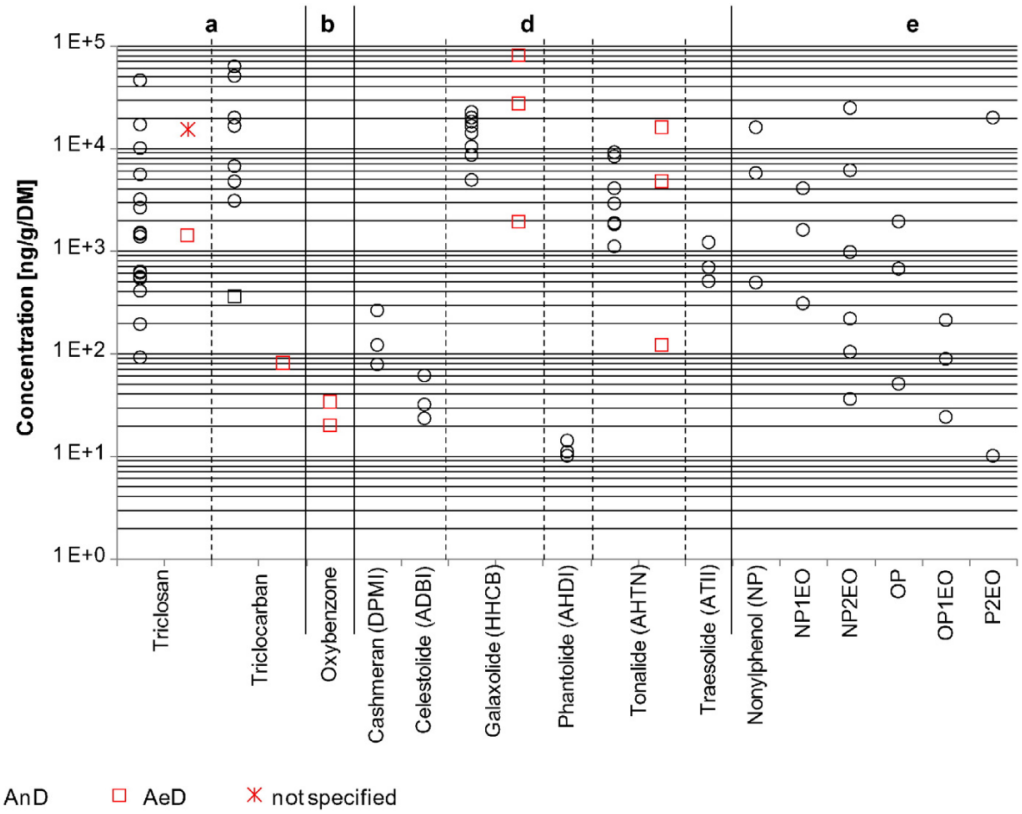


Fig. 2.8: Occurrence of compounds belonging to classes a, b, d and e in biologically digested sludges. [Data from: Carballa et al., 2007c; Clara et al., 2011; Heidler et al., 2006, 2009;Mailler et al., 2014; McAvoy et al., 2002; Osemwengie et al., 2006; Peysson et al., 2013; Stasinakis et al., 2008, 2013; Stevens et al., 2003; Subedi et al., 2014; Ying and Kookana, 2007].

Biosolids, composted, conditioned, dried and differently treated sludge

Fig. 2.9 and Fig. 2.10 report literature data for selected PPCPs in biosolids, composted, conditioned, dried and other kinds of treated sludges, according to the definition in Table 2.1. Referring to biosolids, the most investigated class was antibiotics (27 compounds) and the most studied compounds triclosan (9 values) and triclocarban (7 values). The highest concentrations were due to triclocarban (441,000 ng/g; US, 2009) and tonalide (427,000 ng/g DM; Kinney et al., 2006), galaxolide (177,000 ng/g DM; Kinney et al., 2006), triclosan (133,000 ng/g DM; US, 2009), ofloxacin (58,000 ng/g DMUS, 2009), and ciprofloxacin (47,500 ng/g DM; US, 2009).

With regard to composted sludges, the most investigated classes were analgesics/anti-inflammatories and psychiatric drugs (8 compounds each), followed by antibiotics (5 compounds), hormones and lipid regulators (4). The most studied compounds were carbamazepine (13 values) and acetaminophen (8 values). The highest concentrations were found for galaxolide (6800 ng/gDM; Tavazzi et al., 2013), triclosan (4230 ng/g DM; Peysson and Vulliet, 2013), tonalide (3500 ng/g DM; Kinney et al., 2006) and acetaminophen (920 ng/g DM; Martin et al., 2012a).

Data regarding conditioned sludge is less available and mainly refers to antibiotics (8 compounds), psychiatric drugs (7 compounds) and analgesics/anti-inflammatories (4 compounds). The most studied substances are carbamazepine (4 values) followed by caffeine, galaxolide, and tonalide (3 values each). The highest concentration was found for galaxolide (30,000 ng/gDM; Carballa et al., 2007b), followed by tonalide (7000 ng/g DM, Carballa et al., 2007b) and triclosan (3500 ng/g DM; Kinney et al., 2006).

In dried sludges, the most investigated classes were non-ionic surfactants and psychiatric drugs (6 compounds each), followed by antifungals (4 compounds). NP and NP1EO occurred at the highest concentrations (50,000 and 31,000 ng/g DM respectively, Mailler et al., 2014), followed by diphenhydramine (6000 ng/g DM; Peysson and Vulliet, 2013), tonalide (5000 ng/g DM; Kinney et al., 2006), triclosan (3700 ng/g DM; Kinney et al., 2006), caffeine (2100 ng/g DM; Malmborg and Magnér, 2015).

On the basis of the collected data and its processing (Table 2.5, Fig. 2.6–Fig. 2.10) it emerges that concentrations of selected PhCs and PCPs may be reduced by common treatments. Digestion represents the first step in treatment, and an attenuation occurs for most compounds.

Composting, conditioning and drying may reduce the variability ranges of occurrence of analgesics and anti-inflammatories, antibiotics, antiseptics by about one order of magnitude.

The most recalcitrant compounds seem to be doxycycline and tetracycline, which are still present at concentrations higher than 560 ng/g DM after conditioning; non ionic surfactants which are present at concentrations higher than 30,000 ng/g DM after thermal drying, and antiseptics and fragrances which are detected up to 5000 ng/g DM.

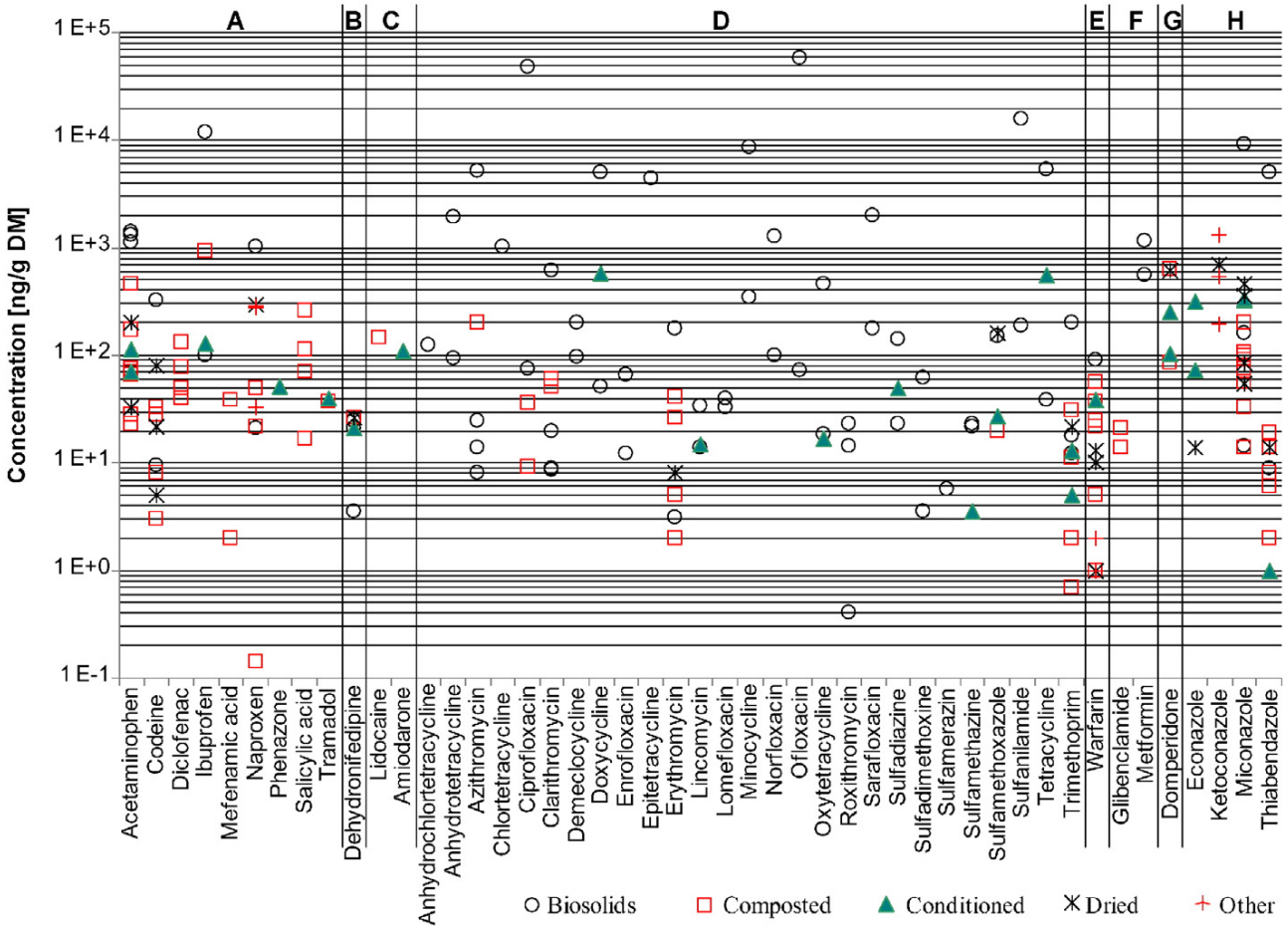


Fig. 2.9: Occurrence of compounds of classes A–H in other types of treated sludge (mainly biosolids, composted, chemically conditioned, and dried). [Data from: Carballa et al., 2007b; Gao et al., 2012b; Jelic et al., 2011; Jones-Lepp et al., 2007; Kinney et al., 2006; Malmborg andMagnér, 2015;Martin et al., 2012a, 2015; Peysson et al., 2013; US, 2009].

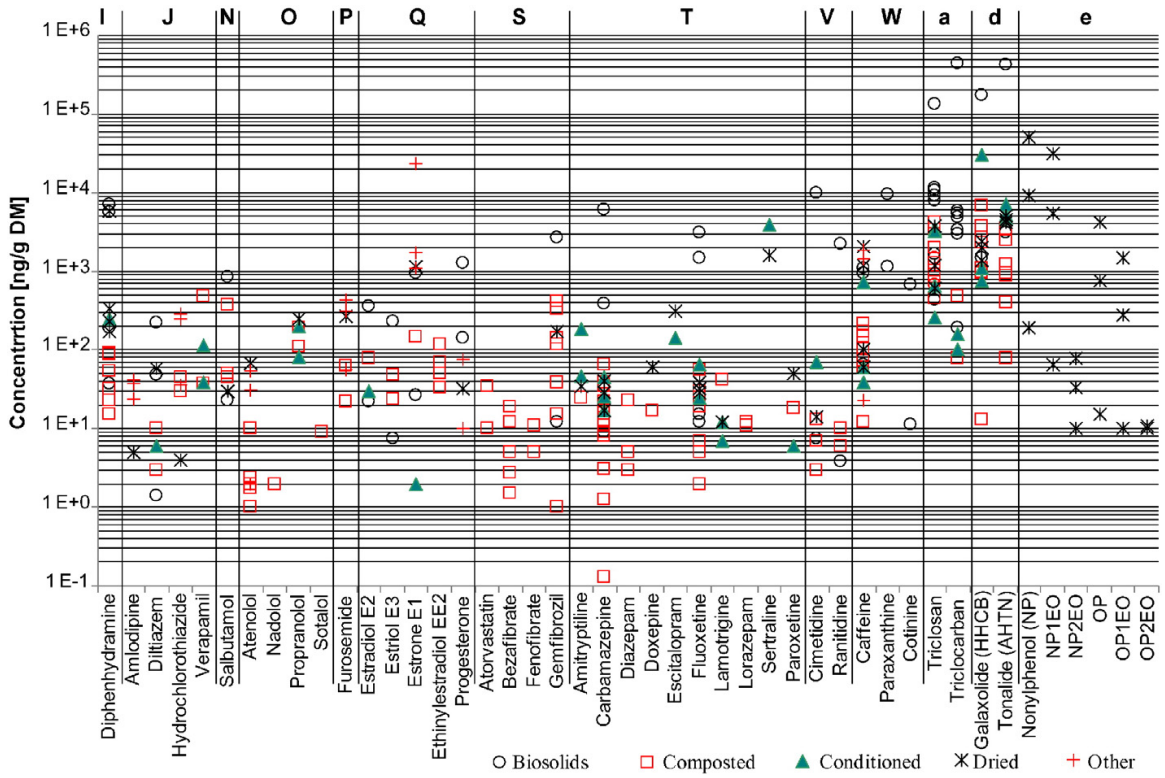


Fig. 2.10: Occurrence of compounds of different classes of PhCs and personal care products in other types of treated sludge (mainly biosolids, composted, chemically conditioned, and dried). [Data from: Carballa et al., 2007b; Chu and Metcalfe, 2007; Gao et al., 2012b; Jelic et al., 2011; Kinney et al., 2006; Mailler et al., 2014; Malmborg and Magnér, 2015; Martin et al., 2012a, 2015; Peysson and Vulliet, 2013; Tavazzi et al., 2013; US, 2009].

Table 2.5: Ranges of observed concentrations for the principally investigated groups (ng/g DM).

Class	Analgesics	Antibiotics	Hormones	Phsychiatric drugs	Antiseptics	Fragrances	Non-ionic surfactants
Primary	$3–10^4$	$5–4\ 10^3$	$4–4{\cdot}10^2$	$5–2{\cdot}10^3$	$40–1.5{\cdot}10^4$	$10^3–10^5$	10^2
Secondary	$1–10^3$	$10^{-1}–7{\cdot}10^4$	$10^{-1}–3{\cdot}10^2$	$1–6{\cdot}10^2$	$10^2–2{\cdot}10^4$	$10–10^5$	–
Digested	$4–10^3$	$1–8{\cdot}10^3$	$1–10^4$	$10^{-1}–3{\cdot}10^3$	$10^2–7{\cdot}10^4$	$10–8{\cdot}10^4$	$10–2{\cdot}10^4$
Composted	$10^{-1}–10^3$	$8{\cdot}10^{-1}–2{\cdot}10^2$	$2{\cdot}10–2{\cdot}10^2$	$10^{-1}–9{\cdot}10^2$	$10^1–8{\cdot}10^3$	–	–
Biosolids	$10–10^4$	$4{\cdot}10^{-1}–6{\cdot}10^4$	$8–10^3$	$1–6{\cdot}10^3$	$10^2–4{\cdot}10^4$	$10^3–4{\cdot}10^4$	–
Conditioned	$1–10^2$	$10–5{\cdot}10^2$	$2–3{\cdot}10$	$10–10^3$	$8{\cdot}10^1–3{\cdot}10^3$	$8{\cdot}10^2–3{\cdot}10^4$	–
Dried	$5–3\ 10^2$	$8–10^2$	$3–10^3$	$1–10^3$	$7{\cdot}10^2–4{\cdot}10^3$	$10^3–7{\cdot}10^3$	$10–5{\cdot}10^4$

Composting

Composting processes aim to accelerate the biodegradation of organic compounds thanks to a high microbial diversity and activity (mainly thermophilic organisms), abundant substrates, changing pH and redox conditions (aerobic and anaerobic microenvironments) (Xia et al., 2005). Martin et al. (2012a) found that degradation of organic matter and, at the same time, enhancement of the degradation of persistent compounds occurs under aerobic conditions. In the composted sludge a general attenuation of all the groups of compounds is observed (see Table 2.5 with regard to the main classes of selected compounds).

The most recalcitrant substances were triclosan, galaxolide and tonalide (up to $4–5\ 10^3$ ng/g DM; Peysson and Vulliet, 2013; Tavazzi et al., 2013; Kinney et al., 2006), and ibuprofen (close to 10^3 ng/g DM; Martin et al., 2012a).

Lagoon sludge

Martin et al. (2015) investigated the sludge from an anaerobic wastewater stabilization pond in Spain and found thatmost compounds occurred in a wide range of concentrations. Those exhibiting the maximum concentration of greater than 100 ng/gDM were: acetaminophen, salicylic acid, ciprofloxacin, gemfibrozil and caffeine, with naproxen, ofloxacin, carbamazepine, bezafibrate showing values of between 50 and 100 ng/g DM. Compounds always found below the corresponding limit of detection were ibuprofen, ketoprofen, norfloxacin, propranolol, ethinylestradiol, estradiol, estriol, estrone and clofibric acid.

Concentration in sediments (in CW)

Investigations of the concentrations of selected PhCs in sediment of subsurface flow constructed wetlands by Zhu and Chen (2014) confirmed the same tendency to sorb onto gravel for the compounds exhibiting high concentration in excess sludge, in particular for trimethoprim and triclocarban. The authors concluded that the risk of these compounds in sediments should not be neglected.

2.5 Attenuation of PPCP concentrations in sludge during treatment

2.5.1 Primary and secondary sludges

Influence of organic matter content - Martin et al. (2012a, 2015) highlighted different behavior of PhCs during sludge treatment and tried to correlate it to the physico-chemical properties (namely chemical structure, pKa, Log Kow) of the compounds, sludge composition and presence of aerobic/anaerobic conditions influencing the rate of biodegradation and its bioavailability. A higher content of organic matter in secondary sludge (see Table 2.3) could explain why most PhCs (naproxen, carbamazepine, the hormones E1, E2, EE2, E3, and gemfibrozil) were found at a higher concentration in secondary sludge compared to primary. The opposite trend was found by other authors (among them Stasikanis et al., 2013, Martin et al., 2015) - diclofenac, ibuprofen, salicylic acid, caffeine, nonilfenol and triclosan were found at higher concentrations in primary sludge than in secondary, probably due to the protonation at lower pH values of primary sludge (around 6.5) compared to secondary sludge (around 7.2) and the formation of electrostatic interactions between these compounds and the solid surface. Degradation and transformation reactions could also occur during biological treatments and contribute to this trend.

Influence of biological reactor characteristics: Jones et al. (2014) found higher concentrations of triclosan, propranolol, ibuprofen, and erythromycin in primary sludge rather than in secondary sludge samples, while they found that the type of secondary treatment (CAS, MBR, BNR, biological filtration) did not affect the concentration in the sludge.

Fernandez-Fontaina et al. (2013) remarked that the better overall performance of MBRs in the removal of PPCPs with respect to CAS is due to the typical higher biomass concentration in MBRs rather than CAS, resulting (generally) in an enhanced biodegradation of PPCPs. Collected data exhibited that PhCs tended to sorb less onto the aged MBR sludge than the primary and secondary activated sludge, possibly as a consequence of the higher biodegradation potential of the biomass within the MBR (Radjenovic et al., 2009b).

With regard to estrogens, concentrations of E2, E3 and EE2 were found to be similar in primary and secondary sludges, 10-13, 2-3 and <3 ng/g DM respectively (Muller et al., 2010), whereas E1 was higher in secondary sludge (43 ng/g DM) than in primary sludge (8 ng/g DM).

This higher concentration may result either from the bacterial transformation of E2 to E1 or the hydrolysis of conjugated E1 forms during biological treatment. Muller et al. (2010) remarked that WWTPs with biological nitrogen treatment, and SRT in the range of 10-15 d enhance the biodegradation of estrogens, and their concentration in the secondary sludge is lower than that detected in conventional activated sludge systems (12 ng/g DW vs. 50 ng/g DM referring to their total concentration).
Li et al. (2014) found that in CASs, a longer SRT may enhance the sorption of quinolones (including ciprofloxacin and norfloxacin) onto secondary sludge, whereas Stasinakis et al. (2010) did not find any improvement in the sorption of triclosan at a longer SRT.

2.5.2 Stabilized and conditioned sludge

Sludge stabilization and conditioning involve physical, chemical, mechanical and biological processes and changes which could affect solid partitioning, degradation, adsorption and, to a lesser extent, volatilization and photolysis pathways of PhCs and PCPs in sludge matrices. Sludge chemical composition may change, resulting in different adsorption behavior of compounds. This was observed by Martin et al. (2012a) for ibuprofen, salicylic acid, caffeine and gemfibrozil, whose concentrations decreased from secondary, to digested and composted sludges, and by Miao et al. (2005) who investigated carbamazepine, whose concentration increased from untreated to treated (digested and thermally dried) sludge, from 69 to 258 ng/g DM.

With regard to fragrances, Clara et al. (2011) remarked that a good level of removal is achieved in activated sludge systems, as sorption is their principal removal method. A comparison between concentrations of fragrances in excess sludge (Fig. 2.5, class J) and AnD sludge (Fig. 2.6, class J) highlights that anaerobic biodegradation is not really effective in reducing the content of this group of compounds (see also Table 2.5).

An interesting analysis was carried out by Martin et al (2015) regarding 7 different kinds of sludge (primary, secondary, mixed, anaerobically digested, aerobically digested, composted, and settled in a lagoon) with regard to eight main therapeutic classes of PhCs. They found that digested sludges showed lower concentrations than untreated sludges, which is often correlated to the loss of lipophilic properties during stabilization treatments (Khan and Ongerth, 2002).

Kimura et al. (2010) found that modest variations in pH may impact the removal of acidic PhCs (among them ibuprofen, naproxen, ketoprofen) by sorption, presumably due to enhancement of the affinity between the sludge surface and the PhCs subjected to protonation.

An increment in the concentrations of E1 and E2 was found during anaerobic digestion of the excess sludge (Andersen et al., 2003), specifically from 7 ng/g DM to 25.2 ng/g DM and 1.7 ng/g DM to 5.1 ng/g DM respectively. Estrogenic compounds are hydrophobic and they have a high tendency to sorb. The increment in the concentration of estradiol in the digested sludge is due not only to its hydrophobic nature, but also to the cleavage of conjugated steroid estrogens (Khan and Orgerth, 2002; Andersen et al., 2005) and to accumulation on the remaining digested sludge (Martin et al., 2012b). During AnD, E1 is reduced to E2 (Paterakis et al., 2012, Carballa et al., 2007c), and biochemical reactions proceed faster in thermophilic than mesophilic conditions. Mesophilic conditions require a higher SRT than thermophilic conditions, in order to guarantee a significant reduction of E1 to E2 (Paterakis et al., 2012).

In the digested sludge, Martin et al.(2012a) found a decrement in the concentrations of most analgesics, antibiotics and lipid regulators. They ascribe this attenuation to the fact that during anaerobic digestion, many PhCs tend to desorb and may then be involved in biodegradation reactions.

In anaerobic digestion, T and SRT greatly affect the biodegradation of NP1EO, while they do not affect the biodegradation of some PhCs, synthetic musks and estrogens (Carballa et al., 2006; Stasinakis, 2012). Biomass acclimatization improved the biodegradation of diclofenac, diazepam and estrogens (Carballa et al., 2006, 2007b).

The lab scale investigation by Carballa et al. (2007c) on the fate of a selected group of PhCs and PCPs by AnD highlights that a significant removal occurred for several PhCs (operating at a SRT equal to 10-20 d). Values were higher than 85 % for naproxen, sulfamethoxazole, roxithromycin, and E1, E2, and EE2; and between 65 and 85 % for galaxolide, tonalide, and diazepam (only mesophilic AnD). Ibuprofen and iopromide exhibited a poor removal (20-40 %) and carbamazepine was recalcitrant to degradation. They did not find consistent differences between mesophilic and thermophilic conditions.

With regard to antiseptics, Heidler et al. (2006) reported that AnD did not promote triclocarban degradation, resulting in an accumulation in the digested sludge, and McAvoy et al. (2002) reported a good level of removal of triclosan in aerobic digestion but not in anaerobic digestion.

Malmborg and Magnér (2015) investigated the correlation between lipophilicity (defined as log P for bases/neutrals and logD for acids) with the persistence of the compounds (expressed as a percentage of remaining substances) during mesophilic and thermophilic AnD and observed a direct proportionality which would correspond to high solid partitioning of lipophilic compounds, resulting in lower availability to degrading microorganisms.

Anaerobic treatments seem to be more efficient than aerobic ones in removing all PhCs, as shown in Fig. 2.6, Fig. 2.7 and Fig. 2.8. Concentrations of PhCs in aerobically digested sludge subjected to compost are similar or higher than anaerobically digested. This could be due to the loss of organic compounds due to biodegradation and in a concentration of the residual persistent compounds. This is the case of estriol (Khan and Ongerth, 2002).

(Mechanical) dewatering treatments (centrifuge, filter press) do not affect the content of PPCPs in sludge, as they aim to reduce the water volume of the sludge and not to remove dry matter. As compound concentrations are expressed in g compound/g sludge DM, its concentration before and after a filter press or a thickener or centrifuge does not change (Mailler et al., 2014).

Braga et al. (2005) investigated concentrations of steroid estrogens (E1, E2, EE2) in excess sludge and dewatered sludge (by filter press). They found that concentrations are slightly higher in dewatered sludge than in excess sludge, but the PhC load in dewatered sludge is lower than its load in excess sludge.

Chemical and thermal treatments - With regard to chemical treatments, when a lime stabilization is performed, the increment in pH causes the desorption of estrogens (Clara et al., 2004b).

Chemical and thermal (pre)treatments have been thoroughly investigated, but results are not always encouraging. Carballa et al., (2006, 2007a and 2008) investigated the influence on the removal of selected Phcs of pretreatments of anaerobic digestion of mixed sludge. They first tested a thermal pretreatment, consisting of an autoclave at 160°C for 30 mins, followed by a cooling step before AnD, and a chemical pretreatment by adding lime (CaO) to the stirred sludge up to a pH over 12, followed by neutralization, first with HCl, then AnD. They found that higher removal efficiencies were observed only for ibuprofen when thermal pretreatments were present and for roxithromycin in the presence of an alkaline pretreatment. No attenuation was found for estrogens, fragrances (tonalide and galaxolide), psychiatric drugs (carbamazepine and diazepam), sulfamethoxazole and iopromide.

They then investigated the effect of ozonation (20 kg O_3/kg TSS) of the sludge before anaerobic stabilization and found that it reduces carbamazepine by up to 60% but it does not affect the removal of other PCPs (Carballa et al., 2007a, 2008). They remarked that neither chemical nor thermal pretreatments of the sludge prior to AnD can greatly improve the sorption potential of PhCs. This could be due to the fact that some pretreatments may decrease the bioavailability of target compounds (as is the case of thermal processes) or that target compounds are strongly adsorbed onto sludge that may not be attacked by oxidizing (as is the case of chemical retreatment).

Final sludge stabilization and dewatering by thermal pressurized treatments tends to increase the estrogen concentration from anaerobic digestion (mainly for E2 and EE2), probably by enhancing their extractability (Muller et al., 2010).

Pasteurization and Fenton's reaction - According to Malmborg and Magnér (2015), pasteurization has a slight effect on the removal of PhCs from the sludge matrix, with thermal hydrolysis reducing the concentrations of estrone (E1), estradiol (E2) and ethinylestradiol (EE2). This leads to the conclusion that in the case of thermal hydrolysis, the end-product of E2 is not E1 (as is often observed).

An attenuation in secondary sludge concentration was observed by Malmborg and Magnér (2015) for amlopidine, atenilol, caffeine, hydrochlorotiazide, and ketoconazole by means of Fenton's reaction, whereas ammonia treatments increased the concentrations of caffeine, furosemide, naproxen and hydrochlorothiazide). An increment in concentrations was also observed in thermophilic dry digestion for caffeine, furosemide and hydrochlorotiazide.

2.6 Predicted concentrations of selected compounds in sludge

Some studies provide models to predict concentrations in sludges, the so-called predicted environmental concentrations (PEC). The authors of these studies include Carballa et al.(2007b), Cunningham et al. (2012), Khan and Ongerth (2002), Jones et al. (2002).

Frequently, PEC in sludge is evaluated on the basis of eq. 2.1.

$$PEC_{i,\,sludge} = C_{i,\,water} \times K_{d,\,i\,sludge} \qquad \text{(eq. 2.1)}$$

where $C_{i,\,water}$ corresponds to MEC or PEC in water.

Another common equation is that proposed in Jones et al. (2002):

$$PEC_{i,\,sludge} = \frac{M_{c\,i}}{V_{ww}/K_d + M_{sludge}} \qquad \text{(eq. 2.2)}$$

where M_{ci} is the annual consumption of the compound i (kg), V_{ww} is the total annual wastewater volume (m^3), M_{sludge} is the annual sludge production (kg of dry matter) and K_d is the solid-water distribution coefficient which describes the ratio between its concentration sorbed onto sludge and its dissolved concentration S at equilibrium.

In both equations, PEC_{sludge} implies knowledge of the coefficient Kd. Some authors have experimentally evaluated K_d values for many compounds in different kinds of sludge. A reconnaissance of these values is reported in Table SD-4 of Verlicchi and Zambello (2015), along with the corresponding references.

Another approach in predicting PhC concentrations in primary and secondary sludge is proposed by Khan and Ongerth (2002) based on the fugacity model. Close correlations were observed between predicted and measured values for naproxen, ibuprofen and paracetamol in primary sludge, whereas for salicylic acid and carbamazepine, measured values were two orders of magnitude higher than predicted values. This could be ascribed to hydrophilic interactions, not included in the model, which considers lipid partitioning the main mechanism for solid sorption. On the other hand, the measured concentration for gemfibrozil was one order of magnitude less than

predicted. This fact could be attributed to incomplete extraction from the solid owing to its very high lipophilicity and also to a higher biodegradation rate than that estimated in the model.

2.6.1 Considerations regarding Kd

The extent of sorption onto a solid (sludge and soil) is generally based on the distribution coefficient (K_d) which implies a linear equilibrium relationship based on the concept of solute partitioning (Sathyamoorthy and Ramsburg 2013).

K_d values are strictly correlated to different operational conditions, namely temperature, pH, SRT, sludge type, and reactor configuration. Table SD-4 in Verlicchi and Zambello (2015) compiles the measured values of K_d for each compound and for the different kinds of sludge (primary, secondary, digested and differently treated). In many cases, a wide range of variability occurs due to the fact that collected Kd values were found in different systems operating at different conditions as discussed herein.

Lower values of K_d were found at a higher temperature for most organic compounds, whose solubility increases with temperature, as reported by Lajeunesse et al. (2012). With regard to compounds presenting basic properties such as the antidepressants fluoxetine, norfluoxetine, and paroxetine, higher pH values will result in higher K_d values. On the contrary, for neutral molecules (such as carbamazepine) no significant variations in K_d were observed in the case of variation in pH.

K_d values were investigated for sludge produced in activated sludge systems with short and long SRTs (Fernandez-Fontaina et al., 2012; Horsing et al., 2011), in anoxic, aerobic and anaerobic compartments. Fernandez-Fontaina et al. (2012) and Hyland et al. (2012) found similar values of K_d in CAS with different sludge ages, while Jia et al. (2012) found that, referring to fluoroquinolone antibiotics, Kd values are slightly higher in aerobic units than anoxic and anaerobic units.

Fernandez-Fontaina et al. (2012) remarked that K_d values obtained in batch experiments are significantly lower than values obtained in continuous reactors and highlighted the importance of measuring sorption coefficients under real operating conditions. Discrepancies could be due to the different acclimatization conditions of the biomass, resulting in different biodegradation rates and bioavailability.

Horsing et al. (2011) experimentally determined the values of K_d for primary and secondary sludge for 75 compounds. For most PhCs, K_d values are higher for secondary sludges than primary ones due to different factors, including better sorption onto the former, higher organic matter content in secondary sludge (Yan et al., 2014) and fast biodegradation which reduces the concentration of the compound in water (Martin et al., 2012b).

Stasinakis et al. (2010) investigated the influence of SRT (3, 10 and 20 d) on K_d values for NP and TCS in an activated sludge system fed with municipal wastewater. They found that the highest K_d values occurred at the shortest SRT.

There have been many attempts to correlate K_d with properties of the compound of interest and the solid phase (sludge types, particles, sediments and soil), from single parameter to multiple parameter models. To evaluate the sorption of lipophilic compounds on secondary sludge, Matter-Muller et al. (1980) proposed the following equation:

$$K_d = 0.39 + 0.67\, K_{ow} \qquad \text{(eq. 2.3)}$$

In the same years, Karickoff (1981) developed a two parameter equation for K_d on the basis of K_{ow} and the fraction of organic carbon in sludge foc:

$$K_d = f_{oc}\, x\, 0.41\, x\, K_{ow} \qquad \text{(eq. 2.4)}$$

Eq. 2.4 was used by many other authors, including Jones et al. (2002). The parameter foc is frequently assumed to be equal to 0.35. Other values have been suggested for foc for different kinds of sludge - for primary sludge 0.30 (Zhu and Chen, 2014), 0.43 (Braga et al., 2005) and 0.49-0.51 (Stevens-Garmon et al., 2011); for secondary sludge 0.27 (Andersen et al., 2003) and the ranges 0.39-0.47 (Stevens-Garmon et al., 2011), and 0.45-0.55 (Hyland et al., 2012); 0.02-0.136

for differently pretreated mesophilic digested sludge and 0.032-0.152 for differently pretreated thermophilic digested sludge (Carballa et al., 2008a).

An in-depth discussion of further semi-empirical expressions suggested for calculating Kd as a function of K_{ow} is reported in Andersen et al. (2005), Pomiès et al. (2013), Sathyamoorthy and Ramsburg (2013) and, as a function of D_{ow}, by Stevens-Garmon et al. (2011).

Yan et al. (2014) remarked that eq. 2.4 leads to an overestimation of several orders of magnitude for hydrophobic compounds and to an underestimation for ionic and polar ones.

Some authors (Golet et al., 2003,Ternes et al., 2004a) remarked that for compounds, including fluoroquinolones, characterized by low K_{ow},(log K_{ow} = -1 for norfloxacin) and high K_d (log K_d = 3.9 for norfloxacin), electrostatic interactions are the main sorption mechanism. On the contrary, non-ionic compounds such as EE2 (log K_{ow} = 4.2, log K_d = 2.8) tend to be sorbed in the lipid fraction or onto organic matter at ambient pH and for them hydrophobic interactions are quite relevant.

For acidic and basic compounds different correlations have been developed. A discussion is reported in Verlicchi et al. (2013c) and Vasquez-Roig et al. (2012). More complex polyparameter models are discussed in Sathyamoorthy and Ramsburg (2013) with regard to negatively or positively charged compounds.

An interesting compilation of literature data of K_d for secondary sludge obtained in different systems (CAS, MBR) can be found in the review by Sathyamoorthy and Ramsburg (2013), which correlates K_d values to pH, biomass concentration in the aeration tank, charge and pK_a of the secondary treatment under consideration.

2.7 Concentration of PPCPs in soil after sludge application

Once the digested sludge is spread onto soil, occurring PPCPs may be subjected to different processes, namely fixation, mobility and transport, degradation and inactivation. Fixation depends on the nature of interaction between PPCPs and the sludge-amended soil characteristics. PPCP concentration in soils depends on many factors that will be addressed in the following section, in discussing measurements and predicted values.

2.7.1 MEC

Data regarding PPCP concentrations in sludge-amended soil are scarce due to the lack of appropriate instrumentation and methods to carry out accurate measurements of compounds occurring at very low concentrations in complex matrices (Li, 2014, Kinney et al., 2008). Table 2.6 reports the range of concentrations found in literature.

With regard to trimethoprim, carbamazepine and triclosan, different ranges of concentrations were found by Kinney et al., (2008) and Li (2014), confirming that many factors may influence their occurrence. These factors include rate of sludge application, frequency, soil conditions and characteristics, chemical and biological characteristics of the compound (Butler et al., 2012), time between sludge application and soil sampling (Jones et al., 2014) precipitation and runoff.

Golet et al. (2002) measured the concentrations of norfloxacin and ciprofloxacin in the topsoil 8 months after sludge application. They found 0.29-0.32 mg /kg DM for norfloxacin and 0.35-0.40 mg/kg DM for ciprofloxacin. They also monitored the sludge-amended soil concentration after 21 months and noticed a slight reduction in the antibiotic levels, demonstrating that traces of fluoroquinolones persist and may accumulate in the terrestrial environment after sludge application. Butler et al. (2012) reported a slight attenuation of triclosan in soil (initially 0.8-1 mg/kg) in the first eight months following the sludge application in three different soil types. The reduction was about 80 % after one year of application. They attribute this reduction to the biodegradation of triclosan to methyl triclosan, whose concentration was found at about 0.4 mg/kg.

Table 2.6: Measured concentrations of PhCs in soil and corresponding references. [a] n.d.= not detected.

Compound	Measured concentrations [ng/g]	References
Diclofenac	n.d.[a] — 1.16	Li (2014)
Ibuprofen	n.d. — 5.03	Li (2014)
Ciprofloxacin	350–400 after 8 months	Golet et al. (2002)
	280–270 after 21 months	
	450 (2.5 cm depth)	Golet et al. (2003)
Norfloxacin	320–290 after 8 months	Golet et al. (2002)
	270–300 after 21 months	Golet et al. (2002)
	350 (2.5 cm depth)	Golet et al. (2003)
Sulfadiazine	n.d. — 3.82	Li (2014)
Trimethoprim	0.64	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
	n.d. — 60.1	Li (2014)
Diphenhydramine	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
Carbamazepine	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
	0.02–7.5	Li (2014)
Caffeine	n.d.	Kinney et al. (2008)
Triclosan	833	Kinney et al. (2008)
	96;160	Kinney et al. (2008)
	n.d. — 16.7	Li (2014)
	774–949	Butler, Whelan, Ritz, Sakrabani and Van Egmond, 2011
Galaxolide (HHCB)	633	Kinney et al. (2008)
	1050; 2770	
Tonalide (AHTN)	113	Kinney et al. (2008)
	287; 773	Kinney et al. (2008)
NP1EO	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)
NP2EO	n.d.	Kinney et al. (2008)
	n.d.	Kinney et al. (2008)

The sorption of PhC to soil depends on the soil pH, soil organic materials and soil minerals (Thiele-Bruhn, 2003). The most important mechanisms are association with organic matter, ion exchange, surface adsorption to mineral constituents, hydrogen bonding and the formation of complexes with ions such as Ca^{2+}, Mg^{2+}, Fe^{3+} or Al^{3+} (Thiele-Brun, 2003; Diaz-Cruz et al. 2003, Xia et al., 2005).

In this context, on the basis of the pK_a value of a compound (see Table SD-2 of Verlicchi and Zambello, 2015), Monteiro and Boxall (2010) propose a scheme to predict its main sorption mechanisms, which include hydrophobic interactions; van der Waals interactions, hydrogen bonds with OM or clay, cation exchange, charge transfer, and ligand exchange with OM.

With regard to the adsorption of antibiotics to organic and mineral exchange sites, this is mostly due to charge transfer and ion interactions and not to hydrophobic partitioning. Strongly adsorbed antibiotics are subjected to transportation processes due to fast leaching through soils by macropores, or to the transportation of the dissolved soil colloids to which they are attached.

In a soil matrix, biodegradation can take place with different kinetics depending on the (micro)environment where they are located. Triclosan and triclocarban, for instance, tend to sorb onto soil and sediment and may be subjected to very low biodegradation in aerobic conditions, whereas in anaerobic conditions they are more resistant (Ying et al., 2007).

The mobility of PPCPs in soil, and consequently their potential for contaminating groundwater and surface waters, is shown to depend on the amount of substance applied, the intensity of the rain events and the soil type. Mobility of a pharmaceutical (or any other organic compound to that

effect) in a heterogeneous porous medium such as soil is also influenced by the soil structure and not simply its composition (Drillia et al., 2005b). Some PhCs may reach surface water due to fast preferential and macropore flow, others due to co-transportation with mobile colloids such as dissolved organic materials (Thiele-Bruhn, 2003). Photodegradation has no significant effect, whereas biodegradation may take place due to the action of enzymatic transformation reactions like oxidative decarboxylation and hydroxylation.

PPCP sorption onto soil organic matter and soil minerals or the formation of complexes may cause a loss of detectability as well as a loss in bacterial activity (Kummerer, 2009). There could be the potential for accumulation of compounds within agricultural soils characterized by very poor biodegradability or biotransformability (as is the case of some benzodiazepines, Redshaw et al., 2008).

2.7.2 Predicted concentrations of PhCs in soil

According to the European Technical Guidance Document on Risk Assessment EUR 20418 EN/2 (EC, 2003), the PhC concentration in soil may be assessed by eq.

$$PEC_{i,\,soil} = \frac{c_{i,sludge}\ x\ APP_{sludge}}{DEPTH_{soil}\ x\ RHO_{soil}} \qquad \text{(eq. 2.5)}$$

where c_{sludge} is the MEC (or PEC) in digested sludge (μg/kg DM), APP_{sludge} is the application rate of the dry sludge onto soil (generally the value of 0.5 kg/m^2 is used for agricultural soil; Stasinakis et al. (2008) adopted 1 kg/m^2), $DEPTH_{soil}$ is the mixing depth (generally 0.20 m is used for agricultural soils. Stasinakis et al., 2008 adopted 0.10 m) and RHO_{soil} is the bulk density of wet soil (1,700 kg/m^3 for agricultural soils; Stasinakis et al. (2008) adopted 1,300 kg/m^3).

The “depth of soil” represents the depth range for the top soil layer which is of interest. The depth of 20 cm is generally taken because this range usually has a high root density of crops, and represents the ploughing depth. For grassland the depth is less, since grasslands are not ploughed. The average period of 180 days for crops is chosen as a representative growing period for crops. For grassland this period represents a reasonable assumption for the period that cattle are grazing on the field. For the ecosystem a period of 30 days is taken as a relevant time period with respect to chronic exposure of soil organisms (EC, 2003).

The model used to evaluate PEC_{soil} is based on the assumption that a complete mixing between sludge and soil occurs. This may not always be verified and the concentration of selected compounds could be higher (accumulation of the substance) or lower. In McClellan and Halden (2010) a different approach for soil prediction concentration which also considers pore water contribution is proposed and discussed.

Table 2.7 reports predicted concentrations in soil for a selection of compounds.

According to Drillia et al., 2005b, the tendency of pharmaceuticals to move through the soil is well correlated with their sorption tendencies and for this objective a rough evaluation could be carried out by using Kd for the different kinds of soil.

Drillia et al. (2005) provide values of K_d for soils with low organic carbon and high clay content and soil with high organic carbon and low clay content.

Sarmah et al. (2008) provided K_d for different soils (in New Zealand) with regard to three estrogens (E2, EE2, and E1) and noted consistent differences in the soil organic carbon content.

Table 2.8 reports the collected data regarding K_d for the different kinds of soils.

Table 2.7: PEC in soil available in literature and PNEC for some compounds.

Class	Compound	PEC soil [ng/g DM]	PNEC soil [ng/g DM]	References
A	Diclofenac	0.14-0.21		Jones et al. (2014)
			0.013	Munoz et al. (2009a)
	Ibuprofen	0.58-1.42		Jones et al. (2014)
			0.73	Munoz et al. (2009)
B	Ciprofloxacin	40 (60 t/ha of sludge)	26000	Eriksen et al. (2009)
		1400–6000 (2.5 cm depth)		Golet et al. (2003)
		180–750 (20 cm depth)	0.29	
	Erythromycin	0.12-0.34		Jones et al. (2014)
			0.0041	Munoz et al. (2009a)
	Norfloxacin	1400–6000 (2.5 cm depth)		Golet et al. (2003)
		180–750 (20 cm depth)		
	Ofloxacin	0.46-1.23 Jones et al. (2014)		
	Oxytetracycline	16.43-91.65		Jones et al. (2014)
	Sulfamethoxazole		0.025	Munoz et al. (2009a)
	Tetracycline	10 (60 t/ha of sludge)	8800	Eriksen et al. (2009)
J	Hydrochlorothiazide		2400	Munoz et al. (2009a)
O	Atenolol		440	Munoz et al. (2009a)
	Metoprolol	20 (60 t/ha of sludge)	58900	
	Propranolol	0.31-0.81		Jones et al. (2014)
	Sotalol	20 (60 t/ha of sludge)	4095000	
S	Atorvastatin	50 (60 t/ha of sludge)	11000	
	Gemfibrozil		0.061	Munoz et al. (2009a)
T	Carbamazepine		0.05	Munoz et al. (2009a)
	Fluoxetine	0.28-0.52		Jones et al. (2014)
			44	Munoz et al. (2009a)
V	Ranitidine	40 (60 t/ha of sludge)	5277	Eriksen et al. (2009)
W	Caffeine		37	Munoz et al. (2009a)
a	Triclosan	80		Stasinakis et al. (2013)
			2.1	Munoz et al. (2009a)
			0.096	Ying and Kookana (2007)

Table 2.8: K_d values in different kinds of soil and corresponding references.

Class	Compound	Kd soil [L/kg]	References
Analgesics/Anti-inflammatories, A	Acetaminophen	32	Barron et al., 2009
	Diclofenac	9 0.45 Low organic carbon and high clay content 164.5 High organic carbon and low clay content	Barron et al., 2009; Drillia et al., 2005b
	Ketoprofen	9	Barron et al., 2009
	Naproxen	11 10.13;252.9	Barron et al., 2009; Monteiro and Boxall, 2010
	Phenazone	8	Barron et al., 2009
	Salicylic acid	82 3.6;397	Barron et al., 2009; Monteiro and Boxall, 2010
	Tramadol	22	Barron et al., 2009
Antbiotics, B	Ciprofloxacin	427 398	Monteiro and Boxall, 2010; Nowara et al., 1997
	Enrofloxacin	260;6310 501;5012	Monteiro and Boxall, 2010; Nowara et al., 1997
	Erythromycin	68 164.8	Barron et al., 2009; Monteiro and Boxall, 2010

Class	Compound	Kd soil [L/kg]	References
	Ofloxacin	1192 Low organic carbon and high clay content 3554 High organic carbon and low clay content	Drillia et al., 2005b; Monteiro and Boxall, 2010
	Oxytetracycline	417;1026	Monteiro and Boxall, 2010
	Sulfamethazine	9 1.68;98.25	Barron et al., 2009; Monteiro and Boxall, 2010
	Sulfamethoxazole	8 0.23 Low organic carbon and high clay content 37.6 High organic carbon and low clay content	Barron et al., 2009; Drillia et al., 2005b; Monteiro and Boxall, 2010
	Sulfapyridine	8 3.47	Barron et al., 2009; Monteiro and Boxall, 2010
	Trimethoprim	26	Barron et al., 2009
Anti coagulant, E	Warfarin	8	Barron et al., 2009
Antifungals, H	Econazole	1029	Barron et al., 2009
Antineoplastic, K	Cyclophosphamide	1626	Barron et al., 2009
B-agonists, N	Salbutamol (Albuterol)	26	Barron et al., 2009
B-blockers, O	Atenolol	15	Barron et al., 2009
	Metoprolol	20	Barron et al., 2009
	Propranolol	58 16.3 Low organic carbon and high clay content 199 High organic carbon and low clay content	Barron et al., 2009; Drillia et al., 2005b;
Hormones, Q	Estradiol E2	3.3 14.1;170.5 Farming soil	Monteiro and Boxall, 2010; Sarmah et al., 2008
	Estrone E1	12.3;50.2 Farming soil	Sarmah et al., 2008
	Ethinylestradiol, EE2	3.35 12.4; 235.9 Farming soil	Monteiro and Boxall, 2010; Sarmah et al., 2008
Lipid regulators, S	Bezafibrate	14	Barron et al., 2009
	Clofibric acid	9 n.d. Low organic carbon and high clay content 5.38 High organic carbon and low clay content	Barron et al., 2009 Drillia et al., 2005b
	Simvastatin	85	Barron et al., 2009
Psychiatric drugs, T	Amitryptiline	138	Barron et al., 2009
	Carbamazepine	13 0.49 Low organic carbon and high clay content 37 High organic carbon and low clay content 4.66;32.78	Barron et al., 2009; Drillia et al., 2005b; Monteiro and Boxall, 2010
	Citalopram	250	Barron et al., 2009
	Diazepam	30	Barron et al., 2009
	Fluoxetine	134.44;234,83	Monteiro and Boxall, 2010
Receptor antagonists, V	Cimetidine	11	Barron et al., 2009
	Ranitidine	50	Barron et al., 2009
Stimulant, W	Caffeine	25	Barron et al., 2009
Antiseptics, a	Triclosan	127	Barron et al., 2009
	Triclocarban	438	Barron et al., 2009

2.8 RQ due to PhCs and PCPs in sludge and in sludge- amended soil

The common equations used for evaluating the environmental risk posed by PPCPs occurring in sludge and after its application to soil for agriculture purposes is based on the risk quotient (RQ) that is the ratio between pollutant concentration and its predicted no-effect concentration (PNEC):

$$\mathrm{RQ}_{i,j} = \left.\frac{C_i}{PNEC_i}\right|_j \qquad i = \mathrm{PhC},\ j = 1\ (\text{digested sludge}),\ 2\ (\text{soil}) \qquad \text{(eq. 2.6)}$$

where C_i represents the concentration of the compound of interest in the solid phase (sludge or soil) and may be directly measured (MEC) or predicted (PEC) by means of literature models as already discussed above. With regard to sludge, PEC is generally evaluated according to eq. 2.1 or eq. 2.2, whereas PEC_{soil} may be predicted after one dose of sludge application, according to eq. 2.5:

Due to the lack of data regarding chronic and acute toxicity for terrestrial dwelling organisms with regard to PhCs and PCPs, many authors (Martin et al., 2012a) evaluate the corresponding PNEC for sludge and soil on the basis of the known PNEC for the water and partition coefficient K_d of the compound of interest, according to eq. 2.7. PNEC reported in Table 2.7 are literature data regarding specific values of PNEC evaluated for soil by the reported Authors. In this study, values of PNEC used for environmental risk assessment are those reported in Verlicchi et al. (2012).

$$\mathrm{PNEC}_{\mathrm{i,\,j}} = \mathrm{PNEC}_{\mathrm{water}} \mathrm{x}\ K_{\mathrm{d,\,i\,j}}\ \mathrm{x}\ 1000 \qquad \text{(eq. 2.7)}$$

where i =PPCPs and j = sludge or soil.

PNEC values refer to the acute toxicity data taken from literature. According to eq. 2.7, PNEC values for soil and sludge refer to aquatic organisms and not to terrestrial ones, as only a little data is available regarding the toxicological effects of PPCPs on terrestrial organisms (Table 2.7). This approach is suggested by the European Commission (EC-TGD, 2003) and is called the equilibrium partition approach.

The criteria usually applied to evaluate the risk by means of RQ values is that proposed by Hernando et al. (2006), which considers a high risk if RQ ≥ 1, medium risk if 0.1< RQ < 1 and low if RQ ≤ 0.1.

An environmental risk analysis was carried out for those PPCPs whose concentrations in digested sludge, Kd values for digested sludge and PNEC (in water) of the compound of interest are known. For this group of PPCPs, the minimum and maximum RQ values have been evaluated (eq. 2.6) on the basis of their minimum and maximum concentrations found in digested sludge (Table SD-3 of Verlicchi and Zambello, 2015) and the average value of K_d among those reported for the compounds in Table SD-4 of Verlicchi and Zambello, 2015.

The results are reported in Fig. 2.11, which provides a snapshot of the current knowledge. It shows that a high environmental risk is posed by antibiotics (sulfamethoxazole, erythromycin, roxithromycin, azithromycin, and ofloxacin), hormones (E1, E2, and EE2), analgesics and anti-inflammatories (acetaminophen, ibuprofen, naproxen and salicylic acid) and the beta-blocker propranolol.

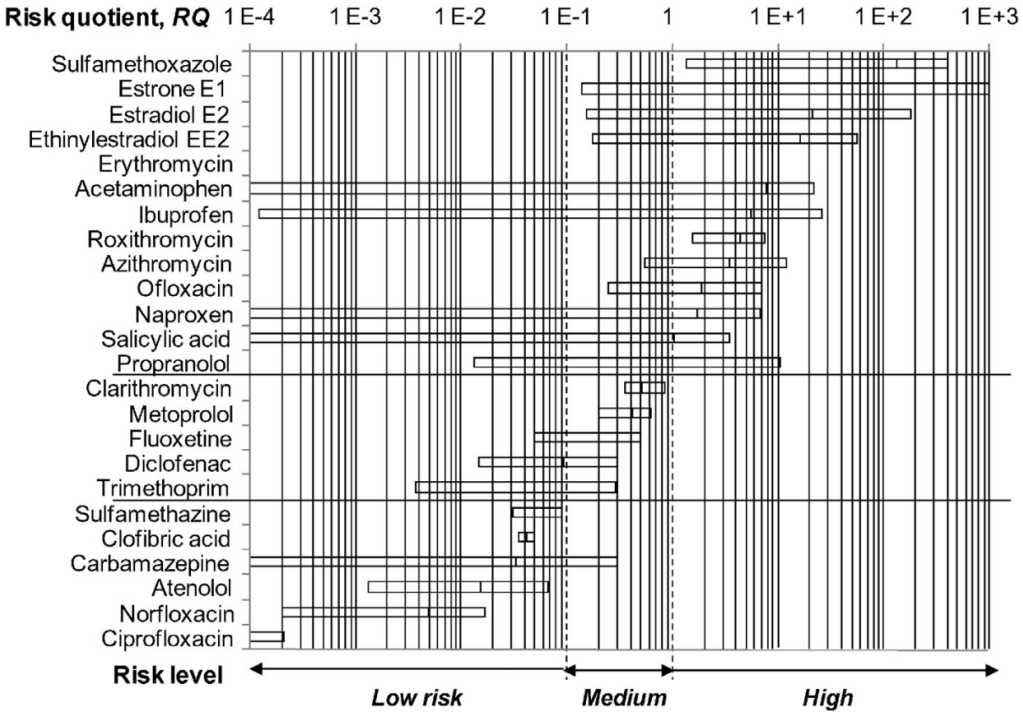


Fig. 2.11: Risk quotient posed by the residue of PhCs in digested sludge

Previous studies provided a risk analysis based on the RQ approach for a limited group of compounds in secondary, digested sludges and after sludge application on soil. These are briefly compiled in Table 2.9. With regard to digested sludge, the most critical compounds are antibiotics (sulfamethoxazole, sulfadiazine, ofloxacine, erythromycin andazithromycin), hormones (ethinyestradiol andestradiol), ibuprofen and triclosan and triclocarban. After sludge application on soil, the high risk is due to the residual of estradiol, ciprofloxacin, ofloxacin, tetracycline, caffeine, triclosan and triclocarban.

Table 2.9: Review of the published investigations on the risk quotient due to PPCPs in sludge and in the case of sludge-amended soil. [a] McClellan and Halden (2010) and Clarke and Smith (2011) follow different approaches in assessing environmental risk.

References	**Sludge**	**Sludge**	**Sludge**	**Dig. sludge-amended soil**	**Dig. sludge-amended soil**	**Dig. sludge-amended soil**
	RQ ≥ 1	**0.1 < RQ < 1**	**RQ ≤ 0.1**	**RQ ≥ 1**	**0.1 < RQ < 1**	**RQ ≤ 0.1**
Martin et al. (2012b) (digested sludge)	Ibuprofen, estradiol, ethinylestradiol	Salicylic acid, carbamazepine	Naproxen, propranolol, caffeine, estriol	Estradiol	Ethinylestradiol	Ibuprofen
McClellan and Halden (2010)a (digested sludge)				Ciprofloxacin, ofloxacin, tetracycline, caffeine, triclosan, triclocarban		
Zhu and Chen (2014) (mixed sludge)	Sulfamethoxazole, Triclocarban, triclosan	Carbamazepine, diclofenac	DEET, trimethoprim, caffeine, ibuprofen			
Yan et al. (2014) (secondary sludge)	Azithromycin, sulfadiazine, sulfamethoxazole, ofloxacine, erythromycin	Norfloxacin, roxithromycin, clfobric acid	Trimethoprim, sulfametazine, diclofenac, bezafibrate, metoprolol, amlodipine, simvastatin, carbamazepine			
Clarke and Smith (2011)a				Triclosan, triclocarban		

Martin et al. (2012b) compare the risk in untreated and treated sludge and remark that the environmental risk due to PhC occurrence in sludge decreases from digested sludge and after application on land (digested sludge-amended soil) and is lower still in the case of compost applied to land. Land application represents a viable environmental route to enter the food chain, even if PPCP concentrations in the sludge remain very low.

Different criteria for environmental risk assessments have recently been proposed and are under discussion. Eriksen et al. (2009) suggest assuming a cut-off of 100 μg/kg as the PNEC of PhCs for soil and that PhC levels below this limit should be regarded by the European Medicine Agency as posing a negligible environmental risk. With regard to hormones, the cut-off is set at 10 μg/kg as this group of compounds is considered to pose a higher environmental risk. This approach implies refining steps in order to identify the group of compounds which requires special attention. Munoz et al. (2009) based their environmental risk assessment on the half-life in soil for the compounds of interest and assume it to be equal to twice the value obtained for the water compartment. In addition, they consider that in 6 half-lives complete degradation of the compound will occur, assuming first-order kinetics.

2.9 Future fields of research

Future investigations should focus on the occurrence of some groups of PPCPs that have a high sorption potential (such as antimycotics) in treated sludge, and their fate in the case of sludge-amended soil. Special attention should also be paid to the ability of the compound to sorb onto the dissolved organic matter fractions. They can affect the mobility of PPCPs in soils influenced by intensive irrigation with reclaimed wastewater or amended with treated sludge (Maoz and Chefetz, 2010).

Moreover, future investigations should also deal with the reduction of the total estrogenic activities measured after treatment due to transformation products, mainly for those treatments able to attenuate the content of the PPCPs of interest.

Improvements in environmental risk assessment are highly recommended in particular research on PNEC referring to soil-dwelling organisms, especially plants and fauna in soil, rather than to aquatic ones, as has already been done for other groups of compounds, including anionic surfactants (LAS) (Kloepper-Sams et al., 1996, Ying et al., 2006).

Very little data is available regarding the chronic toxicity and effects of mixtures of PPCPs on different organisms. Moreover, studies refer to the effects of the contemporary occurrence of sub-therapeutic concentrations of antibiotics on soil microbial community structures, as well as the spreading of antibiotic resistant bacteria.

The environmental risk assessment should be carried out in a global perspective and include potential leaching due to the rain water runoff of sludge-amended soil onto surrounding surface water, the contribution of irrigation by means of reclaimed wastewater reuse, which represents an additional exposure route for the target compounds in terrestrial ecosystems. In this context, Munoz et al. (2009) developed a method to carry out this evaluation and Vasquez-Roig et al. (2012) have already addressed some interesting issues.

There is a further element of risk posed by the wash-off of sewage sludge into water courses. Whilst concentrations of contaminants in sludges reported here were low in relation to the sludge/soil concentration criteria, the presence of a relatively small quantity of sludge in suspension in a watercourse could exceed the much more stringent EQS values that have been set for surface waters.

Reference

- Verlicchi P, Zambello E. Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil – A critical review. Sci Tot Environ 2015;538:750-767

Chapter 3

3 Removal of PCPs in CWs

3.1 Introduction

Every day we use products for our personal care and hygiene, in particular cosmetics (skin care products, hair sprays, and sunscreens), toiletries (bath additives, soaps, hair tonics, shampoos, oral hygiene products) and fragrances (perfumes, aftershaves). These products, commonly called personal care products (PCPs) contain synthetic organic chemicals with a specific function, the ingredients. They may be antimicrobial disinfectants (triclosan, triclocarban), preservatives (methylparaben, ethynilparaben, butylparaben) or sunscreen agents (oxybenzone, avobenzone). In addition, some of them may contain synthetic surfactants (generally anionic and nonionic compounds). These are substances widely used in the formulation of many commercial PCPs not only for their wetting, cleaning, foaming and emollient properties, but also as they can create dispersed systems (suspension or emulsion), modify the cosmetic rheological properties, prolong the durability of the product and control the release of active ingredients (Somasundaran et al., 2006) which greatly improves the quality of the substance.

PCPs are used in the range of several thousand tons per year: parabens are used in more than 22,000 cosmetic products (Andersen, 2008), approximately 350 tons of triclosan are produced annually in Europe (Singer et al., 2002), and in 1998, 1,473 tons of galaxolide, 343 tons of tonalide and 18 tons of celestolide were consumed in Europe (Alder et al., 2007).

These products are disposed of or discharged into the environment on a continuous basis via municipal/industrial sewage facilities and also directly by untreated discharges (Ternes et al., 2003; Kunz and Fent, 2006; Bester, 2007). This means that their exposure potential may reach critical level for the environment, even for those compounds that might have a low persistence.

In recent years, increasing attention has been paid to the occurrence of some of them in aquatic environments, also due to the finding that some PCPs can induce known or suspected undesirable effects on humans and ecosystems (included endocrine disruptions) (Stuart et al., 2012).

Limits of concentrations have been set for surfactants with regard to WWTP discharges into surface water bodies or for the direct reuse of treated effluents. However, limits do not exist for many other PCPs occurring in wastewaters. Environmental quality standards have also been set for some micropollutants in surface water bodies within the EU (Directive 2013/39/UE).

In the European Union, USA and other countries a debate is open regarding the compilation of lists including priority compounds requiring monitoring in the aquatic environment (Directive 2013/39/UE, Richardson and Ternes, 2011; Bottoni et al., 2010; Lapworth et al., 2012). However, due to the lack of information on toxicity and environmental impacts, a large number of contaminants, especially organic compounds, are not included in these lists. The number of compounds which could become priorities is therefore likely to grow.

Recent studies have remarked that due to the wide spectrum of characteristics of emerging contaminants, including PCPs, it is quite difficult to find a treatment able to remove most of them at a high percentage. Recent studies [Verlicchi et al., 2012c, Verlicchi and Zambello, 2014] pointed out that different groups of micropollutants can be removed at a medium-high extent only in those treatment trains where different removal mechanisms may occur. Multi-barrier treatment systems are necessary. As highlighted in [Verlicchi et al., 2013b] constructed wetlands (CWs) are systems where oxic-anoxic-anaerobic environments may coexist, especially in subsurface flow beds or in sequence of different kinds of CW types. In surface flow systems, solar radiation may also contribute to the removal of micropollutants.

Increasing attention is being paid to the investigation of the occurrence and removal of common PCPs from wastewater but only a few studies deal with CWs. This chapter provides an overview of these issues, focusing on the different types of CWs acting as primary, secondary or tertiary steps. Influent and effluent concentrations for 33 PCPs, belonging to 9 different classes were collected and discussed, along with their corresponding removal efficiencies achieved in the investigated types of CWs. The chapter concludes with an analysis of the influence of the main design parameters and operational and environmental conditions on the removal of the reviewed compounds.

3.2 Chapter Framework

This chapter is based on data collected from 36 peer reviewed papers published between 2001 and 2014, referring to 33 PCPs. All compounds are listed in Table 1, grouped according to their class. For each of them, chemical formula, CAS number and molecular structure are reported together with the references of the investigations included in the review dealing with it. A focus on surfactant classes is available in Table 2 where the 9 most common ones are reported. Table 3 reports the schematics to which the investigated wetlands refer (that is if they act as a primary, secondary, or tertiary step) and Table 4 shows the CW types included.

The study continues with an analysis of the occurrence of the PCPs in the influent and effluent of CW acting as a primary, secondary and tertiary step and a discussion of their removal achieved in the three steps distinguishing between the CW types. (Fig. 3.1-Fig. 3.9). The characteristics and performance of restoration wetlands are then discussed and finally data referring to occurrence (Fig. 3.10-Fig. 3.11) and removal (Fig. 3.12) in hybrid systems complete the analysis of the different reviewed configurations. The final part of the chapter discusses how CW type, design parameters and operational and environmental conditions influence the removal of investigated compounds on the basis of the collected literature data.

3.3 Personal Care Products in the environment and compounds included in the study

Table 3.1: List of reviewed PCPs, grouped according to their class.

Anti oxidant	Butylated hydroxyanisole (BHA); Butylated hydroxytoluene (BHT); Ethylenediaminetetraacetic acid (EDTA)
Antiseptics:	Triclocarban; Triclosan;
Deodorant	1,4-Dichlorobenzene *(p*-DCB)
Flame retardant	tris (2-chloroethyl) phosphate (TCEP)
Insect repellent	Diethyl-3-methylbenzoylamide (DEET)
Plasticizer	4,4'-(propane-2,2-diyl)diphenol (Bisphenol A)
Sun screen product	Avobenzone (Parsol); Hydrocinnamic acid; Oxybenzone
Synthetic musk	Cashmeran; Celestolide; Galaxolide (HHCB); Methyl dihydrojasmonate (MDHJ); Tonalide (AHTN)
Anionic surfacants:	Linear alkylbenzene sulfonate (LAS): LAS C10 ; LAS C11; LAS C12; LAS C13; Sulfophenyl carboxylate (SPC): SPC-C9; SPC-C10; SPC-C11;
Nonionic surfacant	Nonylphenol (NP); Nonylphenol mono ethoxylate (NP1EO), Nonylphenol diethoxylate (NP2EO); Nonyl phenol mono ethoxycarboxylic acid (NP1EC) ; Nonyl phenol di ethoxycarboxylic acid (NP2EC); 4-tert-octylphenol (OP); 4-tert-octylphenolmonoethoxylate (OP1EO); 4-tert-octylphenoldiethoxilate (OP2EO); Surfynol 104

Table 3.2: Classes of Surfactants included in the chapter

Anionic surfacants	Methylen Blue Active Subtances MBAS; Linear alkylbenzene sulfonate LAS; Sulfophenyl carboxylate SPC; Linear Alkyl benzene LAB; Alkylethoxy sulfonates AES
Nonionic surfacants	NP(1-3)EO, NP(4-9)EO 4 alkylphenol monoetoxylated APE Alkylphenols AP Triton X 100 (4- octylphenol polyethoxylate)

The chapter refers to 33 PCPs belonging to 9 different classes: 3 antioxidants, 2 antiseptics, 1 deodorant, 1 flame retardant, 1 insect repellant, 1 plasticizer, 3 sun screen products, 5 synthetic musks and 16 surfactants (7 anionic and 9 nonionic ones).

Reviewed compounds are reported in Table 3.1 and classes of surfactants in Table 3.2 and their physico-chemical properties in Appendix A. Their molecular structure is particularly complex due to the presence of aromatic and/or condensed rings, carboxylic and ketonic groups, double or triple bonds and in the case of surfactants, long hydrocarbon chains.

In Italy, NP and *p*-dichlorobenzene have been included among the substances to be monitored in the surface water [D. Lgs. 152/2006]; in Switzerland, EDTA, NP, triclosan, DEET, bisphenol A are included in the list of *relevant micropollutants in wastewater* and they could be considered "target compounds" for which Swiss WWTPs, with a high environmental impact, should guarantee desired removal efficiencies [Kase et al., 2011]. At a European level, NP is included in the list of priority substances Directive 2013/39/UE, requiring monitoring in water, and in the USA, BHA is included in the contaminant candidate List 3 U.S.EPA 2009 (Richardson and Ternes, 2011).

3.4 Classifications of Constructed Wetlands and types included in the chapter

The CWs have been classified according to the treatment step and the main flow direction.

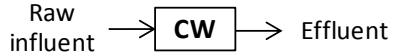
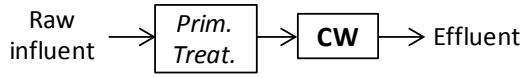
Depending on the treatment level, they have been divided into primary, secondary or tertiary steps (Table 3.3). In cases where they were fed by a river whose water flow is primarily made up of a wastewater treatment plant effluent or even untreated wastewater, the system was called restoration wetland. If the treatment system includes two or three steps relying on CWs, it is called hybrid plant.

Finally, a step may also include more than one stage, either of the same type (monotypic) or of different types (polytypic), thus resulting in a multi-stage system.

Referring to the flow direction, CWs are classified in surface flow systems (SF) and horizontal and vertical subsurface flow beds, H-SSF and V-SSF respectively (Table 3.4). In SF basins, the majority of flow occurs through a water column overlying a benthic substrate, whereas the flow in H-SSF and V-SSF beds is through a porous medium (generally gravel), and classified as either horizontal, if the feed is from one side of the bed to the other part, or vertical, if the feed is spread over the surface of the bed, crossing it from the top to the bottom. Additionally, in H-SSF beds the feed is continuous, while in V-SSF beds it is intermittent. Surface flow systems investigated also include a modified system, [Belmont and Metcalfe, 2003], where the effluent leaves the system after a passage through a stratum of materials at the bottom of the bed, resulting in a combination of surface and subsurface flow systems (Table 3.4).

In addition, there are two systems which are considered non-conventional. They are a pilot system fed by the secondary effluent of Empuriabrava WWTP, Spain, which t operated in parallel with the full scale reclamation plant consisting of surface flow basins [Matamoros et al., 2012a] and a sequence of SF and H-SSF cells [Zhu and Chen, 2014].

Table 3.3: Schematics of wastewater treatments including CWs in different configurations, with the corresponding references

CW acting as	Schematic	References
Primary step		Belmont and Metcalfe, 2003; Carlson et al., 2013; Kadewa et al., 2010; Lishman et al., 2006; Sima et al., 2011; Sima et al., 2013b
Secondary step		Avila et al., 2010; Conte et al., 2001; Hijosa-Valsero et al., 2010b; Hijosa-Valsero et al., 2011b; Huang et al., 2004; Matamoros and Bayona, 2006; Matamoros et al., 2007a; Matamoros et al., 2009; Reyes-Contreras et al., 2012;

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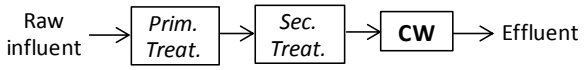
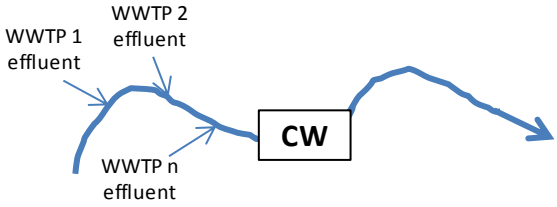
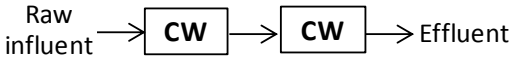
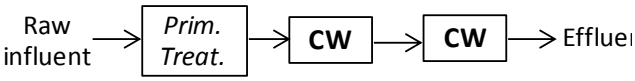
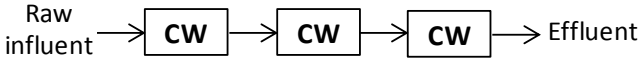
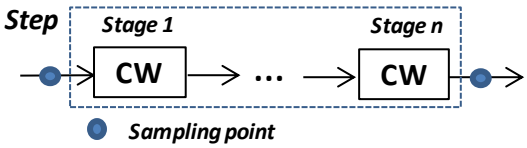
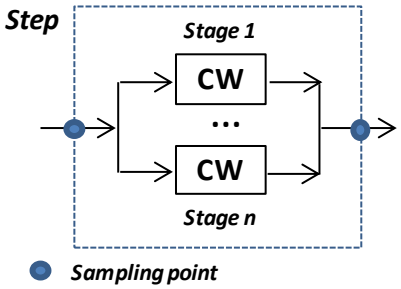
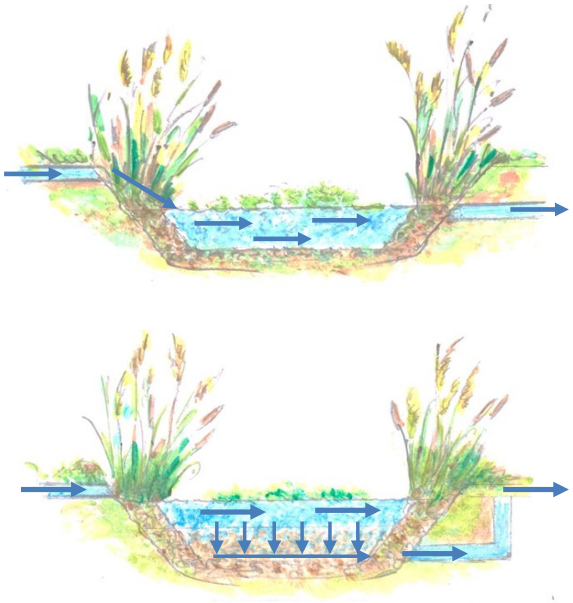
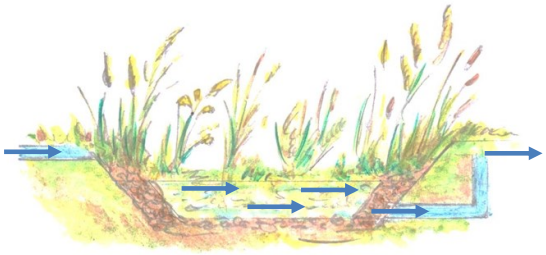
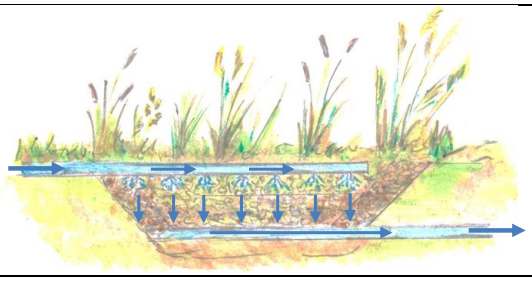
Tertiary step		Barber et al., 2006; Lee et al., 2011; Llorens et al., 2009; Matamoros and Salvado, 2012; Matamoros et al., 2008b; Matamoros et al., 2010; Matamoros et al., 2012b; Park et al., 2009; Reif et al., 2011; Reyes-Contreras et al., 2011; Walman et al., 2006; Zarate et al., 2012; Zhu et a., 2014
Restoration wetland		Matamoros et al., 2012a; Navarro et al., 2011;
Hybrid system		Li et al., 2013bb;
		Avila et al., 2013; Avila et al., 2014; Belmont et al., 2006; Conte et al., 2001; Hijosa-Valsero et al., 2010a; Jorkest et al., 2011;
		Hijosa-Valsero et al., 2010a;
Multi-stage step		Avila et al., 2010; Avila et al., 2014; Barber et al., 2006; Belmont et al., 2006; Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2011b; Kadewa et al., 2010; Matamoros et al., 2010; Maamoros and Salvado; 2012; Matamoros et al., 2012b; Park et al., 2009; Reyes-Contreras et al., 2011; Zhu et a., 2014
		Avila et al., 2013; Hijosa-Valsero et al., 2010a;

Table 3.4: Classification of Constructed Wetlands and corresponding references. The numbers (1,2,3) reported as apex for each reference refer to the treatment steps of the investigated plants while the letter "a" means restoration wetland.

CW Type	Schematic	References
Surface flow (SF): *Classic schematic (A)* *Modified schematic (B)*		Avila et al., 2014 [2+3]; Barber et al., 2006[3]; Belmont et al., 2006[2+3]; Carlson et al., 2013[1]; Conte et al., 2001[2+3]; Hijosa-Valsero et al., 2010a[1+2+3;2+3]; Hijosa-Valsero et al., 2010b[2]; Jokerst et al., 2011[2+3]; Lee et al., 2011[3]; Li et al., 2013b[1+2]; Lishman et al., 2006[1]; Llorens et al., 2009[3]; Matamoros and Salvado, 2012[3]; Matamoros et al., 2008b[3]; Matamoros et al., 2010[3]; Matamoros et al., 2012a[a]; Matamoros et al., 2012b[3]; Navarro et al., 2011 [a(1)]; Park et al., 2009[3]; Reyes-Contreras et al., 2011[3]; Reyes-Contreras et al., 2012[2]; Sima et al., 2011[1]; Sima et al., 2013b[1]; Walman et al., 2006[3]; Zarate et al., 2012[3]
Horizontal subsurface flow (H-SSF)		Avila et al., 2010[2]; Avila et al., 2013[2+3]; Avila et al., 2014[2+3]; Belmont and Metcalfe;2003[1]; Belmont et al., 2006[2+3]; Conte et al., 2001[2]; Hijosa-Valsero et al., 2010a[2+3]; Hijosa-Valsero et al., 2010b[2]; Hijosa-Valsero et al., 2011b[2]; Huang et al., 2004[2]; Jokerst et al., 2011[2+3]; Matamoros and Bayona, 2006[2]; Matamoros et al., 2009[2]; Reyes-Contreras et al., 2011[3]; Reyes-Contreras et al., 2012[2];
Vertical subsurface flow (V-SSF)		Avila et al., 2014[2+3]; Belmont et al., 2006[2+3]; Kadewa et al., 2010[1]; Matamoros et al., 2007a[2]; Matamoros et al., 2009[2]; Reif et al., 2011[3];

3.5 Main features of the investigated plants

The chapter is based on investigations of PCP occurrence and removal in CWs carried out in Europe (64 %: Spain, Denmark and England and Czech Republic), America (28 %: USA, Canada and Mexico) and Asia (8 %: Korea and China).

In the 36 peer reviewed papers, 88 treatment lines were investigated. They mainly include H-SSF beds (49 %) and SF basins (39 %), and in a few cases V-SSF systems (9 %). The types of CW are not well specified in only 3 % of the plants. Of the 88 treatment lines54 refer to pilot plants and 31 to full scale plants, while the remaining 3 refer to full scale plants followed by a pilot plant. Moreover, 12 treatment lines refer to hybrid systems.

In 9 lines the investigated CW acted as a primary step, in 42 as a secondary step, in 16 as a tertiary one and in 9 to restoration wetlands.

The feeding was always a real domestic wastewater, with a few cases where domestic wastewater was injected with selected PCPs at the desired concentration [Avila et lal., 2014, Lee et al., 2011; Avila et al., 2013; Belmont and Metcalfe, 2003] and one more where the influent contained a consistent percentage of industrial wastewater [Navarro et al., 2011]. Two studies [Kadewa et al., 2010; Jokerst et al., 2011] investigated occurrence and removal from greywater. All the treatment trains investigated were outdoor with the sole exception of the one investigated by [Belmont et al., 2006]. In nearly all studies, analyses were processed on grab samples of water.

3.6 Occurrence and removal in the different treatments steps

Fig. 3.1-Fig. 3.12 report concentrations observed in the influent and effluent of CWs acting as a primary, secondary and tertiary step and in the case of hybrid systems. They also report removal efficiencies for the investigated compounds in the systems under study. In the X-axis of each graph, the numbers in brackets after the PCP name correspond to the average values of the collected data for each of the CW types considered. Finally, ranges of concentration data for groups or mixtures of surfactants (MBAS, LAS, LAB, Triton X100) in the influent and effluent of some plants were reported in the discussion.

3.6.1 Primary step: occurrence and removal of selected PCPs

Only a few investigations reported PCP concentrations in the influent and effluent of CWs acting as a primary step. These are reported in Fig. 3.1 and Fig. 3.2, which show ten PCPs in the influent and eight in the effluent. The feeding was always only domestic wastewaters, with the exception of [Navarro et al., 2011] where the influent was a river receiving both untreated domestic as well as industrial wastewaters (see also paragraph 3.9).

[Belmont and Metcalfer, 2003; Sima and Holcova, 2011] investigated subsurface flow beds. All the other studies examined SF basins, which greatly differed for influent flow rate, geometry and size, configuration and environmental and operational conditions. Hydraulic retention time (HRT) varied between 0.4 d [Hijosa-Valsero et al., 2010b] and 5 d [Navarro et al., 2015]. The highest influent concentrations were found for the common nonionic surfactants NP1EO (289 μg/L), NP2EO (168 μg/L) and NP (41.5 μg/L), followed by triclosan (5.44 μg/L). The highest concentrations in the effluent were found for LAS C10 (195 μg/L), NP (28 μg/L), NP1EO (18 μg/L) and LAS C13 (15 μg/L). The same compounds exhibited the highest average values.

Referring to NP, NP1EO and NP2EO, the effluent concentration is always lower than the corresponding influent one, but for NP the reduction is the smallest. This is due to the fact that NP1EO and NP2EO may transform into NP during anaerobic degradation throughout the system.

Classes of surfactants were found at very high concentrations both in the influent and effluent of primary CWs: MBAS (Methylen blue active substances) 1390-17100 μg/L in the influent and 340-4560 μg/L in the effluent [Kadeva et al., 2010]; NP(1-3)EO 441 μg/L in the influent and 13 μg/L in the effluent [Belmont and Metcalfe, 2003]; Triton X100 978 μg/L in the influent and 99 μg/L in the effluent [Sima et al., 2013a; Jokerst et al., 2011]. These data point out that surfactants are present in a wide spectrum of substances commonly used in households, not only PCPs.

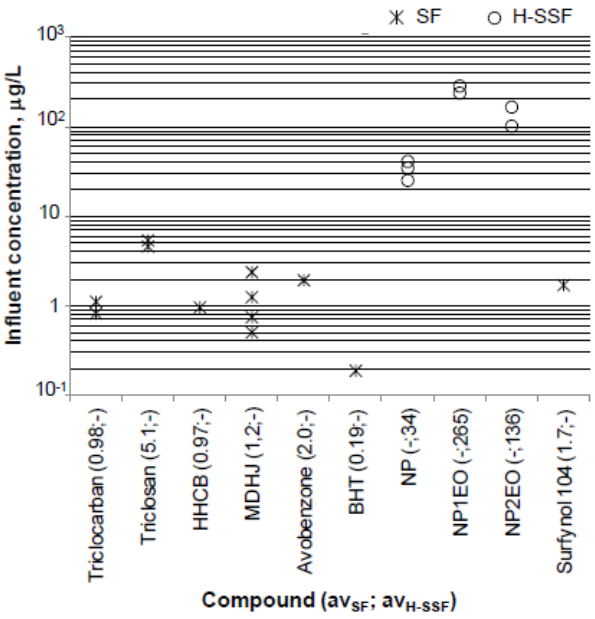


Fig. 3.1: Occurrence of investigated PCPs in the influent of CWs acting as a primary step. Data from: [Belmont and Metcalfe, 2003; Hijosa-Valsero et al., 2010a; Li et al., 2013b; Navarro et al., 2011]

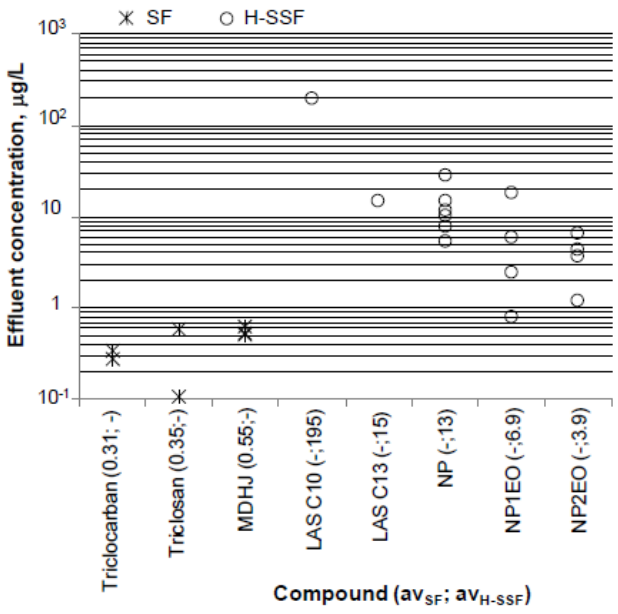


Fig. 3.2: Occurrence of investigated PCPs in the effluent of CWs acting as a primary step. Data from: [Belmont and Metcalfe, 2003; Carlson et al., 2013; Hijosa-Valsero et al., 2010a; Li et al., 2013b; Sima et al., 2011]

Removal - Fig. 3.3 shows the observed removal efficiencies for selected PCPs in SF basins as well as H-SSF beds. In SF systems, high removals were observed for galaxolide and tonalide (both 99 %, [Lishman et a., 2006] and triclosan (98 %, [Li et al., 2013b]), while these were very poor for BHT (less than 30 %). In H-SSF beds, the removal efficiencies for the reviewed compounds were in general lower than in SF systems and the best performances were found for LAS C13 (92.9 %), LAS C12 and avobenzone (both at 83 %).

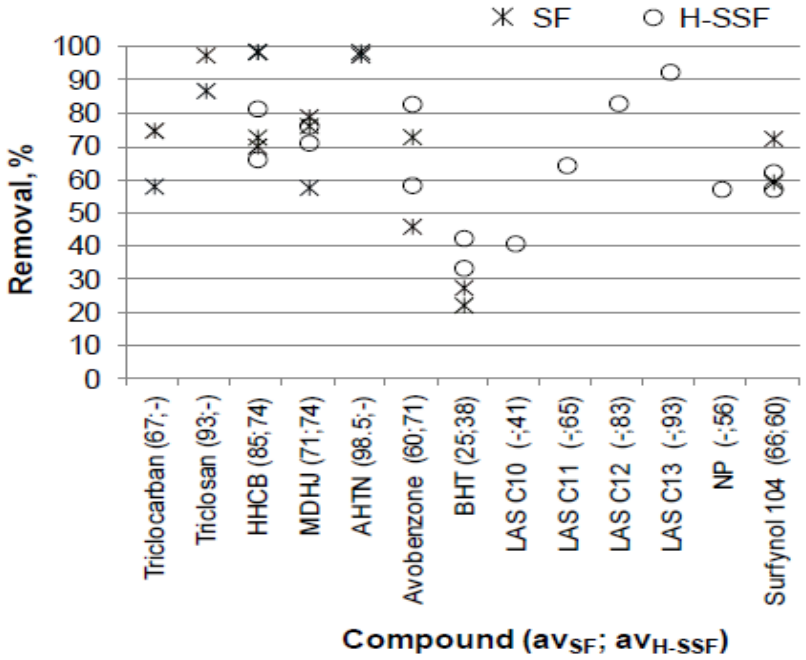


Fig. 3.3: Removal efficiencies observed in primary CWs for selected PCPs. Data from: [Belmont and Metcalfe, 2003; Hijosa-Valsero et al., 2010a; Li et al., 2013b; Lishman et al., 2006; Navarro et al., 2011; Sima et al., 2013]

For the five substances investigated in both systems, higher average removals were observed in SF basins for HHCB and surfynol 104, while avobenzone, BHT and MDHJ removed well in H-SSF beds. APE, AP and LAB were removed to a greater extent in H-SSF beds than in SF systems

[Navarro et al., 2011], suggesting that removal was mainly due to sorption mechanisms. Moreover, APEs exhibited higher removal than APs, around 75 % and 50 % respectively, which is correlated to the fact that APs may form during the biodegradation of APEs [Navarro et al., 2011].

In H-SSF beds, nonionic surfactants were removed to a greater extent than anionic ones [Sima et al., 2013a] and also more quickly [Sima and Holcova, 2011].

Studies of the occurrence and degradation of LAS and SPC in CWs remarked that homologues with an alkyl chain shorter than C10 were rarely detected, as the alkyl chain is first preferably oxidized to carboxylic acid and then it is degraded [Sacco et al., 2006].

3.7 Secondary step: occurrence and removal of selected PCPs

Fig. 3.4 and Fig. 3.5 show concentrations in the influent and effluent of CWs acting as a secondary step and Fig. 3.6 shows the observed removal efficiencies for the reviewed 15 PCPs. Synthetic musks were the most investigated in the influent, followed by sun screen products, while in the effluent the most studied were surfactants followed by synthetic musks.

The highest influent concentrations were detected for the surfactants LAS C11 (2123 μg/L), LAS C12 (990 μg/L), LAS C10 (350 μg/L) and SPC C10 (340 μg/L) [Huang et al., 2004]. It is worth noting that all the investigated surfactants were found at concentrations greater than 100 μg/L (with the only exception of SPC C11). The other PCPs were found below 45 μg/L (the highest values were due to hydrocinnamic acid [Matamoros et al., 2009] followed by the musk MDHJ (39 μg/L) [Matamoros and Bayona, 2006].

Regarding the effluent, the highest concentrations were detected for the same surfactants mentioned for the influent: LAS C11 (1774 μg/L), LAS C12 (731 μg/L), SPC C10 (570 μg/L) and LAS C10 (264 μg/L) [Huang et al., 2004]. All the remaining investigated compounds exhibited concentrations at least one order of magnitude below.

A rapid glance at Fig. 3.4 and Fig. 3.5 shows that for each LAS compound, average effluent concentration is lower than the corresponding influent one, while this does not occur for SPCs as they were formed during the biodegradation of LAS in the system, and their formation was faster than their removal as pointed out in the work by [Huang et al., 2004]. For all the other compounds a reduction of the average concentration was found from inlet to outlet of each type of CW.

Only for MDHJ is it possible to compare performance of the three kinds of CW on the basis of the measured concentrations. The lowest effluent concentrations were found in V-SSF systems leading to the supposition that the aerobic conditions of the bed favor its biodegradation [Matamoros et al., 2009]. Referring to oxybenzone and hydrocinnamic acid, similar performances were observed in H-SSF and V-SSF beds [Matamoros et al., 2007].

As remarked for primary CWs, much higher concentrations were found for classes of surfactants in the influent/effluent of secondary CWs: MBAS were detected around 15000/2500 μg/L [Conte et al., 2001], LAS around 3600/2900 μg/L and SPCs around 500/900 μg/L [Huang et al., 2004]. It is worth noting that only SPCs exhibited an increment in the concentrations due to the biodegradation of LAS, resulting in a formation of PCPs as discussed above.

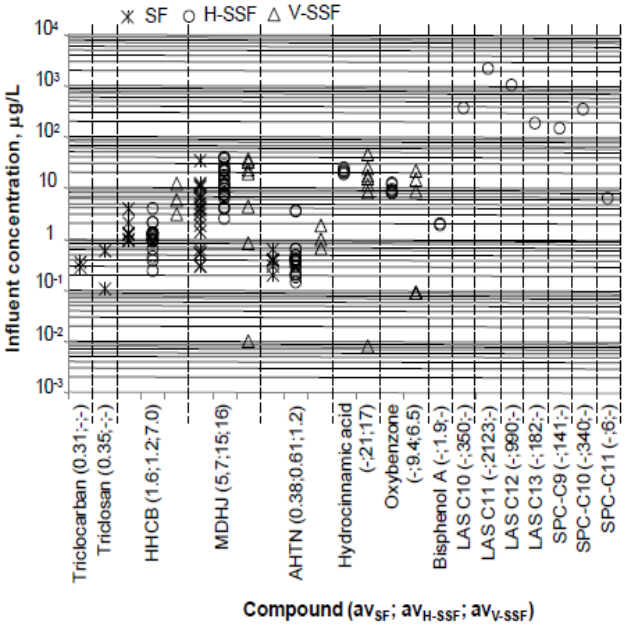


Fig. 3.4: Occurrence of investigated PCPs in the influent of CW acting as a secondary step. Data from: [Avila et al., 2013; Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2010b; Hijosa-Valsero et al., 2011b; Huang et al., 2004; Li et al., 2013b; Matamoros and Bayona, 2006; Matamoros et al., 2007; Matamoros et al., 2009; Reyes-Contreras et al., 2012]

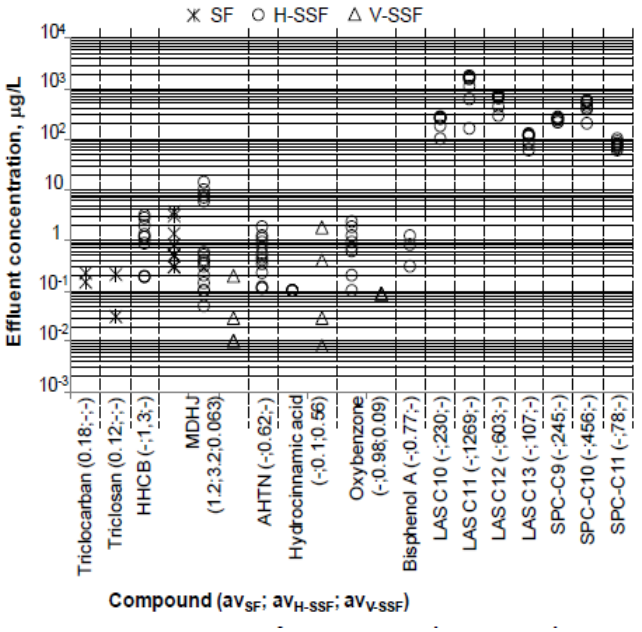


Fig. 3.5: Occurrence of investigated PCPs in the effluent of CW acting as a secondary step. Data from: [Avila et al., 2013; Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2011b; Huang et al., 2004; Li et al., 2013b; Matamoros et al., 2009]

Removals - Regarding collected removal efficiencies (Fig. 3.6), the most investigated compounds were the three fragrances in the SF and H-SSF basins. They exhibited a wide range of variability of removal values. This is also due to the fact that these studies were carried out with the aim of analyzing the influence which different factors have on PCP removal. These factors include design parameters [Hijosa-Valsero et al., 2010b], hydraulic loading rates (HLRs) [Matamoros et al., 2007; Matamoros et al., 2009], operational conditions [Avila et al., 2014; Hijosa-Valsero et al., 2011] and environmental conditions [Hijosa-Valsero et al., 2010b]. In addition, the investigated plants might have different ages, different sizes (lab, pilot or full scale), they may be planted or unplanted, and they may also be affected by clogging, leading to a reduction in the HRT. These factors may greatly influence the removal of PCPs within the system, as discussed in paragraph 3.11. All the investigated compounds were removed up to 95 % with the only exceptions of the antiseptics triclosan and triclocarban and the surfactants.

In SF CWs, the best removals were achieved for the three fragrances. This occurred in the modified SF type reported in Table 3.4 [Hijosa-Valsero et al., 2010b], where the passage of the water through the filling media before discharge into the environment allowed the (lipophilic) pollutants to sorb onto filling materials.

In H-SSF beds, the highest average removals were found for hydrocinnamic acid (99 %), oxybenzone (94 %) and bisphenol A (92 %) and also for fragrances, while surfactants generally exhibited lower removal levels.

In V-SSF beds the best performances were observed for MHDJ (95 %), HHCB (89 %) and AHTN (79 %), suggesting that the intermittent feeding and the aerobic environment are beneficial to the removal of these micropollutants.

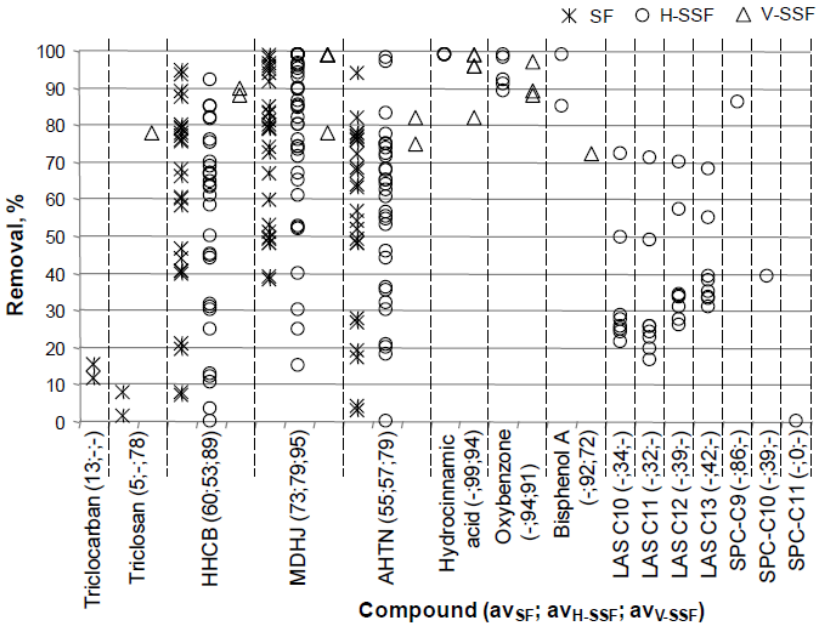


Fig. 3.6: Removal efficiencies for the investigated PCPs in different types of CWs acting as a secondary step. Data from: [Avila et al., 2010; Avila et al., 2014; Hijosa-Valsero et al., 2010a; Hijosa-Valsero et al., 2010b; Hijosa-Valsero et al., 2011b; Huang et al., 2004; Li et al., 2013b; Matamoros and Bayona, 2006; Matamoros et al., 2007; Matamoros et al., 2009; Reyes-Contreras et al., 2012].

Fig. 3.6 does not include negative removal values. These were rarely found were limited to fragrances and SPCs and were due to the internal generation of some compounds following the biodegradation of others (SPCs as intermediates of biodegradation of LAS or longer SPCs, [46], release phenomena of selected compounds (HHTN and AHTN) and clogging conditions, resulting in HRT reduction and malfunctions including the release of compounds that could not be removed from the bed due to lack of time (i.e. MDHJ) [Reyes-Contreras et al., 2012]. Peculiar situations were reported in literature. [Huang et al., 2004], for example, found that in warm periods, suspended solids containing LAS retained within the bed quickly decomposed, resulting in a much higher quantity of SPCs generated compared to cold periods. In contrast, [Reyes-Contreras et al., 2012] found release phenomena for the three fragrances in winter in H-SSF beds but not in summer, perhaps due to an inhibition of the biological activity at low temperatures and a release of the biofilm within the system where fragrance molecules could be present.

3.8 Tertiary step: occurrence and removal of selected PCPs

Fig. 3.7 and

Fig. 3.8 refer to the concentrations of PCPs detected in the influent and effluent of CWs acting as a tertiary step, while Fig. 3.9 shows the removal efficiencies reported by the different authors in the polishing CWs. Twenty PCPs were monitored in the influent, twenty-one compounds in the effluent (the same as the influent plus the fragrance celestolide), and removal values are available for eighteen compounds.

SF systems were the most studied CW type, followed by H-SSF beds. Different authors analyzed multistage polishing systems (see also Table 3.3). The investigated systems consisted of series of SF basins, with the exception of those studied by [Reyes-Contreras et al., 2011; Hijosa-Valsero et al., 2010b], which were sequences of SF and H-SSF CWs. In addition, the multistage polishing plant investigated by [Zhu and Chen, 2014] included 30 cells between SF and H-SSF types; this plant was classified as a non-conventional CW in Fig. 3.7-Fig. 3.9.

The highest influent concentration was detected for EDTA (310 μg/L [Barber et al., 2006]). This surprisingly high value is in accordance with those found in literature in the effluent of secondary WWTPs as reported by [Kase et al., 2011]. The second highest concentrations were for NP2EC with 160 μg/L and NP1EC with 150 μg/L. All the other PCPs exhibited influent concentrations of

two orders of magnitude lower, the highest values being for MDHJ (3.7 μg/L) and galaxolide (2.9 μg/L).

The highest average influent concentrations were found for EDTA (275 μg/L), NP2EC (155 μg/L), NP1EC (145 μg/L), oxybenzone (1.6 μg/L) NP1EO (1.5 μg/L) and AHTN (1.23 μg/L). For the remaining investigated compounds average values were always less than 1 μg/L.

Referring to CW effluent, the highest effluent concentrations were found for NP2EC (135 μg/L), NP1EC (97.5 μg/L), EDTA (87 μg/L), followed by MDHJ (2.2 μg/L) [Hijosa-Valsero et al., 2010].

A comparison between Fig. 3.7 and

Fig. 3.8 highlights that a general decrement in the concentrations occurs from influent to effluent. Referring to cashmeran, average influent concentration is lower than that of the effluent, but an analysis of the investigations dealing with it reveals that some of the reviewed studies only provided effluent values and removal efficiencies, and in all of them a removal was always observed, as reported in Fig. 3.9, and no release occurred. Only DEET exhibited a slight increase in the passage through the polishing system investigated by [Zhu and Chen, 2014], but there is still little available data and it is not possible to conclude that a release would occur.

The only PCP investigated in surface and subsurface flow systems is AHTN - for this all three CW types showed a removal ability.

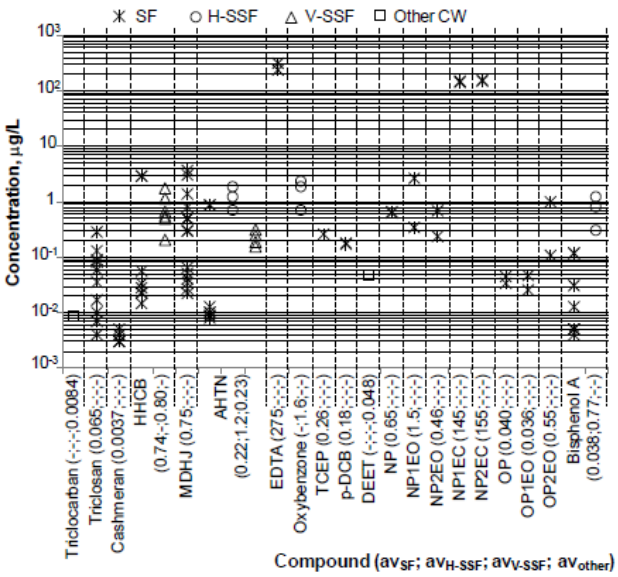


Fig. 3.7; PCP concentrations in the influent of CWs acting as a polishing step. Data from: [Avila et al., 2013; Barber et al., 2006; Hijosa-Valsero et al., 2010a; Lee et al., 2011; Llorens et al. 2009; Matamoros et al. 2008b; Matamoros et al. 2012a;Denmark Reif et al., 2011; Waltman et al., 2006].

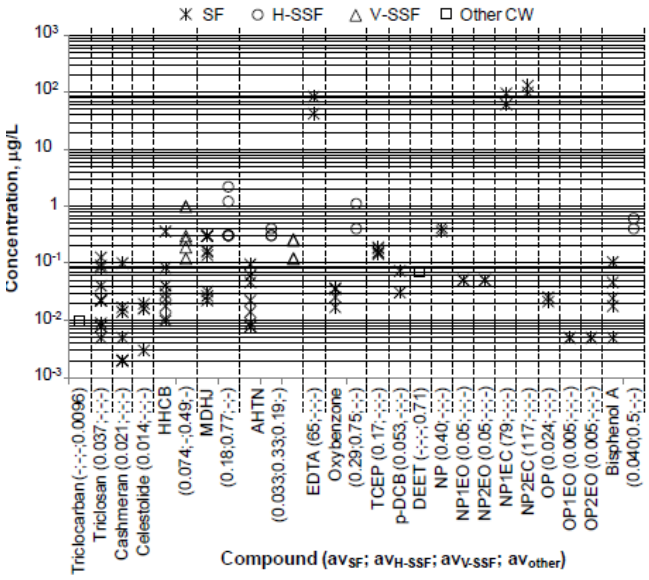


Fig. 3.8: PCP concentrations in the effluent of CWs acting as a polishing step Data from: [Avila et al., 2013; Barber et al., 2006; Hijosa-Valsero et al., 2010a; Lee et al., 2011; Llorens et al. 2009; Matamoros et al. 2012 Spain Matamoros et al. 2012a Denmark Reif et al., 2011; Waltman et al., 2006; Zhu et al., 2013]

Removals – In SF systems, the highest values were found for triclosan (99.99 %, [Park et al., 2009]) and HHCB (99 %, [Matamoros and Salvado, 2012; Matamoros et al., 2010]), AHTN and oxybenzone (both 98 % [Matamoros et al., 2010]), celestolide (97 % [Matamoros et al., 2010]) and cashmeran (95 % [Matamoros and Salvado, 2012]). All refer to two-stage systems. The high attenuation of EDTA (on average 75 %) should be due to photolytic reactions as the compound is quite resistant to biodegradation and has a low affinity for sorption [Barber et al., 2006]. Finally, very low removals are observed for NPs and NPnECs [Barber et al., 2006].

Modest removal values were observed in the V-SSF beds. Based on data reported by [Reif et al., 2011], they ranged between 65 % (HHCB) to 0 % (AHTN). The removals found in H-SSF beds are

even poorer: [Reyes-Contreras et al., 2011] always found them to be less than 20 % for triclosan, HHCB, MDHJ, AHTN, BHA and BHT.

An interesting investigation was carried out by [Sacco et al., 2006] into the removal of the mixture of nonionic surfactants Triton X-100 dosed at 30 mg/L and 300 mg/L in the pilot H-SSF bed. Their mixture contained up to 13 EO groups in different percentages. They found that in the first 40 cm of the bed OP and its monoethoxylate (EO=1) had the biggest increment. The decrease (sometimes also the disappearance) in certain octylphenol ethoxilate (OPEO) oligomers seems to be correlated to increases in others (characterized by a shorter EO chain) and the biodegradation rate of those oligomers with a number of EO greater than 3 is higher than those observed for compounds with shorter chains. Promising results were observed in the (non conventional) biologically-based filtration water reclamation plant investigated by [Matamoros et al., 2012] for oxybenzone, AHTN, HHCB, triclosan and cashmeran, especially in summer time. MDHJ exhibited very high removal in summer (> 96 %) while in winter the removal was nearly absent. Only TCEP was not removed in each season due to its high recalcitrant potential to all removal mechanisms, as also remarked by other investigations [Matamoros and Salvado, 2010; Lee et al., 2011].

In the multistage (SF+H-SSF) systems by [Reyes-Contreras et al., 2011] a consistent increment in the removal efficiencies of MDHJ, triclosan, AHTN, HHCB and BHT was observed during the summer season with respect to the winter one (about 2-8 times higher).

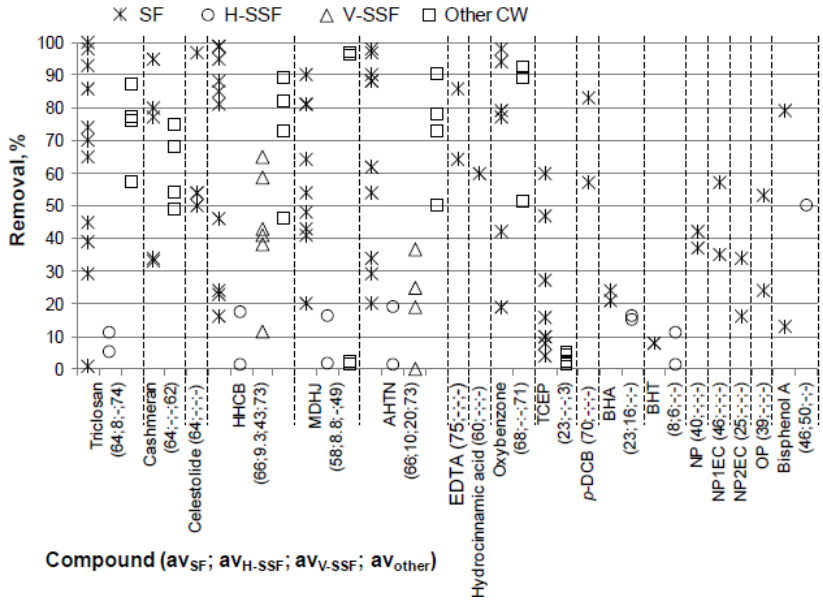


Fig. 3.9: Removal efficiencies for PCPs in different types of CWs acting as a polishing step. Data from: [Barber et al., 2006; Hijosa-Valsero et al., 2010a; Lee et al., 2011; Matamoros et al., 2008b; Matamoros et al., 2010; Matamoros et al., 2012; Matamoros et al., 2012b Matamoros et al., 2012b; Park et al., 2009; Reif et al., 2001; Reyes-Contreras et al., 2011]

The results obtained by [Matamoros et al., 2010] are quite interesting. They compared the removal for a group of PCPs in a tertiary pond and in a conventional tertiary treatment by UV radiation and chlorine disinfection. They found that solar radiation can degrade parental compounds in their intermediates both in the UV reactor and the pond. In most cases these reaction products are more toxic than the parental ones. However, in pond systems other mechanisms including biodegradation, sorption onto solids and sediments and plant uptake may reduce their concentration.

3.9 Restoration wetlands

Two restoration wetlands were included in this study. The first one, described in [Matamoros et al., 2012 a], is located in Denmark and is fed by two rivers - Aarhus (watershed 120 km^2) and Lyngbygaards (watershed 132 km^2) – which are impacted by urban sewage and agricultural runoff. The wetland is interconnected to a lake whose effluent discharges into the sea. The lake is used for recreational purposes and near it there are some of the city's water supply wells. The wetland was created in 2003 to reduce the nutrient concentrations discharged into the lake and then into

the sea and to preserve the downstream water environment conditions. It covers an area of 100 ha and consists of a surface flow basin with an average water depth of 0.5 m and a maximum depth of 2 m, an HRT ranging between 3 and 20 d, on average 7 d. Based on a mass balance between influent and effluent streams to the wetland, a consistent reduction was found in the effluent concentration (mitigation effect passing through the wetland) for most of the investigated PCPs (for triclosan, cashmeran, MDHJ, HHCB, AHTN and bisphenol A it was > 40 %). This reduction was quite scarce (< 15 %) only in the case of TCEP. In winter, due to the low sun-light exposure and cold temperatures, bio and phodegradation processes were limited. It is important to highlight that in the wetland outlet the concentrations of all the investigated PCPs kept quite constant, although the influent values exhibited a wide variability confirming wetland buffer capacity.

The second restoration wetland is a pilot plant fed with the water of the Sordo River (in southeastern Mexico) which receives untreated urban sewage and industrial wastewaters [Navarro et al., 2011]. The CWs consist of 8 cells: four are SF type (substrate upland soils, 0.4 m deep, free water surface flow column, 10 cm high) and four are H-SSF type (filled with 0.4 m of volcanic gravel, water flow 10 cm below the surface). Each of them has an HRT of 5 days. A high attenuation was found for galaxolide, MDHJ, parasol and APE.

3.10 Hybrid systems: occurrence and removal of selected PCPs

Nine compounds were monitored in the influent (Fig. 3.10) and effluent (Fig. 3.11) of different types of hybrid systems and data on observed removal efficiencies were provided for six of them (Fig. 3.12).

The most adopted CW type in the hybrid systems was SF basins, followed by H-SSF beds and the most investigated sequences included SF+H-SSF systems [Hijosa-Valsero et al., 2010a; Jorkest ety al., 2011] and only H-SSF ones [Avila et al., 2013]. All three types were investigated in the hybrid systems by [Avila et al., 2014; Belmont et al., 2006].

A rapid glance at Figures 3.10 and 3.11 highlights that for each substance a reduction was observed. The same was observed for classes of surfactants in the hybrid systems (steps 2+3) investigated by Conte et al. (2001) and Jokerst et al. (2011).

The first found that MBAS decreased from 3,200 and 16,000 μg/L in the influent to 2,000 -2,500 μg/L in the effluent and the second that AES decreased from 50-16,500 μg/L in the influent to 15 - 50 μg/L in the effluent.

Avila et al. (2014) investigated a hybrid system (V-SSF as secondary step and H-SSF +SF as tertiary step) fed by municipal wastewater where PCPs were injected at the desired concentrations. Their investigation also analyzed the operational characteristics inside the tank, in particular redox potential which resulted in the range 110+128 mV in the V-SSF bed, in the range from -59 to -115 mV in the H-SSF bed and between 156 and 171 mV in the SF basin.

Their investigation pointed out that the first stage, a V-SSF bed, was responsible for most of the removal of the selected PCPs, and the following polishing treatment contributed to the removal but to a smaller extent. In particular the effect of the SF stage on the removal of these compounds was quite negligible.

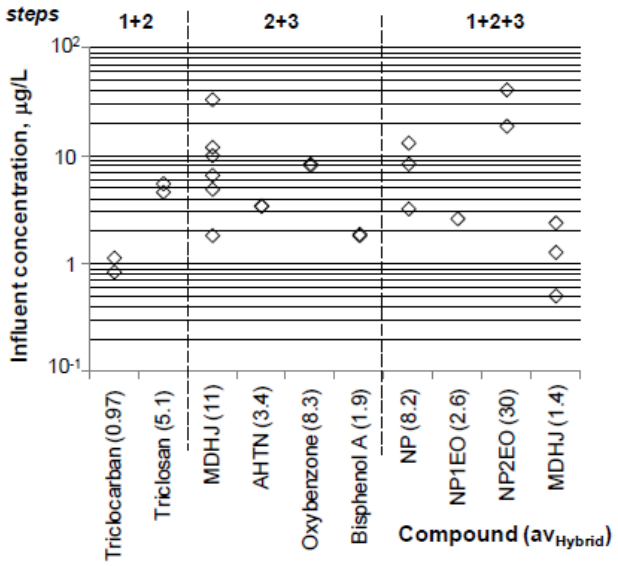


Fig. 3.10: Occurrence of investigated PCPs in the influent of hybrid CWs. Data from: [Avila et al., 2013; Belmont et al., 2006; Hijosa-Valsero et al., 2010a; Jokerst et al., 2011; Li et al., 2013b

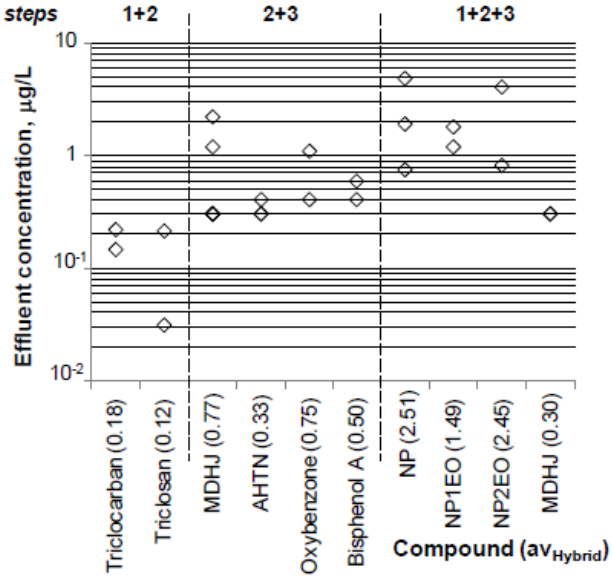


Fig. 3.11: Occurrence of investigated PCPs in the effluent of hybrid CWs. Data from: [Avila et al., 2013; Belmont et al., 2006; Hijosa-Valsero et al., 2010a; Jokerst et al., 2011; Li et al., 2013b]

The highest removal efficiencies were found for triclosan in series of aerated lagoons (on average 97 %, [Li et al., 2013b]) and in a hybrid-polytypic system (V-SSF acting as a secondary step followed by H-SSF+SF as a tertiary step; average removal 91 %, [Avila et al., 2014], for MDHJ (97 %) in the sequence SF+H-SSF beds [Hijosa-Valsero et al., 2010a] and for oxybenzone (97 %) in the sequence of H-SSF beds by Reyes-Contreras et al. (2012).

For triclosan, photodegradation greatly contributes to its removal followed by biodegradation, while for MDHJ photolysis is less important than biodecomposition. This fact is confirmed by the lower removal (81 %) found by the same authors for MDHJ in a series of ponds (steps 1+2+3). Oxybenzone, instead, is mainly removed by biodegradation and then by sorption.

Many investigations confirmed that most of the removal of PCPs occurs in the first step. The comparison provided by Avila et al. (2014) of the contributions in the accumulated average removal efficiencies achieved in each unit of the hybrid system for AHTN, oxybenzone, triclosan and bisphenol A is quite interesting. Referring to bisphenol A, the main removal mechanism is biodegradation and the lowest removal efficiencies (about 65 %) were observed at the lowest redox values (anaerobic conditions in H-SSF beds by [Avila et al., 2013]).

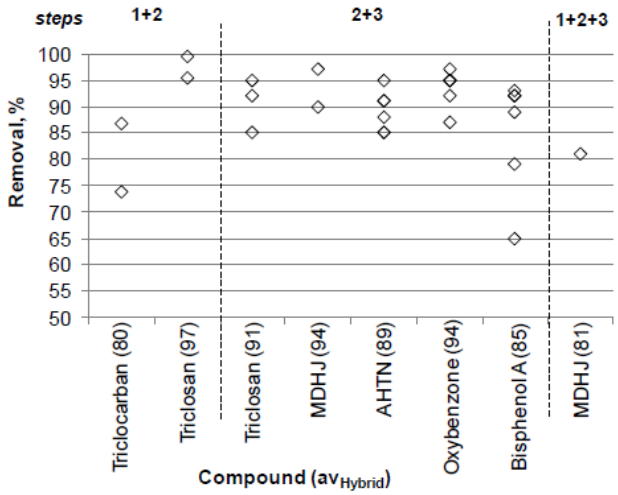


Fig. 3.12: PCP Removal in hybrid CWs. Data from: [Avila et al., 2013; Avila et al., 2014; Hijosa-Valsero et al., 2010a; Li et al., 2013b].

3.11 Discussion of the influence of the main design parameters and operational conditions of PCP removal efficiencies.

As already mentioned, for many reviewed compounds, the removal achieved in CWs exhibited a wide range of variability. In fact, in many cases the studies investigated the influence of some operational conditions (mainly HLR and temperature) and all the removal values observed were reported. As a consequence, the lowest values do not necessarily mean that these systems are not appropriate. In addition, removals are correlated to the influent concentrations. As will be discussed later, higher concentrations generally correspond to higher removal efficiencies.

The following paragraphs analyze the influence of the main design parameters as well as the operational and environmental conditions on the removal of the selected compounds.

3.11.1 Variation in the influent concentrations of PCPs

Higher influent concentrations often correspond to higher removal efficiencies, as reported by some authors (among them Matamoros and Salvado, 2012; Matamoros et al., 2008b; Matamoros et al., 2012). Variations in the influent could be attributed to a different consumption of the compound, infiltration in the sewage network by sea water [Matamoros et al., 2012b] or groundwater, a malfunction in the upstream treatments (if CW acts as a secondary or a tertiary step) [Matamoros and Salvado, 2012], or in the treatment itself.

Reyes-Contreras et al., (2011) found a seasonal variation in the concentrations of the two fragrances: AHTN and HHCB occurred at concentrations three times higher in summer than in winter (tonalide: 1.5 μg/L against 0.44 μg/L and galaxolide 1.2 μg/L against 0.45 μg/L) and their removals were more than twice higher in summer than in winter.

3.11.2 Primary treatment

The influence of two primary treatments - a septic tank and an anaerobic hydrolysis upflow sludge bed (HUSB) - on the removal of PCPs in the following H-SSF bed were compared by Hijosa-Valsero et al., (2011). The former produces an effluent of more constant quality during the year and therefore the effluent of a CW fed by a septic tank is slightly better than the effluent produced by a CW fed by a HUSB system.

Surfactants were removed at a consistent fraction in pretreatments. MBAS, for instance, was removed up to 20 % in screens, horizontal sand traps and sedimentation basins [Sima and Holcova, 2011; Sima et al., 2013].

3.11.3 HLR and HRT

A variation in the influent flow rate may be caused by a different wastewater flow, rainwater, snow melting, and sea water and groundwater infiltration. The main and most frequent disturbance is an increment of the HLR resulting in a shortening of HRT, with respect to the corresponding design values. Prolonged rain events, (together with clean-up or reconstruction of the wetlands) may lead to a pulsed, albeit delayed release of the accumulated PCPs due to desorption.

Many studies agree with the fact that whatever the CW step, the higher the HRT, the higher the removal efficiencies achieved by the system for the investigated PCPs in wastewater (i.e. Matamoros et a., 2008).

Avila et al. (2014) investigated ability in removing a selected group of PCPs (AHTN, oxybenzone, triclosan and bisphenol A) at the three different HLRs (0.06, 0.13 and 0.18 m/d) in their treatment line, consisting of a V-SSF bed, followed by a H-SSF bed and a SF basin as a polishing step. They found that the removal of triclosan decreased with the increase of HLR, while no clear patterns were found for AHTN, bisphenol A and oxybenzone. The same increment of HLR applied to the H-SSF bed only affected the removal of AHTN that decreased, while for bisphenol A, oxybenzone and triclosan no correlation was found between HLR and observed removal.

In V-SSF beds an increment in the HLR (13-70 mm/d) did not result in a decrement of the removal of MDHJ, hydrocinnamic acid, oxybenzone, HHCB and AHTN [Matamoros et al., 2007], while in SF

basins, it resulted in a decrement in the removal efficiencies for oxybenzone and MDHJ [Matamoros et a., 2007] and in H-SSF beds for anionic [Sima et al., 2009] and nonionic surfactants [Sima and Holcova, 2011].

3.11.4 Aging of the CW

The age of the CW may influence the removal of PCPs. In SF basins, biomass growth causes shading of the upper water layer resulting in a reduction of photodegradation processes. Moreover, clogging, matrix saturation and hydraulic conductivity losses may be detrimental for removal mechanisms in (H- and V-) SSF beds, as found by [Matamoros and Bayona, 2006] for MDHJ, HHCB and AHTN. An H-SSF bed could work closer to as a SF basin if surface and volume clogging phenomena occur. In fact they may lead to a flooding of the bed, , with a higher oxygen transfer from the air and a lower HRT, as remarked by [Matamoros and Bayona, 2006; Matamoros et al., 2009]. Removal efficiencies are then affected by these phenomena and organic matter could be mainly removed by aerobic reactions.

3.11.5 Biomass acclimatization

Some long experimental investigations on surfactant removal in H-SSF beds highlighted that microbial flora requires a period of time to adapt itself to the type of pollutant load. [Sacco et al., 2006] reported that in their pilot, H-SSF bed removal of Triton X 100 changed along the 12-month period of observation. A development of new bacteria strains appeared and others increased during the dosage of the mixture, suggesting that these bacteria were adapting to the presence of these surfactants and/or they used them as a source of nourishment.

3.11.6 Redox conditions

The three types of CW differ not only in the main flow direction, but also in their operational conditions. Avila et al. (2014) reported the values of redox potential measured in the three types of systems, confirming aerobic conditions in V-SSF beds and SF basins and anaerobic conditions in H-SSF beds. Hijosa-Valsero et al. (2011) analyzed the seasonality variation of redox potential in H-SSF beds and they found that in summer time redox may increase up to positive values, promoting the development of different microbial communities.

Redox potential within a system may vary during the life of the wetland, due to its aging, clogging phenomena and changes in the influent quality. It mostly influences the removal of PCPs as well as surfactants. [Avila et al., 2013; Navarro et al., 2011; Conkle et al., 2012] remarked that higher redox values promote PCP removal with the exceptions of BHT and AP.

Huang et al. (2004) and Sima et al. (2013a) agreed that anionic and nonionic surfactants can be degraded in a wide range of redox values. Referring to LAS, more oxidized conditions improve their removal and in deeper SSF beds where the environment is characterized by sulfate-reducing methanogenic conditions, low LAS removals were observed [Huang et al., 2004].

In addition, redox conditions can also influence the degradation of PCPs bioaccumulated in sediments or gravel of a wetland. This influence was investigated by [Conkle et al., 2012] who found that DEET is appreciably degraded under aerobic sediments, while in anaerobic conditions this does not occur.

3.11.7 Removal processes along the system

Most of the removal occurs in the first meters of the system for many of the investigated compounds. The fragrances AHTN and HHCB mainly accumulated in the first section of the H-SSF bed investigated by [Matamoros and Bayona, 2006] and a large fraction of nonionic surfactants (about 80 %) and anionic ones (about 50 %) degrade in the first meter of the H-SSF beds investigated by Sima et al. (2009) and Sima and Holcova (2011) respectively. The same profile was confirmed by the investigation of Zarate et al., (2012) into the accumulation of triclosan and triclocarban on the sediments of a polishing SF basin.

Avila et al., 2010; Hijosa-Valsero et al., 2011 investigated the removal of AHTN, HHCB, MDHJ and bisphenol A in secondary multistage CWs consisting of two H-SSF beds in series.

They found that for AHTN, HHCB and bisphenol A most removal occurred in the first stage and near the inlet zone, probably due to the detention of most of the particulate matter with which all these compounds are associated. A different removal pattern was found for MDHJ as its main removal mechanism is biodegradation favored at high temperature.

3.11.8 H-SSF Bed depth

Shallow H-SSF beds (0.3 m water depth) were found to be more efficient than deeper ones (0.5 m) in the removal of LAS due to differently oxidized conditions occurring on the two kinds of wetlands [Huang et al., 2004]. In the first, in fact, denitrification, sulfate reduction and methanogenesis occurred simultaneously, while in the second, the prevailing reactions were sulfate reduction and methanogenesis and denitrification is insignificant. The effect of the depth of V-SSF beds on the removal of anionic surfactants was investigated by Kadewa et al. (2010). They found that in an *acclimatized* and vegetated 0.7 m deep V-SSF bed anionic surfactant removal was in the range of 76-85 %, while in a cascade of three still-ripening and unplanted 0.2 m V-SSF beds it was less , between 37 and 74 %. These findings could be attributed to a more developed microbial community in the ripe higher V-SSF bed which could guarantee a complete biodegradation of the different surfactants, while in the cascade of shallow V-SSF beds, the more oxidized conditions promoted the alkyl chain shortening of the surfactants, but not their complete degradation. Sima et al. (2009) found that the removal of anionic surfactants in an H-SSF bed was faster in the upper 10 cm. At lower depths, anaerobic degradation of LAS occurs where sulphates were shown to be reduced. On the contrary, studies of nonionic surfactants showed that they can be effectively degraded at both depths, independent of aerobic or anaerobic conditions [Sima and Holcova, 2011].

3.11.9 Filling material in SSF beds

Lower effluent concentrations were detected for LAS and SPCs in beds filled with finer gravel (D_{60} = 3.5 mm, C_u = 1.7) than in those containing coarse gravel (D_{60} = 10 mm, C_u = 1.6).

3.11.10 Seasonality and effect of temperature

A seasonal variation was found for the removal efficiency of many compounds, but not for their occurrence. As a rule of thumb, removal efficiencies for dissolved-phase compounds are greatly influenced by temperature as biodegradation is their main removal mechanism, while depletion referring to compounds associated with particulate matter does not exhibit such a pronounced temperature variation since their removals are mainly due to physical mechanisms (sedimentation and adsorption). For compounds such as MDHJ and oxybenzone, whose main removal mechanism is biodegradation, low temperatures directly reduce the physiological activities of the microorganisms themselves, resulting in a slowing down of the degradation reactions that may occur [Matamoros et al., 2012; Reyes-Contreras et al., 2012]. In H-SSF beds, summer removals were generally found to be very high (often greater than 80 %) for HHCB, AHTN and MDHJ, with a few exceptions related to unplanted H-SSF beds, where HHCB and AHTN were not removed at all, while MDHJ had variable removal efficiencies. The first two fragrances present a similar removal pattern as they have a great sorption potential due to their lipophilic properties, while MDHJ is mainly removed by biodegradation. The seasonality variation found in the removal of the investigated hydrophobic compounds can be explained by the release of these compounds in winter and accumulation in summer, when biofilm and plants are more active [Hijosa-Valsero et al., 2011]. In SF basins, HHCB and AHTN exhibited the same (high) removal efficiencies in both seasons at around 85-90 % [Matamoros et al., 2008]. For photodegradable compounds such as triclosan and cashmeran, lower values in their removal observed in SF basins in winter could also be due to lower levels of sun light exposure [Matamoros et al., 2012].

3.11.11 Vegetation

Vegetation can insulate wetland surfaces and thus contribute to maintaining microbial activity; roots provide a surface for the development of microbial colonies and contribute to the creation of aerobic microenvironments within the bed, thus favoring biodegradation. Moreover, vegetation can contribute to the removal of micropollutants by plant uptake. Higher removal levels of anionic surfactants were observed in planted and acclimatized V-SSF beds with respect to unplanted and non acclimatized ones [Kadewa et al., 2010]. In SF basins covered by *Lemna minor*, the removal efficiencies of the photodegradable triclosan were found to be lower than in control unplanted SF wetlands [Matamoros and Salvado, 2012].

Young CWs are more efficient when they are planted. When CWs get older, the efficiency of planted and unplanted systems is similar as many disturbing factors may occur (clogging, shading) causing a performance decrease in the planted CWs. Reinhold et al., (2010) found in their flask scale plants that duckweed can contribute to removing triclosan, while it is not efficient with respect to DEET. [Zarate et al., 2012] investigated bioconcentration patterns of triclosan and triclocarban among three different macrophytes (*Typha latifolia, Pontederia cordata, Sagittaria graminea*) and their concentrations in different sites of the investigated surface flow basin. They found that concentrations of the two analytes were higher in roots rather than in shoots and tended to decrease from the inflow to the outflow. To complete this brief discussion, attempts to correlate observed removal efficiencies of the different PCPs with their $LogK_{ow}$, Log D_{ow} and pK_a were carried by different authors (among them [Park et al., 2009; Lee et al., 2011] but unfortunately no significant correlations were found. Sima and Holcova, (2011) found similar removal efficiencies for BOD_5 and nonionic surfactants.

3.12 Conclusions

It is well known that CWs, if well designed, exhibit a good ability in removing common conventional pollutants. Their potential in removing emerging organic contaminants is, however, still under discussion. This chapter focuses on the ability of CWs in removing common PCPs, substances frequently used worldwide and with increasing levels of consumption. They are quite complex molecules, with different chemical and physical properties and are, in many cases, quite persistent to biodegradation. On the basis of the collated data, in general a removal was observed for each reviewed compound with very few exceptions, mainly referring to groups of surfactants, such as SPCs, as their formation due to LAS degradation is faster than their removal. The highest removal levels were found for the fragrances in all three treatment steps. These compounds were the most studied while for many others there is still little data and further investigations of their removal in the different types of CWs are necessary. The coexistence of different microenvironments within each type of CW which guarantee different redox conditions and the simultaneous occurrence of biological, physical and chemical removal mechanisms make CWs a potentially adequate system for the removal of PCPs, with limited operational costs. The main weaknesses are the wide footprint of these systems - resulting in high investment costs - and the extremely long time required to re-activate the processes within them in the case of malfunctions which are mainly due to clogging phenomena and an influent which accidentally becomes highly polluted. These weaknesses lead to long rest periods (in the first case) or expensive maintenance interventions (in the second). However, CWs, due to their buffer capacity, could represent a barrier to reducing the spread of these types of PCPs into the aquatic environment.

Reference

- Verlicchi P., Zambello E., Al Aukidy M., Removal of personal Care Products in Constructed Wetlands. Chapter in "Personal Care Products in the Aquatic Environment" edited by Dr. Silvia Díaz Cruz and Prof. Dr. Damià Barceló, 2014.

Chapter 4

4 Removal of PhCs by CWs

4.1 Introduction

The ability of constructed wetlands (CWs) to remove conventional (namely COD, BOD_5, nitrogen compounds, phosphorus, microorganisms, etc.) and non-conventional pollutants, such as heavy metals and hydrocarbons, from wastewater (Langergraber, 2013; Zhi and Ji 2012; Galletti et al., 2010, Malaviya and Singh, 2012; Tromp et al., 2012) has been extensively investigated. The main mechanisms by which these pollutants are removed have also been the focus of much research (Saeed and Sun, 2012; Faulwetter et al., 2009; Garcia et al., 2010). Combinations of different kinds of CWs, as well as combinations of conventional and natural polishing systems (Vymazal 2013; Yeh 2008; Verlicchi et al., 2009), have been assessed, not only as regards municipal effluents, but also with other kinds of wastewaters including: diary effluent (Sudarsan et al., 2012), industrial wastewater (Di Luca et al., 2013), agricultural runoff (Maniquiz et al., 2012), livestock farm effluent (Babatunde et al., 2010) and stormwater runoff (Malaviya and Singh, 2012). This research has led to changes in the design of subsurface flow systems to reduce the risk of clogging and to guarantee continuous operation (De Paoli and von Sperling, 2013; Pozo-Morales et al., 2013; Verlicchi et al., 2012b) throughout the entire lifespan of the plant.

Over the last fifteen years, researchers have also begun to investigate the occurrence of the so-called emerging contaminants, including pharmaceutical compounds (PhCs), in wastewater, to discover whether and how they are removed by common treatment systems. Notwithstanding the lack of (legal) limits for such contaminants in effluent and surface water within the European Union, and in other countries, their occurrence in different aquatic environments has been widely investigated. Conventional wastewater treatments (mainly activated sludge systems, membrane bioreactors and advanced oxidation processes), in particular, have been the object of a great number of studies (among them: Verlicchi et al., 2012c and Chelliapan and Sallis, 2013), while the PhC removal efficiencies of CWs have only recently come under scrutiny.

A recent review (Li et al., 2014) compiles removal efficiencies of a selected group of PhCs by means of CWs (mainly pilot and indoor lab scale plants) acting as secondary and tertiary steps, and it discusses the contribution of the three components (substrate, plants and microbes) in the removal mechanisms. In that study the feeding to the CWs included in the review was a real or more often a synthetic urban or rural effluent as well as a swine wastewater.

In the current study, great attention was devoted to the occurrence of a great number of PhCs in the influent and effluent of different types of CWs, fed by only municipal wastewater and acting as primary, secondary or tertiary steps, as well as hybrid systems. The observed removal efficiencies were reported in details for all the selected compounds in the Tables reported in the SD of Verlicchi and Zambello (2014), where for each datum reference is reported) and the main design, operational and environmental parameters that affect them were discussed in details, in order to remark the conditions which improve the removal of the different PhCs. Based on the collated data, an environmental assessment of the risk posed by the residues of selected compounds was carried out and the results are compared with those recently found referring to the risk posed by the same compounds in secondary activated sludge effluents.

The little available data regarding PhCs accumulated in filling media or uptaken by plants is also considered, although we focus in particular on the relative ability of various types of CWs to remove such compounds from the water matrix in the influent (raw or pre-treated wastewater).

In order to provide a snapshot of the potential of such systems to remove PhCs from urban(domestic) wastewater, we report data from investigations carried out on pilot or full-scale plants mainly fed by real wastewater, or real wastewater spiked with compounds of interest. We nevertheless discuss a few investigations into the behaviour of synthetic feed in hydroponic gravel beds, an interesting type of CW, as will be discussed below. It is also important to note that almost all the systems considered operated outdoors in “real environmental conditions”, and that the review is limited to technical aspects rather than economic issues.

4.2 Framework of the study

The study drew data from 47 peer-reviewed papers on the occurrence of 137 PhCs, belonging to 20 different therapeutic classes, in the water phase, as well as in gravel and plant tissues. The removal efficiencies achieved by CWs, specific PhC mass load at their outlets, and the environmental risk posed by the residues of selected PhCs in the effluent of CWs acting as secondary or tertiary steps were analysed. Due to the large amount of information collected, some tables and figures are reported as Supplementary Data in Verlicchi and Zambello (2014); the selected compounds are reported in Appendix A together with their main properties. are cited in the text, in which we focus on what we consider to be the main findings and issues. The schematics of the treatment configurations investigated are the same reported in Chaper 3 in Table 3.3. The analysed flow direction (surface flow system, horizontal and vertical subsurface flow bed or hydroponic gravel bed) are reported in Table 4.2. Several cases of restoration wetlands are also discussed in this review, and a brief description of all the treatment plants investigated in the 47 papers considered can be found in Table 4.3. Table SD-3 of Verlicchi and Zambello (2014) reports more specific details of each of the 136 investigated treatment lines (where available), in terms of matrix type, type of data provided, plant description, design parameters, feeding mode and type, filling media, vegetation, and main investigation fields.

For the full list of Supplementary Data presented in Verlicchi and Zambello (2014) see Appendix B.

4.3 Investigated PhCs

The current review investigates 137 compounds from 20 different therapeutic classes as reported in Fig. 4.1. The chemical formula and physico-chemical properties of each compound is shown in Appendex A togheter with the predicted no-effect concentrations (PNEC), where available.

Table 4.1: Selected compound

Therapeutic class	Compounds
Analgesics/Anti-inflammatories (16)	Acetaminophen; Budesonide; Celecoxib; Codeine; Diclofenac; Fenoprofen; Ibuprofen; Indomethacin; Ketoprofen; Mefenamic acid; Naproxen; Phenazone; Phenylbutazone; Propyphenazone; Salicylic acid; Tramadol
Antibiotics (31)	Amoxicillin; Ampicillin; Azithromycin; Chloramphenicol; Chlortetracycline; Ciprofloxacin; Clarithromycin; Clindamycin; Danofloxacin; Doxycycline; Enoxacin; Enrofloxacin; Erythromycin; Josamycin; Lincomycin; Metronidazole; Nifuroxazide; Norfloxacin; Ofloxacin; Oxytetracycline; Roxithromycin; Spiramycin; Sulfadiazine; Sulfadimethoxine; Sulfamethazine; Sulfamethoxazole; Sulfapyridine; Tetracycline; Tilmicosin; Trimethoprim; Tylosin
Anti diabeticss (2)	Glibenclamide; Glimepiride
Anti-diarrhoeal drugs (1)	Loperamide
Antifungals (3)	Clotrimazole; Fluconazole; Ketoconazole
Anti -hypertensives (8)	Diltiazem; Enalapril; Eprosartan; Hydrochlorothiazide; Irbesartan; Lisinopril; Telmisartan; Verapamil
Anti -neoplastics (1)	Tamoxifen
Antiparkinsons (2)	Bromocriptine; Orphenadrine
Antiseptics (2)	Triclocarban; Triclosan
Anti -spasmodics (1)	Dicycloverine
Beta-agonists (3)	Clenbuterol; Salbutamol; Terbutaline
Barbiturates (2)	Pentobarbital; Phenobarbital
Beta-blockers (10)	Atenolol; Betaxolol; Bisoprolol; Carazolol; Metoprolol; Nadolol; Pindolol; Propranolol; Sotalol; Timolol
Diuretics (1)	Furosemide
Hormones (5)	Estradiol; Estriol; Estrone; Ethinylestradiol; Finasteride
Lipid regulators (8)	Atorvastatin; Bezafibrate; Clofibric acid; Fenofibrate; Gemfibrozil; Mevastatin; Pravastatin; Rosuvastatin
Psychiatric drugs (29)	Alprazolam; Amitriptyline; Buprenorphine; Bupropion; Butalbital; Carbamazepine; Citalopram; Clomipramine; Diazepam; Dilantin; Fluoxetine; Fluphenazine; Haloperidol; Hydroxyzine; Levomepromazine; Lorazepam; Maprotilin; Memantin; Mianserin; Mirtazepine; Naloxone; Nefazodone; Oxazepam; Paroxetine; Perphenazine; Risperidone; Sertraline; Venlavafaxine; Zoldipem
Receptor antagonists (10)	Alfuzosin; Cimetidine; Desloratidin; Diphenhydramine; Famotidine; Fexofenadine; Loratadine; Omeprazole; Promethazine; Ranitidine;
Stimulant drugs (1)	Caffeine
Vasodilators (1)	Dipyridamol

4.4 Classification of the CWs under review

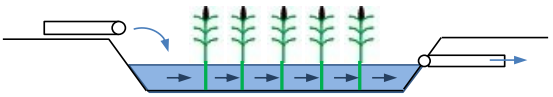
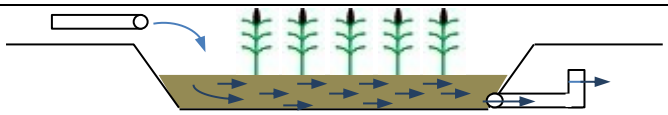
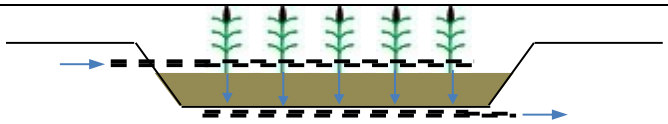
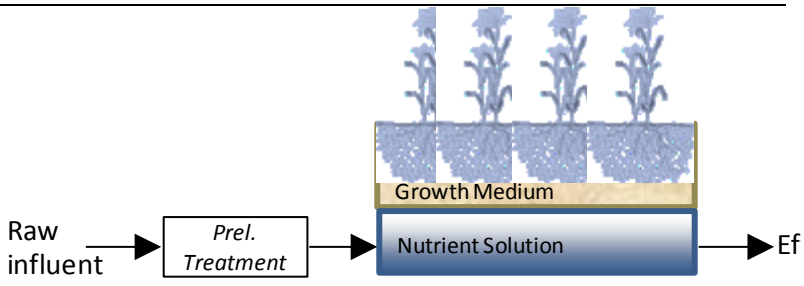
The CW systems reviewed were classified according to the classic nomenclature based on hydrological characteristics (water position and flow direction), distinguishing between: surface flow (SF) systems, horizontal subsurface flow (H-SSF) bed, and vertical subsurface flow (V-SSF) bed. In the first, the majority of flow occurs through a water column overlying a benthic substrate, whereas the flow in the others is through a porous medium (generally gravel), and classed as either horizontal, if the feed is from one side of the bed, or vertical, if the feed is spread over the surface of the bed, crossing it. Additionally, in H-SSF beds the feed is continuous, while in V-SSF beds it is intermittent. To complete the picture, we also include hydroponic gravel beds (HGB) and restoration wetlands (RW), the first referring to systems in which plants may grow using the mineral nutrients dissolved in water, without soil or other media, and the latter to systems generally fed by

a river whose water flow is primarily made up of wastewater treatment plant discharges or untreated wastewaters. Surface flow systems investigated include ponds, lagoons, aerated lagoons and free water basins, but we also include a modified SF system, investigated by Hijosa-Valsero et al. (2010b). The effluent of this so-called free-flow water system leaves through the bottom of the bed, resulting in a combination of surface and subsurface flow systems.

The schematic of each of these types of CW can be seen in Table 4.2. All the systems considered in this review were also classified according to their size (full-scale, pilot) and the treatment step they represent (primary, secondary, tertiary, or polishing and restoration, see Table 4.2). It should be noted that a step may include more than one stage (resulting in a multi-stage system) of the same type (monotypic) or of different types (polytypic). These contrast with a hybrid plant, which consists of two or three steps relying on CWs. Moreover, SF treatments often consist of a series of basins. When this series is fed with a secondary effluent, the system acts as a polishing step and the single basins are considered as different stages of the same step (multi-compartment step). When the reviewed investigation was performed on a hybrid system but provided concentration or removal data for the whole system, the system was considered as a multi-stage step (see for instance Avila et al., 2010).

However, recently Fonder and Headley (2010) revised the usual classification system, proposing a more detailed nomenclature, taking into account not only hydrological characteristics (water position, flow direction, saturation of media, influent loading type), but also vegetation factors (sessility, growth form, emerging variant). This yielded seven “standard types” of CWs with an additional 11 variants. Although this is undoubtedly a valid system of classification, as data regarding vegetation is not always provided in the studies reviewed, we decided to follow the classical approach.

Table 4.2: Schematics of CWs considered in this chapter.

CW Type	Schematic
Surface flow (SF)	
Horizontal subsurface flow (H-SSF)	
Vertical subsurface flow (V-SSF)	
Hydroponic gravel bed	

4.5 Main features of the investigated plants

The current study reviews data on 136 treatment lines adopting CWs collated in 47 papers published between 2004 and 2013 in international journals (only one in the Proceedings of an International Conference on Wetland Systems). The main features of each treatment plant included in the current study are reported in Table 4.3, and more details of each of the 136 investigated treatment lines can be found in Table SD-3 of Verlicchi and Zambello (2014), specifically the: matrix investigated (water, plant tissues, gravel or sediments), type of data provided (concentrations, removal efficiencies, mass loads), sampling characteristics, analytical methods, plant description, feeding mode and type, further design parameters, filling media characteristics, vegetation and evapotranspiration, specific fields investigated, and comparison of CW and conventional WWTP performance. Of the 136 reviewed treatment lines, 38 were full-scale plants and 98 pilot scale. Only two were situated indoors (Dordio et al., 2009c, 2010).

Some pilot-plant investigations were reported to have been carried out on either a microcosm (Dordio et al., 2009c, 2010, Zhang et al., 2011) or a mesocosm system (Hijosa-Valsero et al., 2010b, 2011a,b; Zhang et al., 2012a,b,c, 2013a,b). However, no standard distinction between the two is found in literature. Roughly speaking, microcosm tends to refer to laboratory scale, and mesocosm to pilot-plant scale, although where available we report investigated plant size and dimensions in Table 4.3. Table SD-3 of Verlicchi adn Zambello (2014) (which provides a "qualitative description" of each plant) shows the resulting specific area, i.e., the surface area divided by the person equivalent (PE) served by the plant (m2/PE), when available.

In total, roughly 180 SF basins and filled beds (both horizontal and vertical flow types) have been included in this review. Most of the investigations considered took place in Spain (17 papers), but several were performed in Singapore and the USA (6 papers each); Canada and Denmark (3 papers each); South Korea, England, Portugal and Italy (2 papers each); and Australia, Brazil, Mexico and Sweden (1 paper each).

Redox potential within the investigated systems – Redox potential is one of the most interesting parameters for evaluating the ability of a CW to remove a specific compound. In SF systems, redox conditions are generally found in the aerobic-anoxic range (Hijosa-Valsero et al., 2010b reported values ranging from 50 mV at the surface to -55 mV at the bottom, Navarro et al., 2011 from 300 to 100 mV), but in deep anaerobic ponds, the redox potential is lower at the bottom. In horizontal SSF beds, redox potentials of 22 mV and -91 mV have been documented, confirming that the environment is anoxic-anaerobic. As remarked by many authors, even when anaerobic conditions prevail in a particular treatment system, aerobic microenvironments may be present around the plant roots, around bloom algae, or near the surface. This is confirmed by the higher redox potential values detected by Hijosa-Valsero et al. (2010b) in planted H-SSF beds with respect to similar unplanted ones (-91 mV vs. 22 mV). Although macrophytes may influence redox values in the substrate as they ventilate oxygen through their roots, Navarro et al. (2011) did not find marked differences between planted and unplanted beds. No redox potentials were reported for V-SSF beds in the studies under review, but a comparison of the concentrations of dissolved oxygen measured at the top and bottom of vertical and horizontal SSF beds by Matamoros et al. (2007a, 2009) confirms that an aerobic environment prevails in V-SSF beds.

As previously mentioned, Table SD-3 of Verlicchi and Zambello (2014) completes the "qualitative" analysis of the 136 lines under review. Here we limit ourselves to reporting some considerations on the main aspects relating to the feed and type of sequence investigated, to introduce the subsequent evaluation of their performance. In particular, based on the data in Table SD-3 of Verlicchi and Zambello (2014), it emerges that the type of feed was: real (R) urban wastewater in 61 % of investigated treatment lines (but in a few cases the feed included a portion of industrial discharge, as in Navarro et al., 2011); real wastewater spiked with the PhCs of interest (R+inj) in 18 % of cases, and synthetic feed (S) in the remaining 21 %. Out of the 136 treatment lines, the CWs acted as a primary step in 4 cases, as a secondary step in 47, and as a tertiary step in 35. 10 plants operated as a restoration wetland (Gross et al., 2004; Matamoros et al., 2012a; Navarro et al., 2011), and for 29 sequences it was not possible to define the corresponding treatment level, as they were fed by synthetic water composed of a nutrient solution spiked with the desired PhCs

(Zhang et al., 2011, 2012a, b, c, 2013a,b). Eleven sequences were classified as hybrid types: 2 lines included primary+secondary steps (1+2), 6 lines secondary + tertiary steps (2+3), and 3 lines primary+secondary + tertiary steps (1+2+3).

Among the four CWs acting as a primary step: 2 were SF systems (Carlson et al., 2013) and 2 were H-SSF beds (Ranieri et al., 2011). CWs were rarely adopted as primary treatments due to the high risk of clogging (Hijosa-Valsero et al., 2011b), and instead simple systems like septic tanks (SP), primary clarifiers (PC), Imhoff tanks, and in some cases sedimentation ponds, are used to retain most coarse and suspended materials. Out of the 47 CWs acting as a secondary step, 35 % were SF systems, 52 % adopted an H-SSF bed, and 10 % a V-SSF bed; the remaining 3 % were not specified. In only 6 cases, the step was designed as a multistage system (in all cases monotypic); two H-SSF beds operated in parallel in the treatment train investigated by Avila et al. (2010), and two H-SSF beds operated in series in the five lines assessed by Hijosa-Valsero et al. (2011b). Among the 35 CWs acting as a tertiary step, 35 % were SF, 35 % H-SSF type, 4 % V-SSF and 4 % unspecified. In 7 cases, CWs were designed as a multistage step, six of which were monotypic multistage sequences, i.e., four SF basins in series (Barber et al., 2006); a polishing pond and an SF system (Matamoros and Salvado, 2012 and Matamoros et al. 2010); 4 (not well specified) basins (Matamoros et al., 2012b), and two ponds in series (Park et al., 2009). Only Reyes-Contreras et al. (2011) investigated a polytypic multistage step, which comprised an SF basin followed by an H-SSF bed.

Regarding the 11 hybrid lines, the most commonly adopted CW was the SF system (31 basins out of 42 basins+beds). In fact, 6 lines relied solely on SF basins (27 basins as a whole), while only H-SSF beds (9 beds as a whole) were used in 3 lines, and 2 SF basins and 1 bed were adopted in each of the 2 remaining lines. In only 2 hybrid lines was the primary step designed as a monotypic multistage system, which included 3 aerated lagoons in series (Conkle et al., 2008) and two anaerobic ponds in parallel (Hijosa-Valsero et al., 2010a). In 4 lines the secondary step was a monotypic multistage type, including either 2 H-SSF beds (Avila et al., 2013) or 2 SF systems (Anderson et al., 2013). The 3 multistage tertiary steps found in the literature were: a monotypic three-stage step (3 lagoons in series, Froehner et al., 2011) and two polytypic two-stage steps (SF system+ HSSF bed, Hijosa-Valsero et al., 2010a). Note that 8 out of the 11 hybrid lines reported on are full scale.

Ten of the investigated lines were classified as restoration wetlands, being fed by water from an effluent-dominant river. These are the full-scale Prado SF wetland in California (Gross et al. 2004), the full-scale Aarslev SF wetland in Denmark (Matamoros et al., 2012a), and the 8 pilot lines (4 SF and 4 H-SSF) treating water from Sordo River in Mexico (Navarro et al., 2011). The data reported by Zhang et al. (2013a, b) on two lab investigations on hydroponic gravel beds fed by synthetic wastewater are also considered, specifically the removal efficiencies, plant uptake and their considerations on photo-catalysis.

Table 4.3: Brief description of each treatment plant included in this review and summary of its experimental investigation.

References	Details of treatment plants and summary of experimental investigations under review
1 Anderson et al. (2013)	The full-scale plant investigated (Grand Marais treatment wetland, Manitoba, Canada), has been in operation since 1996, and treats rural wastewater at an average flow rate of about 1700 m3/d. It is a hybrid system that consists of a two-lagoon secondary step (each is approximately 134 m × 134 m × 2.3) followed by a surface-flow polishing treatment (depth between 40 and 60 cm and total volume 23,200 m3) planted with *Typha*. Its peculiarity is that the tertiary step is fed once or twice a year, depending on the lagoon capacity. Five grab and 2 composite water samples were taken between May and August 2012 at the inlet and the outlet of the second step. A Polar Organic Chemical Integrative Sampler (POCIS) was used for continuous time-weighted-average passive sampling. Selected PhCs were carbamazepine, gemfibrozil, sulfamethoxazole and sulfapyridine. Data on PhC occurrence at the different steps are provided, and an evaluation of risk hazards posed by the presence of PhCs is presented.
2 Avila et al. (2010)	The investigated plant is situated in Barcelona (Spain). It consisted of an anaerobic reactor as a primary treatment, followed by two 0.65 m^2 H-SSF beds working in parallel and connected to a 1.65-m^2 H-SSF bed operating in series (one of the same treatment line investigated by Avila et al., 2013). The sequence was not considered as a hybrid, only the inlet of the sequence and the outlet of the last bed were monitored, and no intermediate samples were taken. The total hydraulic retention time (HRT) was 3.5 d. The plant was fed by spiked urban wastewater at a flowrate of 84 L/d. Eight composite water samples were taken at the influent and effluent of each treatment step in May. The removal efficiencies were calculated for ibuprofen, naproxen and diclofenac, and the intermediate degradation products of ibuprofen were studied.
3 Avila et al. (2013)	The investigation took place in Barcelona (Spain) at a pilot station consisting of three treatment lines operating in parallel, each including a primary treatment (an anaerobic reactor or a conventional settler), a secondary treatment (two small H-SSF beds of 0.65 m^2 each, operating in parallel but with a different operational strategies: continuously saturated conditions versus operation in batch with unsaturated periods), and a tertiary step consisting of a wider H-SSF bed of 1.65 m^2. Each line was fed with spiked urban wastewater at a controlled flow rate of 84 L/d. All H-SSF beds were 0.3 m deep and planted with Phragmites australis. The average HRT was 3.5 d per line. Six 12-h composite water samples were collected during December and analysed for three common PhCs: ibuprofen, diclofenac and acetaminophen. Their occurrence and removal are provided.
4 Barber et al. (2006)	The investigated polishing CW is situated near Phoenix, Arizona, and consists of four vegetated 0.89–1.3 ha SF systems, featuring mixed deep-water and shallow-water zones, receiving 7500 m^3/d of secondary treated effluent, with an HRT ranging from 3 to 4 d at average water depth of 0.1–0.5 m. Triclosan and caffeine were monitored at the inlet of the entire system and the outlet of each cell in both summer (July–August) and winter (February). Occurrence and removal efficiency are provided for the entire system.
5 Breitholtz et al. (2012)	The investigation refers to four planted SF systems situated in Sweden and receiving the secondary effluent from a municipal WWTP. The first covers an area of 28 ha and receives about 48,000 m^3/d, has a depth of 1 m and an HRT of 6–7 d. The second covers an area of 28 ha and treats about 5500 m^3/d, with an average HRT of about 10–15 d. The third has a total area of 24 ha, treats about 4000 m^3/d, and has an HRT of about 6 d. The fourth receives 1620 m3/d, covers an area of 6 ha and has a HRT of about 8 d. Grab and composite water samples (n = 6 + 6) were taken at the inlet and outlet of each system in February (temperature ranged between−5 and 7 °C) and processed for 92 PhCs, whose occurrence and removal efficiencies are provided.
6 Camacho-Munoz et al. (2012)	24-h composite flow proportional water samples were taken at the inlet and effluent of two different lagoons (n = 3 and 5) and a CW(not well specified) acting as a secondary step, in different periods of the year, in Spain. Average concentrations and removal efficiencies are reported for 16 PhCs, including analgesics/anti-inflammatories, antibiotics, beta-blockers, stimulants, antiepileptic drugs, estrogens, and lipid regulators.
7 Carlson et al. (2013)	Grab samples and samples collected using POCIS were taken at the effluent of two sewage lagoons acting as a primary step in Manitoba, Canada, during the months of June and July. The lagoons treat wastewater from rural communities and their outfalls are discharged into a river. 33 PhCs were investigated but only 10 of them were detected, including antibiotics, beta-blockers, lipid regulators, analgesics/anti-inflammatories, and psychiatric drugs. An estimate of per capita loads of the investigated compounds in the final effluent is also provided.
8 Conkle et al. (2008)	The investigation took place at the Mandeville WWTP, LA, USA, which treats 7600 m^3/d of municipal wastewater. The plant includes a series of three aerated lagoons (HRT of each lagoon is 9 d) followed by a surface flow system (HRT = 1 d). Three samples were collected at the inlet and outlet of the lagoon step, and after the SF basin. Occurrence and removal efficiencies for 14 PhCs are provided, as is an estimate of their influent and effluent loads.
9 Dordio et al. (2009c)	The investigation was performed on 9 H-SSF microcosms (0.6 m long, 0.5 mwide and 0.4 m deep) using LECA (2/4) as solid matrix and planted with Typha and Phragmites australis. The feed was a solution prepared from secondary municipal wastewater spiked with atenolol at a concentration of 0.78 µg/L. Influent and effluent water samples were collected from each bed, and an analysis of the removal mechanisms of the selected PhC is provided (n = 3).
10 Dordio et al. (2010)	The investigation was performed on 6 H-SSF microcosms (0.6 m long, 0.5 m wide and 0.4 m deep) using LECA (2/4) as solid matrix and planted with Typha. The feed was a solution prepared from secondary municipal wastewater spiked with three PhCs (carbamazepine, clofibric acid and ibuprofen) at concentrations of 1 µg/L. Samples (n = 3)were taken in two different periods (summer, mean T = 26 °C, and winter, mean T = 12 °C) to evaluate the effects of seasonal variability on the performance of the beds.
11 Froehner et al. (2011)	The investigated plant is placed in Cutiriba, South Brazil, and serves 200 000 inhabitants. It consists of a combination of lagoons: first an anaerobic one (46.5 m × 104.5 m × 3.5 m), then a facultative one (92.5 m × 192.5 m × 1.70 m), and finally a polishing step (47.5 m × 112.5 m × 1.70 m). The HRT of the entire plant is equal to 24 d. Samples were taken at the influent and outlet of the hybrid plant and processed for caffeine and 3 hormones (17-β estradiol, estrone and ethinylestradiol). Occurrence and removal data of the investigated compounds are provided.
12 Gross et al. (2004)	The investigation took place at the Prado wetland, California. The plant covers 130 ha of open shallow surface water and consists of two separate sequences of interconnected ponds and channels, with a HRT between 2 and 4 d. It receives the water from an effluent-dominant river, 75% of whose flow rate is due to discharges from WWTPs.

References	Details of treatment plants and summary of experimental investigations under review
	Samples were collected at the inflow and outflow of the Prado wetland every four months from April to December (n=3), and processed for ibuprofen, naproxen, ketoprofen, gemfibrozil and carbamazepine. Occurrence data are provided and considerations about their possible attenuation in surface water systems are discussed.
13 Hijosa-Valsero et al. (2010a)	The investigation took place in Spain, on three full-scale hybrid systems, including different subsystems connected in series (SF and H-SSF) fed with primary-treated urban wastewater. Grab samples (n=4)were collected weekly at the influent and effluent of each plant, on the same day and at the same time, and processed for 5 analgesics/anti-inflammatories, the anti-epileptic carbamazepine, and the stimulant caffeine. Their occurrence in the raw influent and the treated effluent of each plant are provided, together with the observed percentage removal efficiencies. Plant 1 serves 632 inhabitants, has a mean flowrate of 3200 m^3/d and consists of 2 in-parallel anaerobic ponds (335 m^2, 3.75 m depth, HRT = 0.4 d), a facultative pond (8481 m^2, 2 m depth, 4.1 d HRT), and a maturation pond (3169 m^2 surface, 1.5 m depth, 1 d HRT). Plant 2 serves 184 inhabitants, has a flow rate of 20 m^3/d, and includes a facultative secondary pond (1073 m^2 surface, 1.6 m depth, 75.9 d HRT) colonized by Lemna minor, connected to a SF basin planted with Typha (44 m^2, 30 cm layer of 6–8 mm gravel, 40 cm depth, 1.2 d HRT) followed by an H-SSF bed planted with Salix atrocinerea (585 m^2, 55 cm layer of 6–8 mm gravel, 5.7 d HRT). Plant 3 serves 186 inhabitants, has a flow rate of 56.3 m^3/d and consists of a facultative pond (230 m2, 1.5–2 m depth, HRT 4.2 d), connected to an SF basin planted with Typha latifolia (210 m2, HRT 3.53 d) and finally an H-SSF bed planted with Salix atrocinerea (362.5 m^2, HRT=3.16d).
14 Hijosa-Valsero et al. (2010b)	The investigation took place at an open-air pilot station in Spain consisting of seven mesocosm-scale CWs (5 SF systems and 2 H-SSF beds) of the same size, but characterized by certain design differences. They were fed by the primary effluent of a municipal WWTP at a flow rate of 50 L/d (input load 50 mm/d). Grab samples were taken at the influent and effluent of each tank once a week (n = 8 in winter, n = 7 in summer) and processed for 5 analgesics/anti-inflammatories, the antiepileptic carbamazepine and the stimulant caffeine. The relationship between chemical–physical parameters (namely T, pH, dissolved oxygen, and redox potential) and the removal efficiency of the selected compounds were investigated in the two different periods. 2 SF basins were modified SF type.
15 Hijosa-Valsero et al. (2011a)	48-h composite water samples were collected from the influent and effluent of seven open-air pilot scale CWs(5 SF systems and 2H-SSF beds) inside the Léon WWTP (Spain) and processed for 9 antibiotics in the soluble aqueous fraction (n = 5) and in the insoluble aqueous fraction (n = 1). The investigation took place in November. The seven CWs differed by type (SF,H-SSF, vegetation: Typha and Phragmites australis or unplanted, floating or radicated macrophytes), but all were fed with the same primary effluent from the WWTP, each at a constant flowrate of 50 L/d (input load: 50 mm/d). Antibiotic occurrence and removal data are presented, and the latter is discussed with respect to CW design characteristics. 2 SF basins were modified SF type.
16 Hijosa-Valsero et al. (2011b)	The investigation took place in Spain on 5 independent lines of a system including a series of H-SSF beds fed with primary effluent. Each line consists of two tanks connected in series: the first tank is divided into two parallel compartments (0.93 m × 0.55 m × 0.45 m) and filled with 30 cm-depth layer of siliceous gravel; while the second is 1.34 m × 1 m × 0.80 cm and filled with 30 cm of gravel. The water depth was 25 cm in each tank, the flow rate equal to 84 L/d (with a hydraulic loading rate of 28.5 mm/d) and the HRT of 3.5 d. Grab samples were taken, at the same time, in the morning, at the influent and effluent of each line, in winter (n=6) and in summer (n=6) over six consecutive days, and processed for 5 analgesics/anti-inflammatories, the anti-epileptic carbamazepine, and the stimulant caffeine. Their occurrence in the influent and effluent of each line was reported, and the observed removal efficiencies were discussed, taking into account the influence of the adopted primary treatment (sedimentation tank or anaerobic hydrolysis upflow sludge bed hydrolysis upflow sludge bed HUSB), the applied feeding regimes (continuous or batch), the presence of vegetation, environmental conditions and seasonal variations.
17 Lee et al. (2011)	The investigation took place at an SF system, connected to the Damyang WWTP (Korea) and acting as a tertiary step. Grab samples were taken (n = 3)at the SF basin influent and effluent and processed for atenolol, sulfamethoxazole, naproxen, glimepiride, ibuprofen, diclofenac, caffeine, and carbamazepine. Observed removal efficiencies were correlated to the octanol–water distribution coefficient D_{ow}.
18 Li et al. (2013)	The investigated full-scale plant consists of two aerated lagoons in series (5300 m^3 and 3790 m^3), acting as primary + secondary treatment (hybrid system) treating the screened wastewater from a rural area in Illinois, USA. Water samples were taken at the inlet and the outlet of each wetland, and processed for 21 PhCs. Occurrence and removal are provided.
19 Llorens et al. (2009)	A 1-ha full-scale SF system in Catalonia, Spain, receiving the secondary effluent from the Granollers WWTP at a flow rate of about 100 m^3/d (HRT = 1 month, HLR = 10 mm/d) was investigated. It was vegetated with Phragmites australis and Typha latifolia. Grab samples were collected at the influent and effluent of the systemin two sampling campaigns (June and February, n=10), then processed for ibuprofen, naproxen, diclofenac, ketoprofen, clofibric acid, and carbamazepine. Their occurrence and removal efficiencies are reported and discussed.
20 MacLeod and Wong (2010)	The investigation took place at Lac La BicheWWTP (Alberta, Canada),which consists of aerated lagoons (HRT = 90 d) acting as a secondary step for the treatment of rural community effluent (4000 inhabitants, about 2000 m^3/d). A Polar Organic Chemical Integrative Sampler (POCIS), a passive sampling device,was used to obtain time-weighted average loads of 17 PhCs at the outlet of the investigated plant (n = 9) over 10 months (from July to April). PhC occurrence and mass loads are provided.

References	Details of treatment plants and summary of experimental investigations under review
21 Matamoros and Bayona(2006)	An investigation of two in-parallel vegetated H-SSF beds acting as a secondary step, characterized by the same surface area (54 m2) but different water depths (0.27 m and 0.5 m). They are located in the municipality of Les Franqueses del Vallès (Barcelona, Spain) and they treat settled urban wastewater. Influent and effluent grab samples were taken daily in three different periods (n=13 for the dissolved phase, n=5 for the suspended particulate matter), as well as gravel samples with biofilm (n=1) and processed for the analysis of 5 analgesics/anti-inflammatories and the stimulant caffeine. Their occurrence and the observed average removal efficiencies are provided.
22 Matamoros and Salvado (2012)	The investigated pilot plant acts as a tertiary step and consists of two parallel polishing ponds (2 ha surface area and 1 m depth, HRT of 4 d) and an SF system (HRT of 8.5 d) made up of three parallel basins (surface area 0.8 ha each, water depth 0.5 m) and a large shallow pond in series (4.5 ha; average depth 0.2 m). The polishing plant receives the secondary effluent from Empuriabrava WWTP at an average flow rate of 3700 m3/d. Grab samples were collected at the influent of the investigated plant, after the ponds, after the SF system, and at the end of the artificial pond, in four periods (n = 20) and processed for 7 PhCs. Occurrence data and removal efficiencies are presented and discussed.
23 Matamoros et al. (2005)	The behaviour of clofibric acid, ibuprofen and carbamazepine was investigated in two H-SSF beds, each planted with Phragmites australis but characterized by different water depths (0.3 and 0.5 m) and aspect ratios (2:1 and 2.5:1). Each plant operated as a secondary step. Influent flow rates were adjusted to obtain a final influent concentration of 25 µg/L for each selected PhC. Effluent composite samples were collected every 6 h over 21 d. A gravel sample with biofilmwas taken at 2 m from each wetland inlet. The pilot station was located in Spain and the observation period was in April– May. Removal efficiencies are presented and discussed.
24 Matamoros et al. (2007a)	The investigated plant was a pilot vegetated 5-m2 V-SSF bed located near Arhus, Denmark, acting as a secondary step and fed with settled urban wastewater. The filling consists of a 0.20 m bottom layer of coarse gravel (8–16 mm) and a 0.8 top layer of 0–4 mm gravel. Four different hydraulic loading rates (HLR = 13, 30, 70 and 160 mm/d)were applied to the system to evaluate the effect of loading rate on the removal of six selected PhCs. Grab samples were taken daily (n = 15) at the inlet and outlet of the bed, and processed for 4 analgesics and anti-inflammatories, the antiepileptic carbamazepine, and the stimulant caffeine. The observation period was in June–July.
25 Matamoros et al. (2007b)	The investigated plant consists of an H-SSF bed, characterized by an average water depth of 0.33 m, a surface area of 55 m^2, an average grain size of 3.5 mm and an HLR of 36 mm/d, acting as a secondary step. A mixture of 40 L of distilled water spiked with 100 mg of each PhC was homogenized and injected by single shot into the inlet tubing of the plant. Effluent composite samples were collected every 6 h for over 19 d. Two gravel samples with accumulated organic matter and biofilm were sampled at 2 m from the wetland inlet. All samples were processed for clofibric acid.
26 Matamoros et al. (2008b)	The investigated site is a full-scale polishing SF system fed with the secondary effluent from a conventional WWTP, near Barcelona. It is made up of a single cell with a surface area of 1 ha, and treats about 100 m3/d with an HRT of around 30 d. Influent and effluent samples were collected from the wetland daily over 1 week in two different sampling campaigns (June and February, n=10) and processed for 6 PhCs. In addition to removal efficiency data, the study also provides the reduction of the organic micropolutant load achieved by the SF system.
27 Matamoros et al. (2009)	The investigation was performed on nine treatment systems, acting as secondary steps, serving sparsely populated regions in the vicinity of Arhus, Denmark. They include 5 H-SSF beds serving 80–280 inhabitants and having a surface area of between 500 and 4000 m^2, and four V-SSF beds serving 2–4 inhabitants and having a surface area of between 15 and 16 m^2. 16 grab samples were taken at the inlet and outlet of each bed, and processed for salicylic acid, ibuprofen, carbamazepine, naproxen, diclofenac, ketoprofen, caffeine, and furosemide. Occurrence and removal efficiencies are provided.
28 Matamoros et al. (2010)	The investigated polishing treatment consists of two parallel ponds followed by two SF constructed wetlands in series. The total area is 7 ha and the HRT ranged between 5 and 15 d. The feed was effluent from the Empuriabrava WWTP (35,000 PE). Influent and effluent water samples were taken daily over 1 week (n=7), and processed for 10 PhCs from different classes. The occurrence and removal efficiencies observed in the series of ponds and SF systems are reported and discussed.
29 Matamoros et al. (2012a)	The investigation took place from September to December at the Aarslev restored wetland near Aarhus, Denmark. The wetland covers an area of 100 ha, has an average depth of 0.5 m, and a maximum depth of 2 m. Its HRT ranges between 3 and 20 d, with an average of 7 d. It receives the water from two rivers subject to considerable anthropogenic discharges from WWTPs located upstream of the sampling points. Samples were collected at the two influents and effluent of the wetland (n = 6 in each point) and processed for 8 PhCs.
30 Matamoros et al. (2012b)	The investigation took place at the municipal Empuriabrava WWTP (Spain) on a tertiary system including a full-scale pond (2 ha surface and 1 m depth), fed with secondary effluent (on average 3700 m^3/d), followed by two identical (pilot) lines (A and B) working in parallel (one open and one covered), including four 1000 L tanks (0.8 m wide, 1.30 m long and 0.5 m high), each line receiving a flow rate of 994 L/d. Grab samples were collected daily at the influent of the tertiary step and the effluents from the two lines over 1 weekand were processed for 7 PhCs. The effects of different HRTs (1, 2, 3 and 4 d), solar radiation, and seasonality on the removal were also investigated.
31 Navarro et al. (2011)	The investigation took place in Mexico at a pilot station fed with water from the Sordo River, which receives untreated urban sewage and industrial wastewaters. Four SF basins (substrate upload soils 0.4-m deep, water height 0.10 m) and four H-SSF beds (volcanic gravel D60 = 0.04m, depth 0.4 m, water flow 0.20 m). Two SF basins and two H-SSF beds were planted with Typha sp., and the remaining unplanted as control. In each unit HRT was kept constantly equal to 5 d. Four grab samples were taken at the inlet (river) and outlet of each unit nd processed for the stimulant caffeine. Concentrations and removal efficiencies are provided for each CW.
32 Park et al. (2009)	The investigation took place in Korea on a full-scale station consisting of two in-series ponds containing Acorus (the first) and Typha (the second) fed with secondary effluent from the Damyang WWTP. Each pond has an averagewidth, length and depth of 30, 120 and 0.13 m, an HRT of 6 about h, and a flow rate of 1800 m^3/d. Three grab samples were taken at the influent (wastewater effluent) and effluents from the two ponds and processed for 8 common PhCs: atenolol, sulfamethoxazole, dilantin, carbamazepine, diazepam, diclofenac, naproxen, and triclosan.

References	Details of treatment plants and summary of experimental investigations under review
	Removal efficiencies were correlated to pK_a and Log K_{ow}. Samples were also collected from soil and plants to investigate potential removal mechanisms (plant uptake, soil sorption).
33 Ranieri et al. (2011)	Two pilot-scale H-SSF beds, both acting as a primary step, situated near Lecce, Italy, and planted with Typha and Phragmites australis were assessed, together with an unplanted control, for their effectiveness in removing acetaminophen. Each plant has an area of 15 m^2 (3 m × 5 m), and a water depth ranging from 0.6 to 0.65 m. The filling consists of 3 layers: 0.1 m soil (bottom), 0.2 m stones 30–80 mm(medium) and 0.30–0.35 m of 5–10- mm gravel (top).Water samples were taken at the inlet and outlet of each bed every 30 min over a period of 120 d. Removal efficiencies were assessed at two hydraulic loading rates (30 mm/d and 240 mm/d).
34 Reif et al. (2011)	Two vegetated pilot-scale V-SSF beds, designed and developed at Cranfield University (UK), were fed with a secondary effluent (from a conventional activated sludge and a membrane bioreactor) 8 times per day for 10 minutes at 125 mL/min. The beds consisted of a multilayer filter, including from the bottom to the top: 0.6 m of gravel (40–50 mm), 0.05 m gravel (20 mm), 0.15 m pea gravel (10 mm), 0.15 m filter-grade sand. Five discrete samples were taken at the inlet and the outlet of the two V-SSF beds between 8 am and 8 pm on each of three sampling days and processed for ibuprofen, naproxen, diclofenac, diazepam and carbamazepine.
35 Reyes-Contreras et al. (2011)	The investigation took place near Santiago de Compostela (Spain) where the local urban wastewater was pre-treated and then sent to an upflow anaerobic sludge blanket reactor (UASB). A fraction of the UASB effluent (17–20 m3/d) was subjected to a polishing (polytypic) treatment in a pilot station consisting of an SF basin (75 m2,water level of 0.5 m) followed by an H-SSF bed (75 m2, gravel depth of 0.5 m). The average HRT for the series of CWs was 55 h. Integrated water samples (every 4 h over a 24 h period)were taken at the influent of the polishing sequence, after the SF basin, and after the H-SSF bed for three weeks, in 2 sampling campaigns per week (n = 6). The selected PhCs were ibuprofen, naproxen, ketoprofen, caffeine, carbamazepine, salicylic acid, and triclosan. Data are provided for their occurrence in the influent and their removal, considering only the dissolved contribution.
36 Reyes-Contreras et al. (2012)	Seven mesocosm-scale (1 m2) open-air CWs of different configurations (3 SF basins and 4 H-SSF beds) were investigated in Spain over a period of 39 months (May 2007 to September 2010) under the same operational and environmental conditions, to assess their ability over time to remove 5 common analgesics/anti-inflammatories, the antiepileptic carbamazepine, and the stimulant caffeine. All the CWs were fed with the same feed (primary clarifier effluent from an urban WWTP) at a flow rate of 50 L/d (influent load about 50 mm/d). Influent and effluent grab samples were collected once a week during 3 summer and 2 winter campaigns (n = 31). Concentrations and removal data are provided, and the factors affecting the observed removal are discussed.
37 Verlicchi et al. (2013b)	4-h composite water samples (n = 4)were taken at the inlet and outlet of a long, narrow H-SSF bed (28 m × 1m × 1.2 m) acting as a tertiary step, and processed for 73 common PhCs. The bed was filled with gravel (8/10) and planted with Phragmites australis. Concentrations and removal efficiencies of selected compounds in the beds are provided, and the possible removal mechanisms and main factors affecting them are discussed.
38 Waltman et al. (2006)	A 24-h cycle of three 8-h composite samples was collected from the influent and effluent of an SF system (n = 19) acting as a tertiary step, and processed for triclosan. The system receives a small fraction of secondary effluent from Denton municipal WWTP. Its surface area is 0.21 ha and its depth ranges from a few centimetres at the inflow to 0.6 mat the wetland outflow. Occurrence data and observed removal efficiencies are provided.
39 Ying et al. (2009)	In South Australia, occurrence and removal of 8 selected compounds (caffeine, carbamazepine, triclosan, gemfibrozil, diclofenac, ibuprofen, ketoprofen and naproxen) were investigated in a hybrid plant consisting of ten lagoons: 2 parallel anaerobic lagoons followed by 8 aerobic lagoons. The flow rate was 760 m3/d. 24-h composite time-proportional water samples were taken at the inlet and at outlet of each lagoon.
40 Zarate et al. (2012)	The study area was a pilot-scale SF CW, located at the Pecan CreekWWTP in Denton, Texas, and fed with a fraction of the WWTP secondary effluent at an average flow of 71 m3/d. The mean HRT was 4.3 d. The wetland featured a loamy substrate and was dominated by Typha, P. cordata, and S. graminea. Plant tissues and sediments were sampled at the inlet, in the centre, and at the end of the wetland, to investigate the bioaccumulation of triclosan and triclocarban, two antimicrobial agents contained in a great variety of consumer products.
41 Zhang et al. (2011)	Six microcosm H-SSF beds (planted and unplanted) were investigated in Singapore to evaluate their ability to remove carbamazepine, diclofenac, ibuprofen and naproxen under different hydraulic regimes (HRT = 2 and 4 d). Beds were 1.20 m long, 0.6 m wide and 0.60 m deep, three were vegetated with Typha angustifolia, and the remaining three unplanted. The gravel layer was 0.3 m deep and the water height was 0.25 m. All beds were fed with synthetic wastewater with the same organic load, and operated for 4 weeks before sampling. HRTs were 2 and 4 days. The influent concentration of each PhC was 25 µg/L. Water samples were taken at the exit of each bed, and occurrence and removal efficiencies are provided.
42 Zhang et al. (2012a)	Six mesocosm-scale H-SSF beds (1.20 m × 0.60 m × 0.60 m) were investigated in Singapore, to evaluate the influence of feeding mode (batch vs. continuous) on the removal of ibuprofen, naproxen, diclofenac and carbamazepine from synthetic wastewater. The beds were filled with 30 cm of 4–10-mm gravel, vegetated with Typha Angustifolia. They were tested at two different HRTs: 2 and 4 d. Batch and continuous modes were operated, with HLRs of 56 and 28 mm/d respectively. Grab samples were taken at the influent and exit of each bed.
43 Zhang et al. (2012b)	Six in-parallel mesocosm-scale H-SSF beds were investigated in Singapore to evaluate the fate and transport of 8 PhCs. Three vegetated (with Typha augustifolia) and three unplanted beds were continuously fed with synthetic wastewater at different HRTs (2–6 d). Each bed was 1.2 m long, 0.6 m wide, and 0.6 m deep, and filled with 4–10 mm gravel up to 0.30 m (porosity 0.45). Grab samples were collected at the influent and the 6 effluent points, and processed for carbamazepine, naproxen, ibuprofen, diclofenac, salicylic acid, ketoprofen, clofibric acid and caffeine.
44 Zhang et al. (2012c)	Three in-parallel mesocosm-scale H-SSF beds were investigated in Singapore to evaluate the influence of the feeding mode (continuous and batch) on the removal of 8 PhCs (carbamazepine, naproxen, ibuprofen, diclofenac, salicylic acid, ketoprofen, clofibric acid and caffeine) from synthetic mesocosm-scale H-SSF beds. Both loading modes were operated at the same HLRs of 56 mm/d and 28 mm/d
45 Zhang et al. (2013a)	The fate and removal of carbamazepine and naproxen were investigated in a mesocosm scale CW (4 L vessels) planted with 4 Scirpus validus plants and fed with a modified Hoagland nutrient solution, spiked with the two PhCs at concentrations of 0.5, 1.0 and 2.0 mg/L. This system acts as a hydroponic gravel bed. The nutrient solution remaining in the vessels was collected and analysed for the different compounds after four different exposure

References	Details of treatment plants and summary of experimental investigations under review
	periods: 3, 7, 14 and 21 days. A study on the ability of Scirpus validus to take up and translocate these compounds was carried out, as was an evaluation of the roles of photodegradation and biodegradation in their removal.
46 Zhang et al. (2013b)	Removal efficiency and fate of caffeine in a nutrient solution spiked with the selected compound at concentrations of 0.5, 1.0 and 2.0 mg/L were investigated in the same lab-scale plants (hydroponic gravel beds) investigated in Zhang et al., 2013a, under the same operational conditions. Photodegradation biodegradation and uptake by the plant roots and shoots were assessed.
47 Zhou et al. (2009)	In West Sussex, UK, samples were taken at the influent and outlet of a lagoon acting as a secondary step, receiving the effluent from a primary treatment at a flow rate of 19,900 m^3/d. Six PhCs: tamoxifen, propranolol, sulfamethoxazole, carbamazepine, indometacin and diclofenac were monitored. Their occurrence and removal efficiencies were reported. The daily variation in the selected compounds was also investigated.

4.6 Characteristics of the experimental investigations and critical analysis of the plants described

As already reported, Table SD-3 of Verlicchi and Zambello (2014) gives a “qualitative” summary of the investigations included in the review and the type of data they provide. In addition, Table SD-2 shows the number of influent and effluent concentrations and removal values reported for each compound in the different studies. This table clearly shows that one group of compounds was most frequently monitored and investigated in CWs. These compounds include the (common) analgesics and anti-inflammatories ibuprofen, naproxen, ketoprofen and salicylic acid, the psychiatric drug carbamazepine, and the stimulant caffeine. Data collated include PhC concentrations in the influent and effluent of the different types of CWs acting as primary, secondary, and tertiary steps, as well as hybrid systems; removal efficiencies in the different types of CWs, referring to the step, PhC concentration in plant and filling media; and specific PhC mass load expressed as g/(inhabitants day) or g/(1000 inhabitants day). The descriptions of the investigated plants generally contain plenty of design and operational details, but several important design parameters are sadly lacking in some cases, especially in pilot plants (average influent flow rates, water losses and HRT are not always provided). Furthermore, some papers provide data for the CW effluent, but not for the influent, and sometimes concentration values are only reported in graphs (not tables), making it rather difficult to determine their exact values.

4.7 Quality assurance data and sampling mode recommendations

To decide which studies to include in this review, an in-depth analysis of quality of data presented and discussed was carried out. According to the EC Technical Guidance Document on risk assessment (EC, 2003), and as remarked by many Authors (among them Liebig et al., 2006), references had to feature a description of the analytical methodology used for the assessment of measured concentrations, and the quality assurance programme adopted for sampling, analysis and elaboration (see Table SD-3 of Verlicchi and Zambello, 2014). Only papers reporting reliable concentrations and removal data were included, enabling us to depict the state-of-the-art as regards the occurrence and removal of PhCs in the influent and the effluent of CW systems fed with municipal wastewater. It is, however, important to note that reported PhC concentrations refer to the aqueous phase, since all samples were filtered to remove particulate material prior to analysis. Only in a few exceptions was suspended particulate matter considered (mainly Hijosa-Valser et al., 2011a, Matamoros et al., 2005, 2007b; Matamoros and Bayona 2006; Park et al., 2009; Zarate et al., 2012). Nevertheless, Matamoros and Bayona 2006 state that the greatest quantity of their investigated PhCs were found in the dissolved phase, and further studies have confirmed that PhCs sorbed onto particulate matter represents only a small fraction of the total load (among them Miao and Metcalfe, 2007). As regards sampling mode, most of the investigations used grab samples or flow-proportional composite water samples. However, Carlson et al. (2013), comparing PhC concentrations detected in grab samples with those obtained by Polar Organic Chemical Integrative Sampler (POCIS), showed that the latter revealed several compounds, including gemfibrozil, ethinylestradiol and naproxen, that were not detected by solid phase extraction (SPE) of grab samples. This demonstrates the limitations of grab samples in detecting compounds that are less common in the system and have intermittent detection patterns in aquatic ecosystems receiving effluents, indicating that more frequent sampling is necessary to model a system receiving continuous or pulse inputs over time.

4.8 Results

From Fig. 4.1 to Fig.4.16 summarize the collated data on influent and effluent concentrations, as well as the removal efficiencies of the different types of CWs used as primary (Fig 4.1-Fig.4.3), secondary (Fig. 4.4- Fig. 4.6) tertiary (Fig. 4.7-4.15) steps and in hybrid systems (Fig. 4.16). The different symbols indicate the different types of CWs: a star for SF systems, a circle for H-SSF beds, a triangle for V-SSF beds and a square for non-specified CWs. Where more than one type of data is presented in the graph, a legend is provided. The figures do not include hydroponic gravel beds or non-specified treatment systems (fed by synthetic influent, which is not comparable to raw, primary or secondary effluent), which have, however, been discussed in dedicated subparagraphs. Note that the PhC concentrations reported below their corresponding limit of detection (LOD) are taken as their LOD in the graphs.

As regards the removal efficiencies, Fig.4.3, Fig.4.6, 4.13, 4.14 and 4.15 report only positive observed values, while negative values, along with the name of the corresponding compounds, are listed below each figure, as well as in Table SD-1 of Verlicchi and Zambello (2014), which also reports the corresponding references. Likewise, the average removal efficiencies presented and discussed below were calculated using positive values alone. In the X-axis of these graphs, a superscript asterisk after the name of a compound indicates that release phenomena occurred for that compound in the step under consideration. The average values of removal efficiencies are also reported after the name of each PhC, with reference to the different CW types. When an effluent concentration was detected below its corresponding LOD, the removal efficiency were evaluated assuming the LOD value as effluent concentration.

As previously mentioned, in only 34 out of the 47 investigations was the feed real wastewater; in a further seven investigations it was a real wastewater spiked with the compounds of interest at the desired concentrations, and in the remaining six the feed was synthetic. Nonetheless, in all cases of spike or synthetic feed, the influent pharmaceutical concentrations were within the range observed in other studies for raw municipal wastewater, as documented by Verlicchi et al. (2012b). Some differences were, however, found with respect to real wastewater fed to secondary and tertiary treatment steps, as discussed below.

4.8.1 Occurrence in the treatment step influent and effluent – Removal efficiency by CW

The following figures show PhC concentrations in the influent and effluent of each step (primary, secondary and tertiary). For each of them, any data provided for the influent and effluent of the same step of the hybrid lines are included. The number of values collated for each PhC at each different treatment step and for each type of CW in this review are reported in Table SD-2 of Verlicchi and Zambello (2014).

Primary treatment

Influent and effluent concentrations - In CWs acting as a primary step, 23 PhCs were monitored both in the influent (Fig. 4.1) and effluent (Fig. 4.2), 6 analgesics/anti-inflammatories, 5 antibiotics, 2 antiseptics, 5 beta-blockers, 1 lipid regulator, 2 psychiatric drugs, 1 receptor antagonist and 1 stimulant drug. All CWs were SF types. Table SD-1 of Verlicchi and Zambello (2014) reports all the collated average values of influent and effluent concentrations of each compound, alongside the corresponding references.

The substance most frequently monitored in the influent was caffeine (7 values), followed by ibuprofen, naproxen and carbamazepine (6 values). Carbamazepine (8 values) was the substance most frequently monitored in the effluent, followed by ibuprofen, naproxen and caffeine (6 values). In the influent, the highest concentrations were detected for salicylic acid (78 μg/L) and caffeine (58 μg/L), followed by acetaminophen and naproxen (39 μg/L), while in the effluent, ibuprofen (14 μg/L), caffeine (7.3 μg/L) and naproxen (6.9 μg/L) were detected at the highest concentrations. The average highest concentrations in the influent were detected for acetaminophen (39 μg/L), salicylic acid (36 μg/L) and caffeine (25 μg/L), and in the effluent for caffeine (4.1 μg/L), ibuprofen (3.3 μg/L) and gemfibrozil (1.9 μg/L). Only salicylic acid was found in the influent at a value (78

μg/L) greater than those observed and reported in the review by Verlicchi et al. (2012b) (maximum of 32 μg/L). The average values detected in the influent and effluent are reported after the name of the compound along the X-axes of Fig. 4.1 and Fig. 4.2.

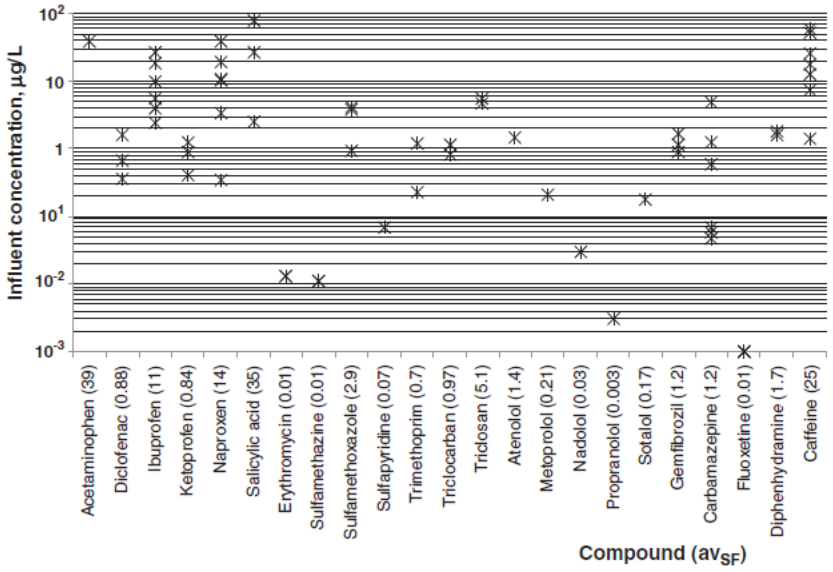


Fig. 4.1: Reported concentrations of PhCs in the influent to CWs (all SF systems) acting as a primary step.

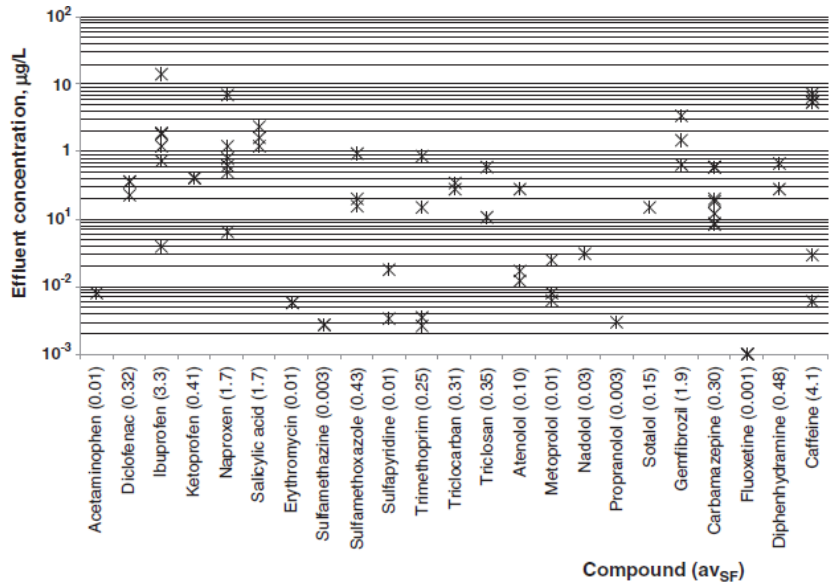


Fig. 4.2: Reported concentrations of PhCs in the effluent from CW (all SF systems) acting as a primary step.

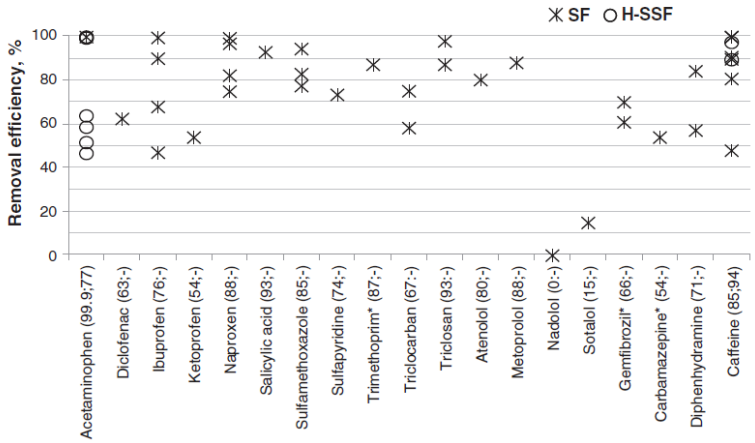


Fig. 4.3: Reported removal efficiencies in CWs acting as a primary step (circle = H-SSF bed), asterisk = SF. *Negative percentage removal efficiencies reported for trimethoprim(−283%), gemfibrozil (−68%) and carbamazepine (−44%, −177%, −316%).

Removal efficiencies - Removal efficiencies for 19 out of the 23 PhCs reported in Fig. 4.1 and Fig. 4.2 are shown in Fig. 4.3. Most data refer to SF systems, but for acetaminophen and caffeine, data were also available for H-SSF beds (Ranieri et al., 2011 and Navarro et al., 2011). In the influent and effluent, erythromycin, sulfamethazine, propranolol and fluoxetine were detected below their corresponding detection limit (Li et al., 2013b; Conkle et al., 2008), so for these compounds no removal data are available for inclusion in Fig. 4.3. Negative removal values were reported for trimethoprim, gemfibrozil and carbamazepine. Considerations about the possible causes of such phenomena are reported in the Discussion subsection: PhC release phenomena during treatment.

Considering the SF systems alone, the highest removal values were found for caffeine (> 99.9 %100 %), acetaminophen (99.98 %), ibuprofen (99.6 %), naproxen 99.4 % and triclosan (98 %), while the lowest were found for nadolol and sotalol. The highest average values of removal were for acetaminophen (99.98 %), salicylic acid and triclosan (both 93 %). In the two multistage primary steps included in the review (Conkle et al., 2008 and Hijosa-Valsero 2010a), the best removal efficiencies, for naproxen, caffeine and ibuprofen, were observed in the three lagoons in series investigated by Conkle et al. (2008). This was ascribed to the high HRT (9 d in each lagoon) of this system, as discussed by the same authors. Regarding the H-SSF bed investigations, the highest removal efficiencies were observed for acetaminophen (99.9 %), while the highest average value was for caffeine (93.6 %). A comparison of the performance of the two types of CWs shows that SF systems are more efficient at removing acetaminophen, while caffeine removal is high in both systems. An analysis of the main removal mechanisms at work is reported in the Discussion.

Secondary treatments

Influent and effluent concentrations – Fig. 4.4 and Fig. 4.5 report the concentrations of 31 PhCs in influent and 37 in effluent of CWs acting as a secondary step. Monitored compounds in the effluents were the same as those investigated in the influent, plus an additional 6, namely celecoxib, codeine, fenoprofen, citalopram, paroxetine and omeprazole. The fourth column of Table SD-1 of Verlicchi and Zambello (2014) reports the average influent and effluent concentrations of each compound in secondary step CWs, alongside the corresponding references.

The X-axes of Fig. 4.4 and Fig. 4.5 show the name of each PhC investigated, followed by the average concentrations detected in the influent (Fig. 4.4) or effluent (Fig. 4.5) of the four types of systems investigated, in the order: SF, H-SSF, V-SSF systems and non-specified CWs. The monitored compounds were: 6 analgesics/anti-inflammatories, 11 antibiotics, 2 antiseptics, 5 beta-blockers, 1 diuretic, 1 hormone, 1 lipid regulator, 2 psychiatric drugs, 1 receptor antagonist and 1 stimulant drug in the influent, and 9 analgesics/anti-inflammatories, 11 antibiotics, 2 antiseptics, 5 beta-blockers, 1 diuretic, 1 hormone, 1 lipid regulator, 4 psychiatric drugs, 2 receptor antagonists and 1 stimulant drug in the effluent. The most commonly monitored compounds in the influent were naproxen and ibuprofen (60 values), and in the effluent naproxen and carbamazepine (42 values). The highest influent concentration was reported for ibuprofen (132 μg/L, Avila et al., 2010), while the highest average influent concentrations were detected for amoxicillin (46 μg/L), acetaminophen and caffeine (34 μg/L), and salicylic acid (31 μg/L). The highest effluent concentration was also reported for ibuprofen (55 μg/L, Avila et al., 2013), whereas the highest average concentrations were detected for amoxicillin (37 μg/L).

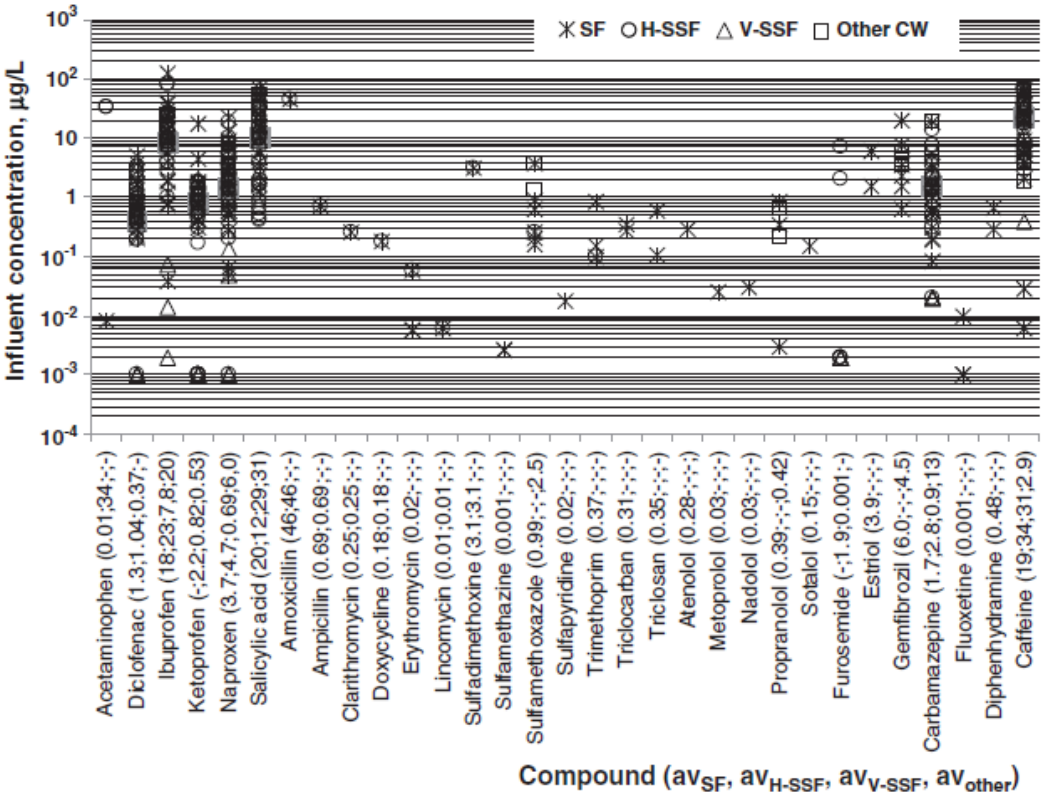


Fig. 4.4: Reported influent concentrations of PhCs in CWs acting as a secondary step.

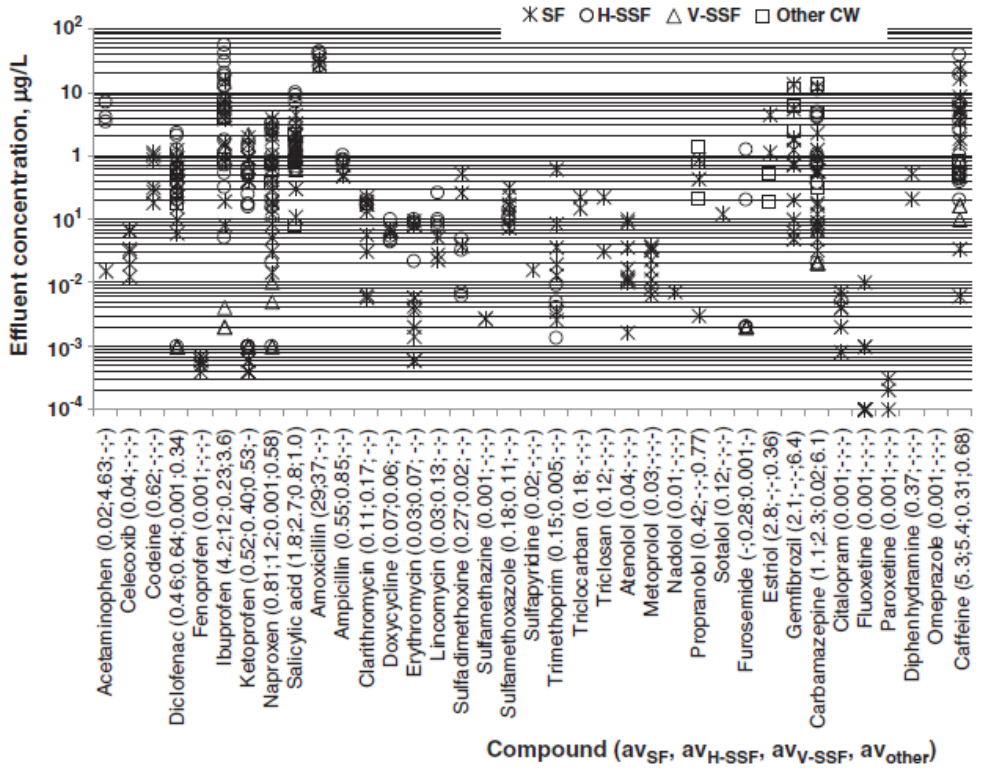


Fig. 4.5: Reported effluent concentrations of PhCs in CWs acting as a secondary step.

Fig. 4.6 reports all collated data regarding removal of the 29 investigated compounds, grouped according to their therapeutic class, in all types of secondary CWs and the main issues raised are discussed below. Removal data are available for 26 compounds in SF systems, 15 in H-SSF beds, 6 in V-SSF beds and 7 in other non-conventional CWs. A wide range of variability was observed for the majority, in particular diclofenac, ibuprofen, ketoprofen, naproxen, salicylic acid, carbamazepine and caffeine. Investigations into the removal of ibuprofen, naproxen, salicylic acid,

carbamazepine and caffeine have been conducted in all the four types of CW. Limiting the analysis to the three known CWs, it is possible to seven compounds – the abovementioned five and diclofenac and ketoprofen. Collated, the data reveal that:

- Diclofenac, ibuprofen and naproxen exhibit the highest average removal values in V-SSF beds (63, 85 and 84 % respectively), while in SF and H-SSF systems they had lower but similar average removal efficiencies: 28-35 % for diclofenac, 52-60 % for ibuprofen and 51-57 % for naproxen in both systems.

- Salicylic acid had a higher average removal in V-SSF beds (88 %), and lower but similar average values in SF and H-SSF systems (79 %). Removal ranges in SF and H-SSF systems were also very similar (78–79 %), in marked contrast to the discrepancy found for the other three analgesics and anti-inflammatory drugs mentioned in the previous point.

- Carbamazepine had quite similar (but low) removal efficiencies in all three types of systems, although the values observed in the SF basin were slightly higher (36 % vs. 23–25 %).

- Caffeine was more efficiently removed by SSF beds (in vertical flow type 89 % and in horizontal 84 %), while in SF systems its removal was, on average, 62 %.

- Average removal efficiencies higher than 75 % were reported for salicylic acid, sulfadimethoxine and nadolol in SF systems; salicylic acid, sulfadimethoxine, trimethoprim and caffeine in H-SSF beds; and ibuprofen, naproxen, salicylic acid and caffeine in V-SSF beds.

- Poor removal (< 25 %) was reported for clarithromycin, sulfamethoxazole, sulfapyridine, triclocarban, triclosan, sotalol, gemfibrozil and diphenhydramine in SF systems; amoxicillin and clofibric acid in H-SSF beds; and carbamazepine in V-SSF beds.

Two-stage secondary steps were investigated by Avila et al. (2010) and Hijosa-Valsero et al. (2011b), but data provided did not permit evaluation of the influence of each stage in the removal process. Release phenomena were reported for diclofenac, ibuprofen, ketoprofen, naproxen, carbamazepine, gemfibrozil and. The highest release values were found for naproxen and gemfibrozil (Conkle et al., 2008) in a SF system, and for naproxen in an H-SSF bed (Reyes-Contreras et al., 2012). Release phenomena and the forces behind them are analysed in the Discussion.

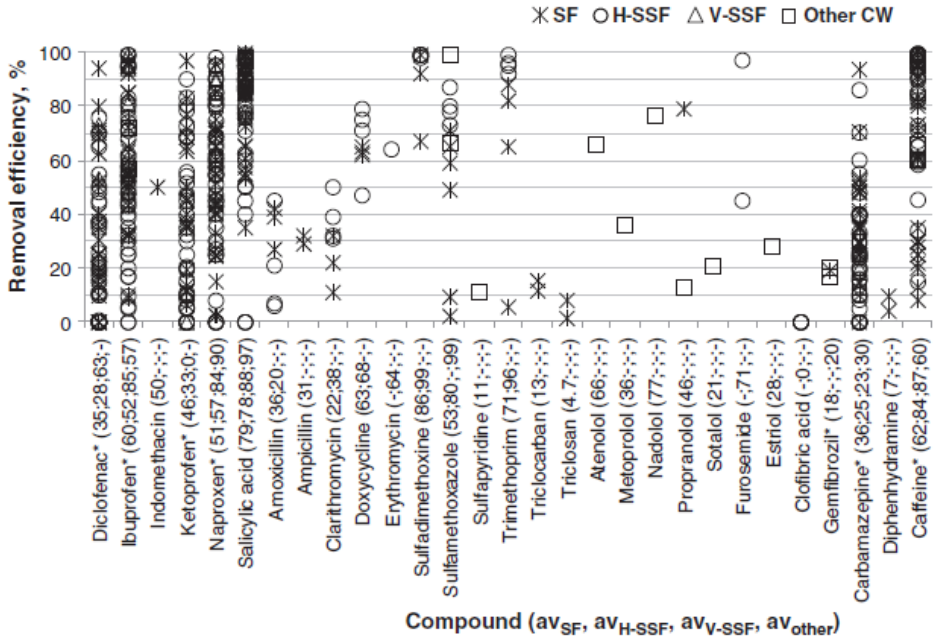


Fig. 4.6: Reported removal efficiencies of PhCs in CWs acting as a secondary step. *Negative removal observed for: Diclofenac:−1,−6, −12,−13, −14,−22, −24, 29,−49, −62,−77; Ibuprofen:−13,−46,−48,−105; Ketoprofen = −6,−31,−83,−80; Naproxen = −78,−103,−202; Carbamazepine:−6,−7,−9,−15,−16,−24,−40,−46,−49,−73,−164; Gemfibrozil: −26,−182; Caffeine: −14.

Tertiary treatments

Influent and effluent concentrations – Fig.4.7–4.9 report the concentrations of the 102 monitored compounds in the influent of tertiary steps, while Fig. 4.10–4.12 collate the concentrations of the 99 compounds monitored in their effluents. 96 compounds were detected in both influent and effluent, while 6 compounds were detected in the influent alone, namely the analgesic budesonide, the antiparkinson bromocriptine, the psychiatric drugs clomipramine, haloperidol and risperidone, and the receptor antagonist promethazine. In contrast, the antispasmodic dicycloverine and the psychiatric drugs fluphenazine and nefazodone were only detected in the effluent. Table SD-1 of Verlicchi and Zambello (2014) provides the average influent and effluent values found for each PhC in the 4 types of CWs. Most data are from SF systems and H-SSF beds, and only a limited number refer to V-SSF beds (investigations by Reif et al., 2011). The most commonly monitored compounds were ibuprofen (45 values in the influent and 37 in the effluent), naproxen (42 values in the influent and 35 in the effluent), diclofenac (40 influent and 32 effluent values), carbamazepine (38 influent and 30 effluent values) and then ketoprofen (35 influent and 27 effluent values). The reported influent and effluent concentrations of some compounds, namely ibuprofen, naproxen, triclosan, carbamazepine and caffeine, varied over a wide range. The highest values were reported for ibuprofen in both the influent and effluent (55.2 μg/L and 40 μg/L, respectively); these were documented in the injection experiments reported by Avila et al. (2013).

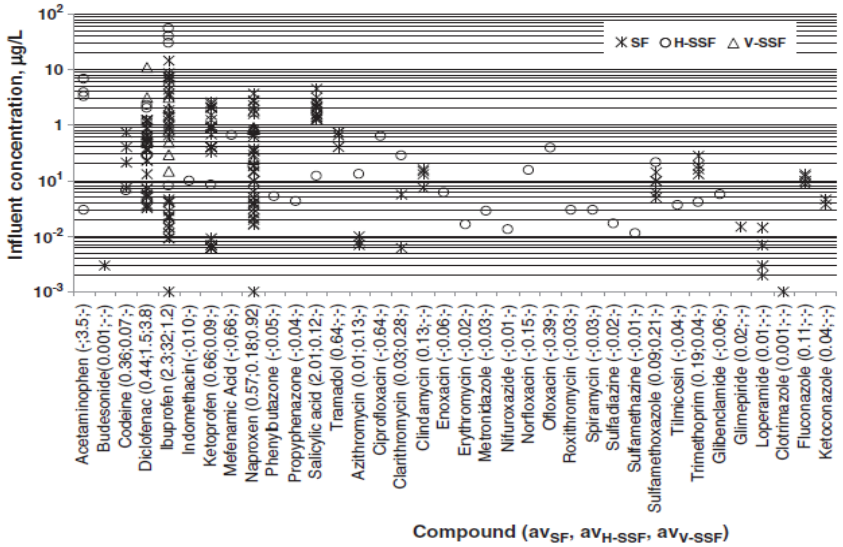


Fig. 4.7: Reported influent concentrations of selected PhCs in CWs acting as a tertiary step (1/3).

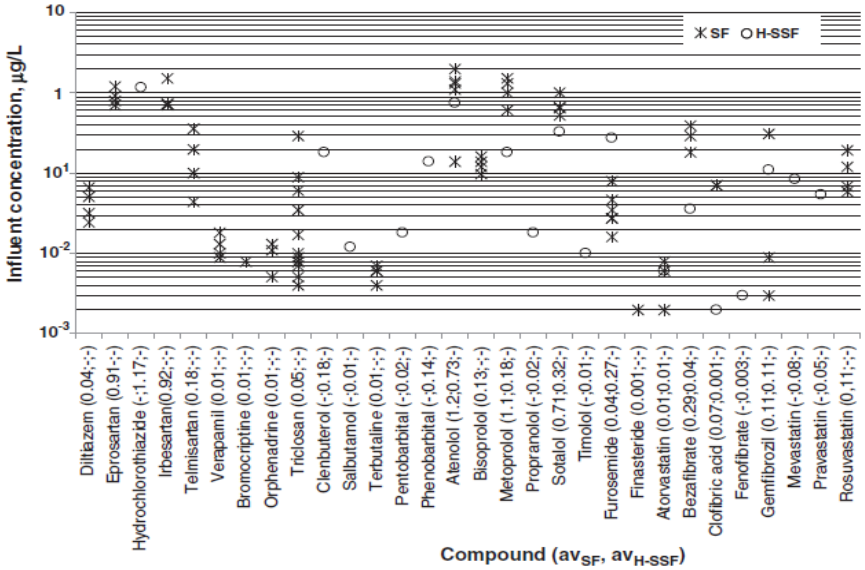


Fig. 4.8: Reported influent concentrations of selected PhCs in CWs acting as a tertiary step (2/3).

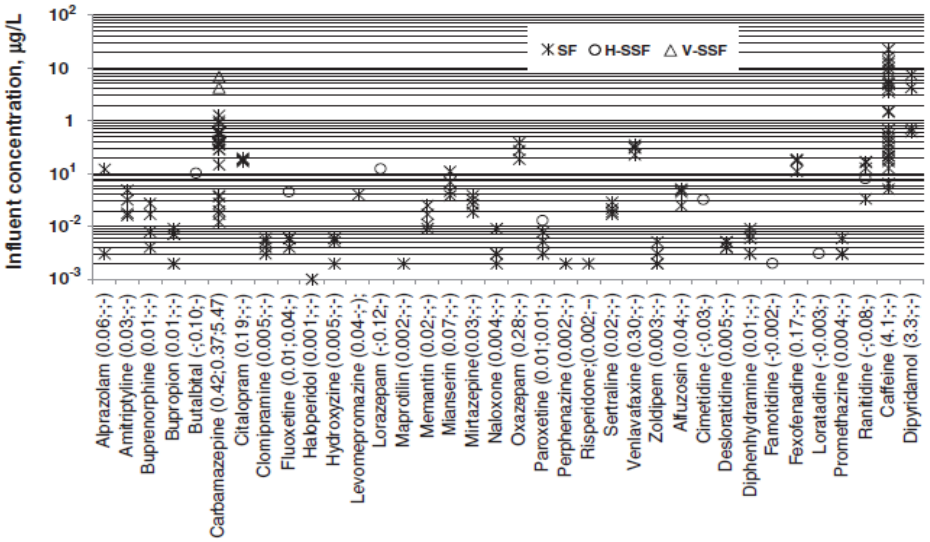


Fig. 4.9: Reported influent concentrations of selected PhCs in CWs acting as a tertiary step (3/3).

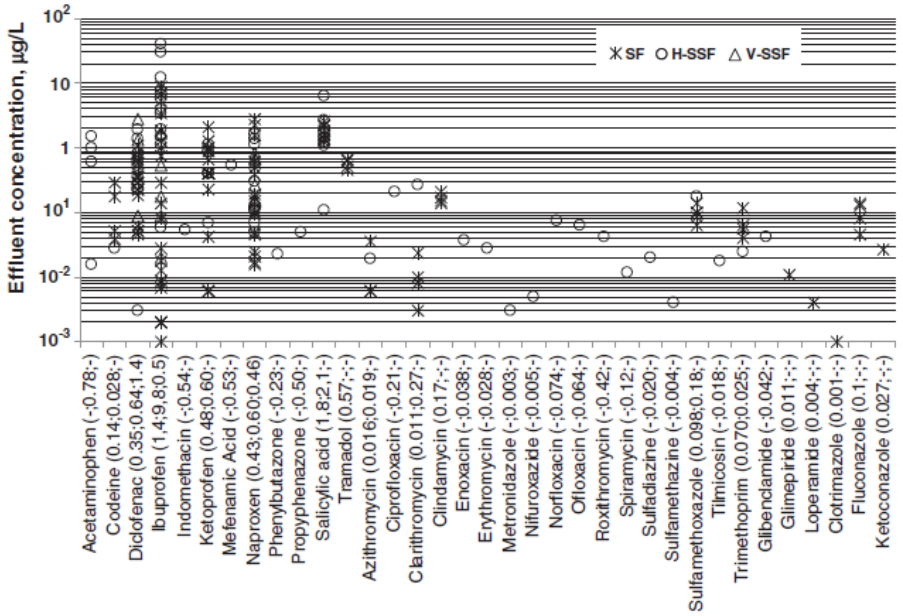


Fig. 4.10: Reported effluent concentrations of selected PhCs in CWs acting as tertiary steps (1/3).

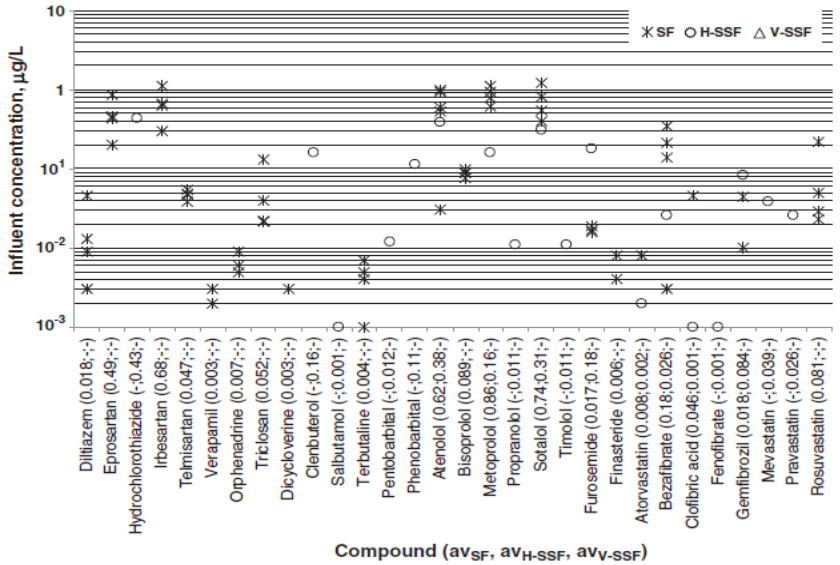


Fig. 4.11: Reported effluent concentrations of selected PhCs in CWs acting as a tertiary step (2/3).

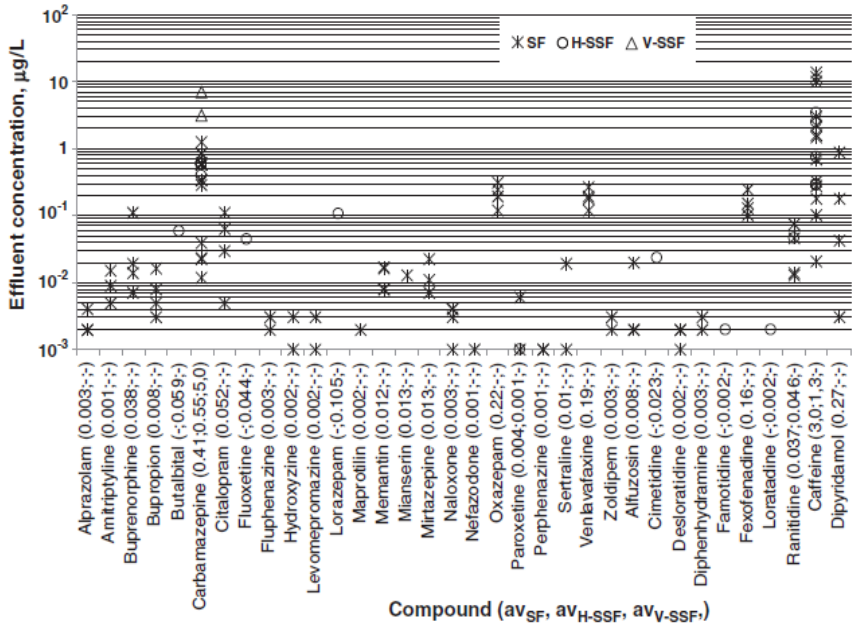


Fig. 4.12: Reported effluent concentration of selected PhCs in CWs acting as a tertiary step (3/3).

Figures 4.13–4.15 collate the removal efficiencies reported for 87 PhCs monitored in CWs acting as a tertiary step. Collated literature data refer to all four types of CWs. In particular, 59 compounds were monitored in SF systems, 50 in H-SSF beds, 4 in V-SSF beds and 7 in non-specified CWs. The only compounds monitored in all were diclofenac, ibuprofen, naproxen and carbamazepine. The collated data reveals that:

- SF basins exhibited the highest average removal for naproxen (52 % against 41% in non-conventional CWs, 42 % in V-SSF basins and 14 % in H-SSF beds).
- H-SSF beds removed carbamazepine more efficiently than other CW systems (60 % vs. 12 % in non-conventional CWs, 25 % in SF systems and 26 % in V-SSF beds).
- V-SSF beds removed more diclofenac than other CWs (79 %, vs. 69 % in CWs, 58 % in SF systems and 7.5 % in H-SSF beds).
- The non-conventional wetland investigated by Matamoros et al. (2012b) removed ibuprofen better than other CWs (76% vs. 69 in V-SSF beds, 60 % in SF basins and 48 % in H-SSF beds).

The highest average removal efficiencies (greater than 75 %) were found for:

- 8 compounds in SF systems: verapamil, furosemide, alfuzolin and ranitidine, mianserin, levomepromazine, dipyridamol and alprozolam.

- 11 compounds in H-SSF systems: metronidazole, nifuroxazide, sulfamethazine, salbutamol, atenolol, atorvastatin, fenofibrate, paroxetine, famotidine, azithromycin and ofloxacin,

- diclofenac in V-SSF beds

- Ibuprofen and ketoprofen in the non-conventional CW investigated by Matamoros et al. (2012b)

Very poor average removals (< 25 %) were found for:

- Azithromycin, clindamycin, perphenazine, sotalol, maprotilin, glimepiride, oxazepam, irbesartan, tramadol, sulfamethoxazole, atorvastatin, memantin, fexofenadine and metoprolol in SF systems

- Sotalol, clarithromycin, diclofenac, ketoprofen, clenbuterol, metoprolol, lorazepam, salicylic acid, naproxene, caffeine, sulfamethoxazole, phenobarbital, mefenamic acid, gemfibrozil, glibenclamide and cimetidine in H-SSF beds

- Carbamazepine in the non-conventional CW investigated by Matamoros et al. (2012b).

As reported in Figures 4.13–4.15, release phenomena occurred for several compounds. Negative removal values are reported below each Figure caption. Factors implicated in these phenomena are discussed in the Discussion subsection: PhC release phenomena during treatment.

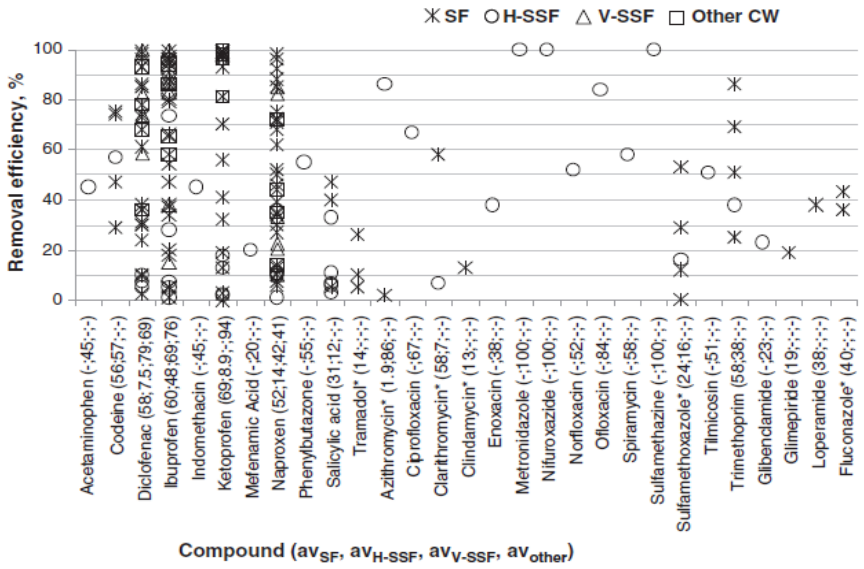


Fig. 4.13: Reported removal efficiencies of selected PhCs in CWs acting as a tertiary step (1/3).
*Negative removal values for: propyphenazone: −19; tramadol: 12; azithromycin: −350; clarithromycin:−58; clindamycin:−27,−62,−103; sulfamethoxazole:−2,−7,−104; erythromycin:−78; roxithromycin:−43; sulfadiazine:−17; clotrimazole:−18; fluconazole: −8; −40.

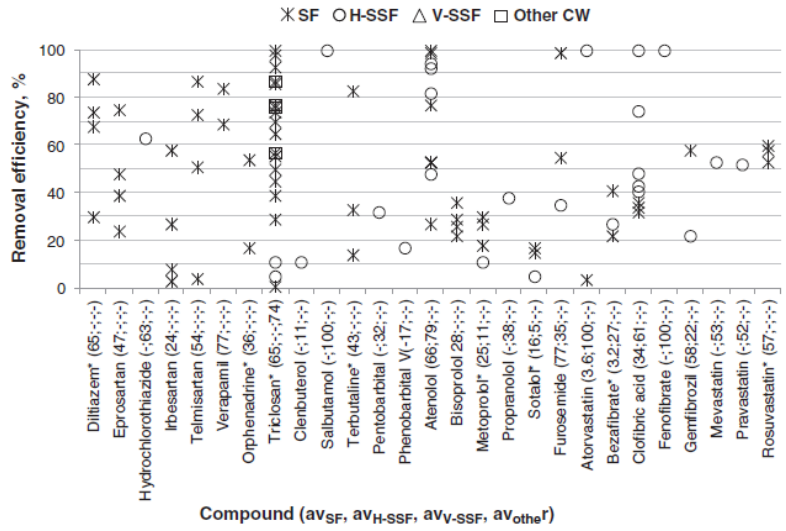


Fig. 4.14: Reported removal efficiencies of selected PhCs in CWs acting as a tertiary step (2/3).
*Negative removal values for: diltiazem:−5; orphenadrine:−19; triclosan:−6; terbutaline:−11; finasteride:−141,−394; metoprolol:−3; sotalol: −18,−21; timolol:−8 bezafibrate:−21; rosuvastatin: −16.

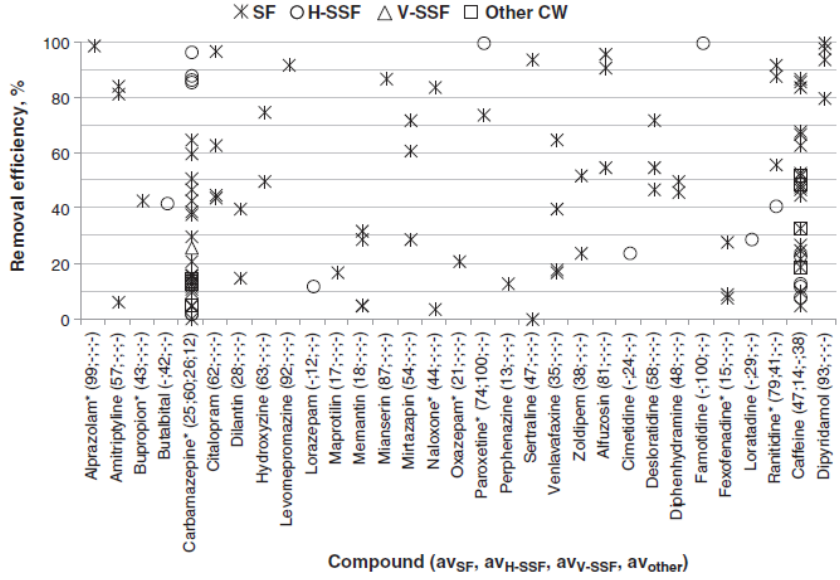


Fig. 4.15: Reported removal efficiencies of selected PhCs in CWs acting as a tertiary step (3/3).

*Negative removal values for: alprazolam: −12, −130; bupropion: −23, −81,135; carbamazepine:−1,−4,−6,−19; diazepam: −6; fluoxetine: −1; naloxone:−28, −116; oxazepam: −26; paroxetine:−83 fexofenadine: −26, ranitidine: −39.

Lessons learned from multi-stage tertiary systems -The treatment lines included in this review featured different types of multi-stage tertiary steps (Table SD-3 of Verlicchi and Zambello (2014)): Froehner et al. (2011), Matamoros and Salvado (2012), Matamoros et al. (2010, 2012b) and Park et al., (2009) analysed parallel and/or in-series surface flow systems, while Hijosa-Valsero et al. (2010a) and Reyes-Contreras et al. (2011) investigated the polytypic multi-stage sequence SF + H-SSF. Although thirteen compounds were monitored overall, namely: diclofenac, ibuprofen, ketoprofen, naproxen, salicylic acid, sulfamethoxazole, triclosan, atenolol, furosemide, carbamazepine, diazepam, dilantin and caffeine, the types of data collected varied. Indeed, only Hijosa-Valsero et al. (2010a) reported the influent and effluent concentrations in addition to the removal efficiencies for each stage of the two investigated treatment lines, while other studies only reported removal efficiencies for each stage (Park et al., 2009 and Reyes-Contreras et al., 2011). Several limited themselves to providing overall removal efficiencies for the whole tertiary step (Matamoros and Salvado 2012 and Matamoros et al., 2010, 2012b), rather than for each of its component parts, and Froehner et al. (2011) merely reported the removal efficiency of the entire treatment line, comprising primary, secondary and tertiary steps. The highest removal efficiencies (> 99 %) for most investigated compounds were found in the SF-system series described by Matamoros et al. (2010) and Matamoros and Salvado (2012). The sequence SF + H-SSF, investigated by Hijosa-Valsero (2010a) and Reyes-Contreras et al. (2011), exhibited lower removal efficiencies than the series of SF basins investigated by the other research groups for all monitored compounds, namely diclofenac, ibuprofen, ketoprofen, naproxen, salicylic acid, triclosan, carbamazepine and caffeine. Reyes-Contreras et al. (2011) and Hijosa-Valsero et al. (2010a) confirmed previous observations by Conkle et al. (2008) that most removal takes place during the first stage, regardless of its nature, and that the extent of removal depends on the concentration of the pollutant in question. Some exceptions were observed for caffeine, naproxen and ibuprofen, whose removals were higher in the second stage. Another factor affecting removal mechanisms was lower temperatures, whose influence was more evident in surface flow systems than in H-SSF beds, as noted by Reyes-Contreras et al. (2011) for ketoprofen, triclosan, ibuprofen and carbamazepine.

Hydroponic gravel beds

Zhang et al. (2013a, b) investigated lab-scale hydroponic gravel beds laid in 4 L vessels and planted with 4 exemplars of Scirpus validus. These beds were fed by a modified Hoagland nutrient solution spiked with naproxen and carbamazepine (Zhang et al., 2013a) or caffeine (Zhang et al., 2013b) at concentrations of 0.5, 1.0 and 2.0 mg/L. The nutrient solution was left in the vessels and PhC concentrations were measured at 3, 7, 14 and 21 days. Removal from the liquid phase and uptake by aboveground and underground parts of the plants were investigated as a function of exposure time, revealing that the highest removal values invariable corresponded to the highest initial PhC concentration (equal to 2.0 mg/L). The observed removal ranges were: for naproxen, between 84 and 92 % after three days, and between 97 and 99 % after 21 days; and for carbamazepine, between 53 and 60 % after 3 days and 64–74 % after 21 days; while between 52 and 93 % of caffeine was removed after 3 days of exposure, and over 99.7 % at 14 days and over. However, it is important to note that these investigations were conducted on extremely high PhC concentrations that are not found in real municipal wastewater.

Hybrid systems

Fig. 4.16 reports the overall removal efficiencies of each hybrid sequence investigated (Table SD-3), grouped according to the step sequence (I+II; I+II+III; II+III). Only three compounds were investigated in all three types of step sequence, namely ibuprofen, naproxen and caffeine. 99 % removal of ibuprofen was observed in the I+II lines (including only SF systems) investigated by Conkle et al. (2008) and Li et al. (2013), and in the I+II+III line (including only SF systems) studied by Hijosa-Valsero et al. (2010a). Up to 99 % of naproxen was also removed by the I+II-hybrid system assessed by Li et al. (2013), a slightly lower figure (85–90 %) was reported by Ying et al. (2009) for their I+II+III system (a series of SF systems), and 73–82 % was removed by the II+III system documented by Hijosa-Valsero et al. (2010a) (all SF systems). In all the hybrid systems bar

one, more than 95 % of caffeine was removed. The exception was the I+II+III line investigated by Hijosa-Valsero et al. (2010a), where it was approximately 83 %.

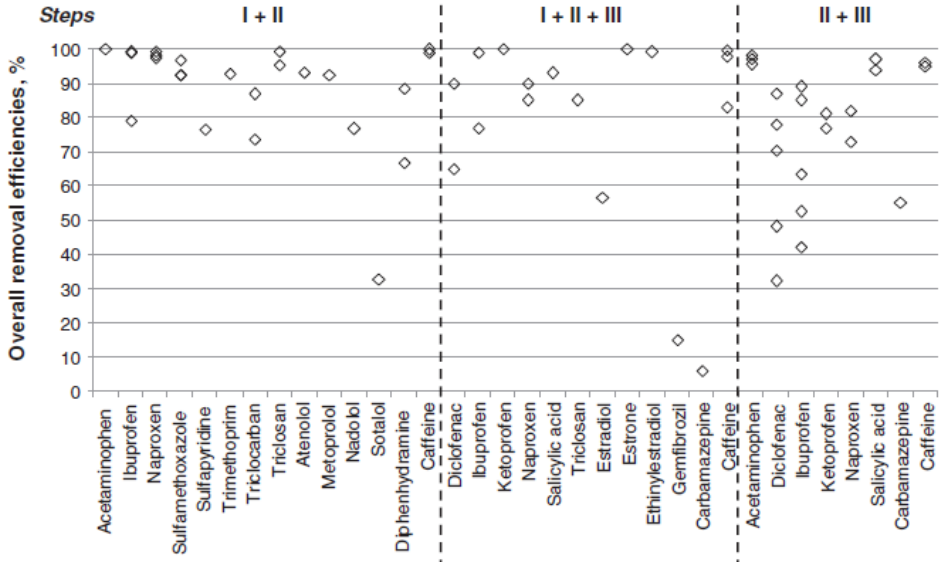


Fig. 4.16: Overall removal efficiencies observed in the different kinds of hybrid systems under review (I + II, I + II + III, II + III).

It is worth noting that the most commonly investigated hybrid systems were series of SF basins, and this type of hybrid system provided the highest removal of the monitored compounds.

Restoration wetlands

Reports of three different restoration wetlands were considered in order to evaluate the attenuation of selected PhCs during river transport and their passage through a constructed wetland. They detailed:

- The Prado wetland in California, USA, fed by different rivers whose flow is mainly due to WWTP effluents (Gross et al., 2004);

- The Aarslev restoration wetland near Aarhus in Denmark, which receives flow from two rivers, impacted by urban and agricultural runoff (Matamoros et al., 2012a);

- A pilot CW in Mexico fed by the Sordo River, which receives untreated urban sewage and industrial wastewaters (Navarro et al., 2011).

Further details of these systems are reported in Table 4.3.

Overall, 9 PhCs were investigated in one or more of the three wetlands, namely: ibuprofen, naproxen, ketoprofen and carbamazepine (Prado and Aarslev), gemfibrozil (Prado), triclosan, diclofenac and furosemide (Aarslev), caffeine (Aarslev and Mexico). All three investigations found that attenuation efficiency is compound-dependent, ranging from zero to 84 % at Aarslev, zero to 58 % at Prado, and up to 97 % in Mexico. According to Navarro et al. (2011), the most significant factors affecting pollutant removal are their conductivity and initial concentration. However, the presence of plants and a porous substrate did have a positive influence on the removal of emerging contaminants, leading all of these authors to conclude that a restoration wetland represents a good means of improving water quality in polluted rivers. They also emphasized that reducing the abundance of emerging pollutants should be considered a priority if the good chemical and ecological status of natural surface waters is to be restored.

4.8.2 Occurrence in sediment and gravel

Suspended particles from wastewater are retained in a wetland bed, in which sorption of dissolved contaminants in organic matter and on the biofilm coating the grains in the bed can be significant removal mechanisms (Matamoros et al., 2005). As most of the organic matter is retained in the first meters of the bed, substrate samples were generally taken near the inlet (Matamoros et al., 2005, Zarate et al., 2012).

Adsorption of a compound onto a solid matrix (that is gravel, soils or sediments) depends on its chemical nature and is correlated to Kow and Kd (see "rules of thumb" in Table SD-3), pH, ionic strength and the presence of cations in the medium. Table 4.4 reports the concentrations of selected PhCs investigated in gravel samples (Matamoros et al., 2005, 2006, 2007b, Matamoros and Bayona, 2006), sediments (Zarate et al., 2012) and pond soil (Park et al., 2009). Conkle et al. (2010) also remarked upon sorption in wetland soil, highlighting its importance as a removal pathway for ciprofloxacin, ofloxacin and norfloxacin (fluoroquinolone antibiotics), and in mitigating any downstream release during wastewater treatment.

The mean values reported only provide a rough estimate of PhC retention by the filling medium, and need to be interpreted with great caution, as they depend on many factors, including influent PhC concentration, operational period, pH, presence of other sorbable PhC competitors. An in depth analysis of the mechanisms involved in sorption and the factors that influence them has been discussed in depth by Dordio and Carvalho (2013).

Table 4.4: Average concentrations (μg/kg) of selected PhCs in gravel, sediment and soil samples, and corresponding literature reference. Matamoros et al. (2005): inlet samples of gravel filling an H-SSF bed planted with Ph. australis. Matamoros and Bayona (2006): inlet samples of gravel filling an H-SSF bed planted with Ph. australis. Matamoros et al. (2007b): samples of gravel in an H-SSF bed. Zarate et al. (2012): concentrations in sediment samples at the inlet/outlet of an SF system. Park et al. (2009): samples of soil in SF systems planted with Acorus or Typha

Compound	Average values [μg/kg]	Reference
Diclofenac	Gravel: <0.3	Matamoros and Bayona (2006)
	Soil: <2.5	Park et al. (2009)
Ibuprofen	Gravel: 15	Matamoros et al. (2005)
	Gravel: 6.5	Matamoros and Bayona (2006)
Ketoprofen	Gravel: <1.2	Matamoros and Bayona (2006)
Naproxen	Gravel: <0.26	Matamoros and Bayona (2006)
	Soil: <5	Park et al. (2009)
Salicylic acid	Gravel: b0.18	Matamoros and Bayona (2006)
	Soil: <5	Park et al. (2009)
Sulfamethoxazole	Soil: <2.5	Park et al. (2009)
Triclocarban	Sediments (in/out): 17/50	Zarate et al. (2012)
Triclosan	Sediments (in/out): 12/29	Zarate et al. (2012)
	Soil: 0.07; <10	Park et al. (2009)
Atenolol	Soil: 2.5	Park et al. (2009)
Clofibric acid	Gravel: 14	Matamoros et al. (2005)
	Gravel: <0.12:	Matamoros et al. (2007b)
Carbamazepine	Gravel: 97	Matamoros et al. (2005)
	Soil: 0.03; 0.5; 0.06; <2.5	Park et al. (2009)
Dilantin	Soil: <10	Park et al. (2009)
Caffeine	Gravel: 69	Matamoros and Bayona (2006)

4.8.3 Contribution of plants in removal of pharmaceuticals

Plants may contribute to the removal of micropollutants in different ways, either by directly taking up and assimilating contaminants, or by creating conditions favourable for their removal within the systems. The latter involves acting as suitable surface for biofilm anchorage, promoting the development and growth of different microbial species within the systems, secreting exudates, pumping and releasing oxygen at the bottom, retaining suspended solids particles and insulating against low temperature. That being said, data on tissue concentrations of PhCs in common plants used in CWs are sparse and somewhat varied, as shown in Table SD-3 of Verlicchi and Zambello (2014). The most commonly used and investigated species are: Phragmites australis and Typha, followed by Acorus, Gliceria, Scirpus, Lemna and Carex. A limited number of PhCs have been investigated in stems, leaves and roots of emerging species. Zarate et al. (2012), for example, investigated the presence of triclosan and triclocarban in the tissues of the macrophytes Typha,

Pontederia cordata and Sagittaria graminea, radicated in an SF system. They showed that PhC accumulation is consistently greater in roots than in shoots, is species-specific, and declines from the inflow to the outflow of the basin. Zhang et al. (2013a) used a lab-scale vessel to investigate the uptake of naproxen and carbamazepine from a nutrient solution by Scirpus validus, also showing that these compounds accumulate in the roots rather than the shoots, but that such uptake is not the predominant removal mechanism for such compounds. In stark contrast, Zhang et al. (2013b) found that caffeine can be efficiently eliminated from solution by Scirpus validus grown hydroponically, and the main mechanism behind the removal of this compound does seem to be assimilation by plants.

In this case, however, shoot concentrations were found to be far higher than those in the roots; after 14 days of exposure, the shoot concentration was 13 μg/g and root concentration was 0.3 μg/g. These authors concluded that for highly polar and water soluble organic compounds like caffeine, direct uptake, accumulation and translocation in plant tissues can be very useful means of phytoremediation.

4.9 Discussion

4.9.1 Removal: mechanisms, modelling and influence of main CW design parameters

Most studies provide an explanation for the behaviour of their selected compounds in the system investigated, attempting to pinpoint and analyze the main removal mechanisms that could be occurring within. An extremely thorough analysis of all possible mechanisms behind micropollutant removal, particularly PhCs, in CWswas made by Imfeld et al., (2009). They describe all the destructive (phytodegradation and microbial degradation) and non-destructive processes (volatilization and phytovolatilization) that affect organic pollutants within CW systems, analysing in depth the redox processes occurring at constructed-wetland-system scale and processes at the rhizosphere scale. The complex behaviour of such systems when removing organic chemicals from the water phase is illustrated, and the authors emphasize the difficulty in predicting the fate of a specific compound within a CW, as this will be influenced by many different factors (CW design characteristics, operational and environmental conditions, nature of influent wastewater, vegetation, chemical properties of the compounds, T, insolation, and so on). Here we discuss the main factors affecting the performance of SF and SSF systems, and how they influence the major findings of the investigations under review.

Influence of PhC chemical structure - No relationship between PhC chemical structure and functional groups and removal efficiency has been observed. Camacho-Munoz et al. (2012) remarked that even though ibuprofen and ketoprofen have quite similar chemical structures, ibuprofen is more efficiently removed from wastewater. One possible explanation for this was offered by Matamoros et al. (2009), who pointed out that the recalcitrance to biodegradation of PhCs can be ascribed to specific groups within the molecule, for instance the presence of two extended aromatic rings (as in the ketoprofen molecule) or the chlorine atom (as in diclofenac).

Influence of PhC physical-chemical properties – There have been several attempts to define rules of thumb for predicting the potential behaviour of a compound during its passage through a treatment plant. An in depth discussion regarding prediction of the potential sorption of PhCs onto solids and sludge and/or their degradation in different treatments from their physical chemical properties, in particular pK_a, Log K_{ow} , Log D, and Log K_d is provided in Verlicchi et al. (2013). Some of these rules are reported here in Table 4.5, but unfortunately have not been confirmed by the specific experimental investigations. In fact, Breitholtz et al. (2012), Lee et al. (2011), Park et al. (2009), Verlicchi et al. (2013) and Zhang et al. (2012b,c) have all tried to determine whether there is a correlation between removal and hydrophobic characteristics by plotting observed removal efficiencies vs. Log K_{ow} for a large number of PhCs, but none of them found any evident relationship. Park et al. (2009) also tried to correlate removal values observed in SF systems with the corresponding pK_a of selected PhCs to determine the effect of electrostatic interactions, but found no distinct relationship.

Lee et al. (2011), on the other hand, found an inversely proportional relationship between removal efficiency and octanol-water partition coefficients (Log K_{ow}), after taking into account the presence or absence of ionizable functional groups (log D_{ow}). This implies that compounds with greater hydrophilicity (for example atenolol and naproxen) could be more efficiently removed by CWs than hydrophobic compounds (including carbamazepine). Dordio et al. (2010) postulated that the moderate lipophilicity (corresponding to 0<Log K_{ow} <3) of carbamazepine, clofibric acid and ibuprofen was behind their plant uptake, as it enables them to easily pass through cell membranes and enter the plant's transpiration stream. It is also likely that an increase in the amounts of oxygen released by the plant's roots into the rhizosphere favours the occurrence of aerobic biodegradation processes, which are more efficient than anaerobic ones. This may also be responsible for enhancing the removal efficiencies of the most biodegradable pharmaceuticals.

Table 4.5: Rules of thumb for predicting potential behavior of pharmaceuticals during treatments

Parameter	Conditions	Rule of thumb	Reference
pK_a	2-12	Low acidity,	
pK_a	< 2	High acidity,	
Log K_{ow}	< 2.5	Low sorption	Rogers, 1996
Log K_{ow}	> 4	High sorption	Rogers, 1996
Log D_{ow}	< 1	Low sorption	Cunningham, 2008
Log D_{ow}	>3	High sorption	Cunningham, 2008
K_d	> 500 L/kg	High sorption	Ternes and Joss, 2006
Log K_d	> 2.67		
K_d	< 500 L/kg	Low sorption	Ternes and Joss, 2006
Log K_d	< 2.67		

PhC removal modelling in CWs and kinetics studies – As shown in Table SD-3 of Verlicchi and Zambello (2014), many authors have investigated the kinetics of selected compounds in CWs. For the most commonly investigated PhCs, removal processes follow a kinetic behaviour that fits first-order equations well in the period 6-96 h (Dordio et al., 2010, Matamoros et al., 2012b, Ranieri et al., 2011, Zhang et al., 2012b). In particular, studying H-SSF beds, Zhang et al. (2012b,c) found that the values of the area-based decay constant (k) were within the range 0.01-0.1 m/d. Higher values for this constant rate have also been found in planted beds than in unplanted ones (Dordio et al., 2010 and Zhang et al., 2012b), confirming that plants contribute to the biodegradation of micropollutants in CWs. This may also explain the higher removal rates seen in the summer, when a more active vegetation stage, higher plant transpiration rates, higher microbiological activities and faster physical sorption may all increase removal (Dordio et al., 2009c, 2010, Matamoros et al., 2012b)

The k-values for most of the compounds tested under batch operation mode were found to be higher than under continuous mode (Zhang et al., 2012b). Additionally, k was found to increase as influent PhC concentrations of the increases (Avila et al., 2013). Accordingly, the higher the HRT, the greater the removal efficiency. In general, in CWs the half-lives of the different PhCs varied between 0.6 and 42 days (Matamoros et al., 2012b and Ranieri et al., 2011). Interestingly, Matamoros and Bayona (2006) found that ibuprofen and caffeine removal had comparable kinetics to the biodegradation of organic matter. Indeed, for the PhCs they investigated, the values of the areal kinetic constant were found in the range 0.04-0.21 m/d, and in the range 0.06-1.0 m/d for BOD_5.

Influence of vegetation – The relative importance of the presence of plants and the ability of particular species to improve the removal efficiency of PhCs in CWs is still unclear. This is because many other factors, namely their related microorganism communities and the nature of the wastewater, as well as environmental and operational conditions may all be acting in concert. Indeed, Hijosa-Valsero et al. (2011a) found that root-related biofilm, plant exudates and microenvironment modifications near plant tissues could play a role in the removal of tetracycline. Nevertheless, Hijosa-Valsero et al. (2011b) also found that despite the greater evapotranspiration water losses in planted CWs, the effluent concentration of PhCs are lower in planted than in unplanted systems during summer time. Indeed, the presence of plants improves the degradation of naproxen, ibuprofen, diclofenac and caffeine, with Phragmites australis displaying better

performance than Typha, at least in the summer months (Hijosa-Valsero, 2010b). However, unvegetated SF systems exhibited higher removal of clarithromycin and trimethoprim (Hijosa-Valsero et al., 2011a) than vegetated ones, presumably mainly due to more direct insolation and higher concentrations of Chlorophyta algae in the former. That being said, the same unvegetated SF basins displayed lower amoxicillin removal than their planted equivalents, but significantly lower effluent concentrations of erythromycin and trimethoprim were found in the planted, with respect to the unplanted, H-SSF bed.

Various authors have postulated that more developed aerial and underground parts of plant species can improve the removal of PhC in a CW. Indeed, a slight difference in acetaminophen removal between two H-SSF beds planted with Phragmites australis and Typha has been documented (Ranieri et al., 2011), although this difference was not statistically significant at the 95 % confidence level. However, Phragmites does have a larger rhizome volume, providing a greater specific surface for biodegradation process and potentially a greater biofilm. In contrast, Dordio et al. (2009c) found that atenolol was better removed in the presence of Typha rather than Phragmites in two similar H-SSF beds, although this could be ascribed to the fact that the aerial parts of Typha were slightly better developed than those of Phragmites. That being said, Reyes-Contreras et al. (2012) found that young systems are more efficient if they are vegetated, but this advantage is lost when they get older, as clogging or shading phenomena may reduce performance.

Evaporation and evapotranspiration – Some studies have investigated the amount of water loss during their campaigns, finding that evaporation and evapotranspiration may greatly influence the results. To counteract this influence, some authors (for instance Dordio et al., 2010) checked evapotranspiration in the beds every day, restoring the lost water volumes with distilled water, while others take these losses into account when evaluating the removal efficiencies of the selected compounds (Avila et al., 2010) Table 4.6 shows water loss values for the types of CW investigated, expressed as percentage or flow rates (mL/d), alongside the corresponding reference.

In unplanted beds, the rate of evaporation is mainly dependent on the temperature and relative air humidity and is therefore highest in the summer season. In planted beds this water loss is greatly increased by plant transpiration, and evapotranspiration rates depend heavily on the type of the plants and their vegetative stage. Indeed, greater plant size and their more intense activity during the summer also contribute to the increased evapotranspiration rate of microcosm wetlands in this season.

Table 4.6: Water losses (% or mL/d) in different CWs by season and corresponding references.

CW type	Vegetation type	Water losses (period)	Units of measure	References
SF	Floating macrophytes	16 (winter); 29 (summer)	%	Hijosa-Valsero et al. (2010b)
		54 (summer)	%	Li et al. (2013)
	Planted macrophytes	15–19 (winter); 25 (summer)	%	Hijosa-Valsero et al. (2010b)
	Unplanted	9 (winter); 6 (summer)	%	Hijosa-Valsero et al. (2010b)
H-SSF	Planted macrophytes	13 (winter); 28 (summer)	%	Hijosa-Valsero et al. (2010b)
		1–2 (winter)	%	Verlicchi et al. (2013b)
		50 (winter); 550 (summer)	mL/d	Dordio et al. (2010)
		16–24	%	Ranieri et al. (2011)
	Unplanted	3 (winter); 6 (summer)	%	Hijosa-Valsero et al. (2010b)
		35–45 (summer)	%	Avila et al. (2010)
		45 (winter); 110 (summer)	mL/d	Dordio et al. (2010)

Extensive evaporation in CW systems decreases outflow volume and concentrates effluent (as documented by Li et al., 2013b for gemfibrozil, carbamazepine, naproxen in the effluents in SF systems), but also increases retention time. This provides longer times for the PhCs to interact with the wetland ecosystem, increasing the removal rate of specific compounds (Kadlec and Wallace, 2009). Clearly, therefore, evaporation and evapotranspiration rates should be accurately estimated and carefully considered.

Clogging and flooding – Clogging phenomena are more likely to occur on the surface of (H- and V-) SSF beds following flooding. This will inevitably reduce redox potential, due to the rapid consumption of the oxygen trapped in the medium or present in water by microbes. Indeed, during flooding periods, Navarro et al. (2011) registered a reduction in redox potential from 300 to 120 mV in their H-SSF systems, which corresponded to a drop in caffeine removal efficiency. Matamoros and Bayona (2006) also noted lower removal efficiencies for ibuprofen and naproxen, as well as caffeine (all readily biodegraded), during flooding, which they ascribed to a reduction in bed residence time and hence the biological processes responsible for (ibuprofen) degradation.

Filling materials – The H-SSF and V-SSF beds under review were mainly filled with gravel (Avila et al., 2010, 2013; Reif et al., 2011, Reyes-Contreras et al., 2011, Hijosa-Valsero e al., 2011c; Matamoros et al., 2008a; Matamoros and Bayona 2006; Verlicchi et al., 2013), and, in some cases, LECA (light expanded clay aggregate). Dordio et al., in particular, investigated the influence of LECA beds on atenolol (2009c) and ibuprofen, carbamazepine and clofibric acid (2010) removal. Gravel media generally had a particle size of 8–15 mm, and porosity 30-40 %. The effective particle size d10 and uniformity coefficient U = d60/d10 are provided in a few studies, including those by Matamoros et al. (2005) (d_{10} = 2 mm and U= 1.7) and Dordio et al. (2010) (d10 = 1.22; 1.4, 3 and U = 2.21; 2.36; 1.32). An in-depth analysis of the role of the support matrix on the removal of xenobiotics (not only pharmaceuticals, but also personal care products, polycyclic aromatic compounds, pesticides and flame-retardants, etc.) is provided in a recent review by Dordio and Carvalho (2013). They compare the removal observed, generally in lab experiments with synthetic wastewater (tap water spiked with a few compounds, not always at realistic conditions) when using traditional materials such as sand and gravel, with less common media such as LECA or activated carbons, and more recent innovations like kaolinite, diatomite, cork, perlite and zeolites.

We limited our focus to the performance of the most common filling media (mainly gravel, followed by LECA), making only brief reference to other substrates. Indeed, in many of the studies reviewed by Dordio and Carvalho (2013), the tested PhC concentrations were extremely high and not consistent with values observed in untreated or (partially) treated wastewater. We therefore give more weight to the findings of investigations performed using real or spiked wastewater, with PhC concentrations within the typical range, as the influent concentrations and the matrix effect are essential to defining the main mechanisms responsible for PhC removal.

According to recent studies, PhCs generally behave as follows:

- Non-polar organic pollutants can be preferentially adsorbed via hydrophobic process to substrate materials especially rich in organic matter such as soil, compost and agricultural wastes. Polar or ionic pollutants are predominantly adsorbed to substrates (e.g., some kinds of clay) by electrostatic interactions or ionic exchange (Dordio and Carvalho, 2013).
- LECA is a good sorbent for acidic compounds such as clofibric acid and ibuprofen, as well as neutrals such as carbamazepine (Dordio et al., 2009b). Considering the alkaline nature of LECA, electrostatic interactions with acidic pharmaceuticals are likely to be responsible for the affinity of these compounds for LECA's surface. For the neutral compounds, van der Waals interactions may be more influential. That being said, Dordio et al. (2009a) found that LECA also strongly sorbed atenolol, an alkaline, positively charged molecule. This affinity cannot be explained by electrostatic interactions, alhough perhaps ion exchange may be responsible to some extent.
- High removal efficiencies (88 % in winter and 97 % in summer) were seen in a Typha microcosm (HRT = 7 d) featuring a matrix of expanded clay aggregates, materials known for their adsorption properties (Dordio et al., 2010).

Influence of primary treatment – Various primary treatments have been studied and included in this review. In particular, Hijosa-Valsero et al. (2011b, c) compared the performance of a primary clarifier with an anaerobic hydrolysis upflow sludge bed (HUSB). These systems produce effluents of differing redox potential and dissolved oxygen concentrations, which may affect processes occurring within the CWs. Specifically, the sequence fed with primary clarifier effluent exhibited slightly higher removal efficiencies for ketoprofen and ibuprofen than that fed by HUSB effluent.

Influence of loading mode – Avila et al., 2013 and Zhang et al. (2012a,b) observed a considerable enhancement in PhC removal, for ibuprofen, diclofenac and naproxen, when H-SSF CWs were fed in batch mode. This they ascribed to the higher redox status caused by alternating cycles of saturation and unsaturation. In contrast, the differences between batch and continuous loading noted by Hijosa-Valsero et al. (2012b) were very small.

Influence of HRT – As previously mentioned, HRT is a key parameter in PhC removal in CWs. According to Matamoros et al., (2008b, 2012b), the greater the HRT, the higher the removal efficiencies for most of the selected compounds, in particular hydrophobic compounds such as hormones (Froehner et al., 2011). Significant correlations ($p < 0.05$) were also found, by Zhang et al. (2012b), between HRT and the removal of caffeine, salicylic acid, ketoprofen and clofibric acid, and they reported Pearson correlation values of 0.99 for all 4 compounds.

Influence of fill depth in SSF beds – Matamoros et al. (2005) found that H-SSF depth is a key design parameter for the removal of PhCs. However, for fairly unrecalcitrant compounds such as ibuprofen, shallow SSF beds are more efficient, presumably due to the more oxidized conditions. More refractory PhCs such as clofibric acid or carbamazepine showed poor removal in those systems, however, in line with the limited removal observed in conventional WWTPs.

Influence of specific surface area (m^2/PE) – An interesting parameter to use in comparison between the performances of different CWs is the specific surface area defined as the ratio between the area of a single step and the number of PE corresponding to the applied hydraulic load (assuming a specific water consumption per person per day). Unfortunately, this parameter has only been evaluated in a few cases and data is difficult to interpret, as it all refers to the surface of the whole treatment line and not to the specific step under evaluation.

Influence of redox potential – The redox potentials observed in the different CWs are included in the description of the different types of CWs in Section 0. It seems that anoxic (−100 mV < RP <100 mV) and aerobic (RP > 100 mV) conditions favour the biodegradation of organic micropollutants through the promotion of biogeochemical reactions (Matamoros et al., 2008a). However, ibuprofen, naproxen, salicylic acid and caffeine were all better removed under aerobic conditions (Matamoros and Bayona, 2006; Hijosa-Valsero et al., 2010b). Schwarzenbach et al. (2003) stated that polyhalogenated compounds, such as diclofenac, can easily be degraded by reductive dehalogenation in anaerobic degradation. This was confirmed by Avila et al. (2010), who revealed very high removal efficiencies for diclofenac. Hormones (17-β-estradiol, <17-α-ethinylestradiol and estrone), on the other hand, can be degraded in both aerobic and anaerobic environments, as demonstrated in the series of anaerobic-facultative-aerobic ponds investigated by Froehner et al. (2011).

Influence of seasonal variability – High temperature generally promotes biodegradation of PhCs. This has been documented in many studies, including Matamoros et al. (2012b), Hijosa-Valsero et al. (2011b), and Reyes-Contreras et al. (2011). The latter in particular investigated the contribution of a two-stage polishing treatment, consisting of an SF followed by an H-SSF, in removing 7 PhCs (salicylic acid, caffeine, naproxen, triclosan, ketoprofen, ibuprofen and carbamazepine) in both winter and summer. They found that the SF system contributed to the removal of all the compounds, but this contribution was particularly marked in the hot season for ketoprofen, triclosan and carbamazepine.

In the winter, SF systems predominantly or completely freeze. The low temperature fewer hours of sunlight (blocking sunlight from the water column) result in a poor or lack of removal of some PhCs, by either microbial activity or photodegradation, until the spring melt, as noted in various studies. Among them Matamoros et al. (2008b) reported lower removal efficiencies in winter than in summer for naproxen (52 % vs. 92 %) and diclofenac (73 % vs. 96 %), and MacLeod and Wong (2010) detected higher concentrations of the antibiotics clarithromycin and erythromycin, the analgesics and anti-inflammatories codeine, diclofenac and naproxen, and the beta-blocker propranolol in the effluent of a lagoon system in winter with respect to the summer time. Likewise, Reyes-Contreras et al. (2012) found that salicylic acid and caffeine are more easily removed in summer than in winter. Seasonality mainly affects biodegradation processes, and to a lesser extent chemical-physical removal processes like adsorption. This is mainly controlled by interactions

governed by isotherms, in which low temperature can have a negative effect (Reyes-Contreras et al., 2011).

Influence of photodegradation – Direct photolysis and indirect photodegradation may be important processes for most PhCs, as they generally contain aromatic rings, heteroatoms and/or other functional groups that can either directly absorb solar radiation or react with the photogenerated transient species in water. Nonetheless, thus far there is no reliable rule of thumb for predicting the photodegradation behaviour of PhCs. An attempt to find one was made by Kim and Tanaka (2009), who observed that several PhCs bearing an amide group ($RCONR_2$) are not easily photolysed. However, high photodegradability has been observed for clarithromycin and diclofenac, which both contain amide groups. It follows, therefore, that the amide bond is not always the main site affected by UV energy during PhC photodegradation.

Nevertheless, Matamoros et al. (2012b) revealed that photodegradation is key in removing triclosan, ibuprofen and diclofenac in their thorough investigation into the effect of solar radiation on PhC removal. These authors compared two similar SF system sequences fed with the same influent, one of which was completely covered, and also found that diclofenac, ketoprofen and triclosan were removed at similar rates as the advanced oxidation processes such as ozone and photo-Fenton or high pressure driven membranes (nanofiltration and reverse osmosis) investigated by Kimura et al. (2009) and Rosal et al. (2010). Many authors (Llorens et al., 2009; Hijosa-Valsero et al., 2010b, Matamoros et al., 2008b, 2012b, Conkle et al., 2008) have concluded that the elimination of some recalcitrant compounds, including clofibric acid and carbamazepine (up to 34 % and 39 %), in SF systems has to be correlated to high HRT and exposure of the water to sunlight, as previously postulated by Andreozzi et al. (2003) and Doll and Frimmel (2003), Lin and Reinhard (2005), who cited photodegradation as a potential means by which PhCs are removed from aquatic environments. In particular, photodegradation has been ascribed an important role in the removal of sulfamethoxazole and sulfapyridine (Anderson et al., 2013). The authors of this study stated that when these compounds were detected, it may have been the result of light attenuation and shielding due to turbidity and dissolved organic carbon within their lagoon cells. However, Bonvin et al. (2013) observed a photolytic transformation of one of the metabolites of sulfamethoxazole back into the parent compound, indicating that this metabolite may serve as an environmental source of the drug. Zhang et al. (2013a, b) confirmed that photodegradation plays a minor role in the removal of caffeine and carbamazepine, in accordance with Andreozzi et al. (2003) and Doll and Frimmel (2003). They also ascribed it a major role for naproxen removal, supporting previous reports by Lin and Reinhard (2005). According to Yamamoto et al. (2009), the beta-blocker propranolol is removed by photodegradation, although mechanism had no effect whatsoever on the removal of atenolol. Nevertheless, photodegradation and biotransformation are typically the most influential processes for the attenuation of organic micropollutants in effluent-receiving waters. Therefore, optimization of conditions (i.e., using extended periods of aging in sewage lagoons or additional steps such as bioreactors or oxidation ditches) can affectively minimize or prevent environmental exposure to biologically active concentrations of these chemicals.

PhC release phenomena during treatment – As reported in previous sections, negative removal values have been observed for various compounds in different treatment steps. While in some substances this phenomenon is clearly ascribable either to the presence of deconjugates interfering with biological transformation of the deconjugated compounds or to the release of PhC sorbed onto the particulate dissolving after the biological treatment, in others further investigation is required.

It is, however, important to note that at the low concentrations detected for some PhCs in the influent and secondary effluent, instrumental errors may lead to apparent releases of the investigated substance rather than negligible removal during their passage through the treatment plant. Sampling variation may also contribute to apparent negative removal, as reported by Clara et al. (2005b), if the collection of effluent samples is not time-adjusted to account for long HRTs. Collecting composite samples over a period longer than plant HRT may improve the comparability between influent and effluent (Roberts and Thomas, 2006).

4.10 Occurrence and removal of pharmaceutical enantiomers

Very little literature data is available regarding enantiomers of pharmaceuticals or the enantiomer composition of chiral drugs released by CWs. As remarked by Fono and Sedlak (2005) and MacLeod et al. (2007), enantiomers of pharmaceuticals are affected identically by abiotic processes, but may be affected differently by biologically mediated processes in wastewater treatment. Fundamentally, such chemicals may also exhibit differential toxicity to aquatic life (Lopez-Serna et al., 2013). That being said, only a limited number of chiral drugs have been investigated to date. MacLeod and Wong (2010) studied atenolol, citalopram, fluoxetine, metoprolol, nadolol, pindolol, propranolol, salbutamol and sotalol in the effluent of an aerated lagoon treatment, and Hijosa-Valsero et al. (2010a) investigated ibuprofen in a full-scale hybrid pond CW. Different removal efficiencies were observed for the S- and R-enantiomers of most of these compounds, but little else is known. Hence it is crucial that new analytical methods to detect not only new PhCs, but also their metabolites and transformation products (Lopez-Serna et al., 2011), not to mention chiral drugs with enantiomer-specific toxicity to aquatic life, are soon developed and validated. This will greatly contribute to further investigations in the so-called research “unknown unknown” and “unknown known” fields, as remarked by Daughton, (2014).

4.11 Comparison with conventional treatment plants

Many studies have compared the removal efficiencies of CWs with those of corresponding “conventional” secondary or tertiary treatments. Here a discussion of the main results of the different investigations reported.

CWs acting as a secondary step – Variability ranges of PhCs in the effluents of CWs acting as a secondary step are similar to those found in the effluents of conventional treatments (CAS and MBR), as reported in Verlicchi et al. (2012b). Higher values were only found for ibuprofen in the H-SSF bed investigated by Avila et al. (2013) (55.2 μg/L, while the maximum concentration reported in Verlicchi et al. (2012b) was 44.2 μg/L), salicylic acid (1–9.92) as compared to at maximum of 0.391 μg/L in CAS effluent) in the investigations by Camacho-Munoz et al. (2012), Hijosa-Valsero (2010a, 2011b) and Matamoros et al. (2009), amoxicillin (33–43 μg/L vs. 0.007 μg/L), estriol (4.4 μg/L vs. 0.0084 μg/L) and gemfibrozil (11-13 μg/L vs. 5.2 μg/L). Conkle et al. (2008) and Llorens et al. (2009) demonstrated that a series of SF basins characterized by a high retention time (up to 30 d) guarantee better removal of common PhCs (mainly analgesics and anti-inflammatories and carbamazepine) with respect to conventional treatment plants including a range of different technologies (clarification, activated sludge, P removal), due to their lower retention times (10-60 h depending on the technology). Camacho-Munoz et al. (2012) also compared the average removal of a selected group of common PhCs by conventional techniques (activated sludges, oxidation ditches, trickling filters) and by the so-called low cost techniques (CWs). They, however, found that removal is slightly higher (64 %) in the latter than in the former (55 %), concluding that this may be due to both aerobic degradation promoted in conventional techniques by forced aeration, and the removal of PhCs by sorption onto the large amounts of sludge generated.

Matamoros and Bayona (2006) investigated the capacity of a H-SSF bed to remove common analgesics and anti-inflammatories (ibuprofen, salicylic acid, naproxen, diclofenac and ketoprofen) and the stimulant caffeine from municipal wastewater, and detected average values very close to those found in conventional activated sludge systems. However, in a V-SSF bed, Matamoros et al. (2007a) found that diclofenac was removed to a much greater extent (up to 73 %) with respect to conventional WWTPs, where it is generally among most recalcitrant. Likewise, Zhang et al., 2011 found that, in a tropical environment, removal of common PhCs (naproxen, diclofenac and ibuprofen) can be even higher in constructed wetlands than in conventional systems. This was true for naproxen and carbamazepine, while little difference between the two systems was found for diclofenac and ibuprofen.

CWs acting as a tertiary step – Hijosa-Valsero et al. (2010a) found good agreement between conventional plants and CW systems in the removal achieved for selected analgesics and anti-inflammatories, carbamazepine and caffeine. Macleod and Wong (2010) also found similar concentrations of several common PhCs in the tertiary effluent of an aerated lagoon and the

tertiary effluents from municipal WWTPs including an UV treatment as a third step. The exceptions to this rule were gemfibrozil, carbamazepine, clarithromycin, diclofenac, erythromycin, propranolol and sotalol, which occurred at higher concentrations in the UV-treated effluents. These discrepancies may, however, be explained by differences in drug uses and water consumption in different communities as well as differences in the treatment. As reported in Table SD-3, the series of aerated lagoons investigated featured a retention time of 90 days. This gave the drugs in these plants far greater exposure to sunlight, and therefore more time for photolysis, than allowed in the plants in the CAS system (including at least 8 seconds of low-pressure UV exposure), where sunlight exposure was less than 24 h. MacLeod and Wong (2010), however, found similar average removal efficiency values for a large group of PhCs when comparing the effluents from a similar sequence (CAS followed by UV treatment) and from another conventional WWTP, with effluent from their aerated lagoon effluent. Numerous common PhCs were also studied in a tertiary pond and after UV treatment by Matamoros et al. (2010), who found higher removal efficiencies in the pond system (80 % on average) than in the conventional system (40 % on average). The same authors remarked that they detected the intermediate degradation product of diclofenac (1-chlorocarbazole-1-acetic acid) in the UV effluent, but not in the pond effluent. It is known that this compound can be formed by photodegradation in UV reactors, although it could also be formed through solar radiation in the pond system, where other removal mechanisms may result in its elimination.

Similar removal efficiencies were found for caffeine (98 and 96 % respectively), ketoprofen (90 and 98 % respectively),, and triclosan (85 and 89 %, respectively) in a series of ponds (acting as primary, secondary and tertiary steps) and bioreactors followed by UV reactor by Ying et al. (2009) However they also found higher average removal values for diclofenac (90 % vs. 61 %), but lower for carbamazepine (6 vs. 15 %), gemfibrozil (15 vs. 72 %) and ibuprofen (77 vs. 96 %) in the pond series.

4.12 Specific mass loads of selected PhCs

MacLeod and Wong (2010) compared per capita time-weighted average daily loads (μg/person day) of a wide spectrum of PhCs in the tertiary effluent from a lagoon series treating a rural settlement and the effluents from a CAS-UV system fed with an urban wastewater. They found no statistically significant differences for atenolol, clarithromycin, codeine, gemfibrozil, metoprolol, naproxen, triclosan or trimethoprim, but greater values in the urban effluent of carbamazepine, diclofenac, propranolol, erythromycin, sotalol. The Authors remarked that although the differences in per capita loading were statistically significant, they may not be so in practice, as all were less than one order of magnitude (two to eight times) greater, and the absolute differences ranged from 2 to 398 μg/person day. Verlicchi et al. (2012b) evaluated and compared the specific mass loads (expressed in terms of g/(1000 inhabitants day)) of 73 PhCs, grouped by therapeutic class, in raw domestic wastewater, CAS effluent (treated by nitrification and denitrification) and polished effluent from an H-SSF. They found that the total specific load was 4.38 g/100 inhabitants day in the raw influent, 1.86 in the secondary effluent and 1.16 g/inhabitants day in the polished effluent, with analgesics, anti-inflammatories and antibiotics predominating at all three sampling points. These results highlight the fact that the internal and (often) persistent (micropollutant) mass load of PhCs from a secondary effluent can be reduced by a polishing treatment able to favour different removal pathways, which are necessary due to the great variability of the contaminants in question. This is of particular importance if the receiving river is an effluent-dominant water body, as it becomes necessary to improve the quality of the discharge in order to reduce its long-term environmental impact.

4.13 Environmental risk assessment

Safety threshold values for PhCs are seldom available and often related to single compound-single organism toxicity studies. Cleuvers (2003) studied the toxicity on Daphnia magna of diclofenac, carbamazepine and propranolol, revealing that the EC50 values for each compound (68, 72 and 7.5 mg/L , respectively) were higher than the observed concentration detected in surface water.

However, it is vital to bear in mind that the impact of a mixture of different persistent compounds, such as PhCs, is likely to be more toxic than the individual compounds alone.

A known tool we have to date for assessing the potential risk posed by individual PhCs in the effluent from the different types of CWs, acting as a secondary or tertiary steps, is their risk quotient (RQ). This is calculated as the ratio between measured environmental concentrations (MEC) of a PhC and its predicted no-effected concentration (PNEC). PNEC values can estimated on the basis of toxicity data, which is available for several aquatic organisms, namely bacteria, algae, invertebrates and fish. PNEC values are generally (EC, 2003; Tauxe-Wuersch et al., 2005) estimated as 1000 times lower than the most sensitive species assayed, so as to take into account the effect on other, potentially more sensitive, aquatic species to those used in toxicity studies (compiled in Verlicchi et al., 2012c) The values of PNEC adopted for the selected compounds are reported in Table 4.7. PhCs can be ranked by means of the commonly used risk criterion as follows: RQ<0.1, minimal risk to aquatic organisms, 0.1≤RQ<1, moderate risk; RQ≥1, high risk (Hernando et al., 2006).

Table 4.7: PNEC values used in the environmental risk assessment (from Verlicchi et al., 2012c)

Classe	Composto	PNEC [µg/L]
Analgesic/Anti-inflammatory	Acetaminophen	1
Analgesic/Anti-inflammatory	Codeine	16
Analgesic/Anti-inflammatory	Diclofenac	9.7
Analgesic/Anti-inflammatory	Ibuprofen	1.65
Analgesic/Anti-inflammatory	Ketoprofen	15.6
Analgesic/Anti-inflammatory	Naproxen	2.62
Analgesic/Anti-inflammatory	Salicylic acid	1.28
Antibiotics	Amoxicillin	0.0037
Antibiotics	Ampicillin	0.075
Antibiotics	Clarithromycin	0.07
Antibiotics	Doxycycline	0.3
Antibiotics	Erythromycin	0.02
Antibiotics	Lincomycin	82
Antibiotics	Sulfadimethoxine	3.5
Antibiotics	Sulfamethazine	4
Antibiotics	Sulfamethoxazole	0.027
Antibiotics	Sulfapyridine	21.61
Antibiotics	Trimethoprim	2.6
B-blockers	Atenolol	30
B-blockers	Metoprolol	8
B-blockers	Nadolol	110
B-blockers	Propranolol	0.244
Hormones	Estriol	0.0008
Lipid regulators	Gemfibrozil	0.9
Psychiatric drugs	Carbamazepine	13.8
Psychiatric drugs	Fluoxetine	0.05
Stimulant drug	Caffeine	182

For each compound listed in Table 4.7, MEC was assumed to be equal to the average concentrations for secondary or tertiary effluent on the basis of all collated data. It is important to note that not all compounds were investigated in all the three CW types or in both the two steps, and that the results are obviously dependent on the data collated and used to evaluate the

average values and RQ. The analysis performed is reported in detail in Figures 4.17-4.18, which refer to the different types of CWs acting as a secondary and a tertiary step. The main findings of the environmental risk assessment where CW acted as a secondary step are:

- Amoxicillin, ampicillin, sulfamethoxazole, clarithromycin, erythromycin, salicylic acid and ibuprofen pose a high risk (RQ>1) in the effluents from both SF systems and H-SSF beds.
- In addition, in the effluent from SF systems three further PhCs pose the same risk level: estriol, gemfibrozil and propranolol, and in the effluent from H-SSF systems this occurs for acetaminophen.

Naproxen and doxycycline present a moderate risk in SF and H-SSF systems, as does carbamazepine in H-SSF systems, and salicylic acid and ibuprofen in V-SSF beds.

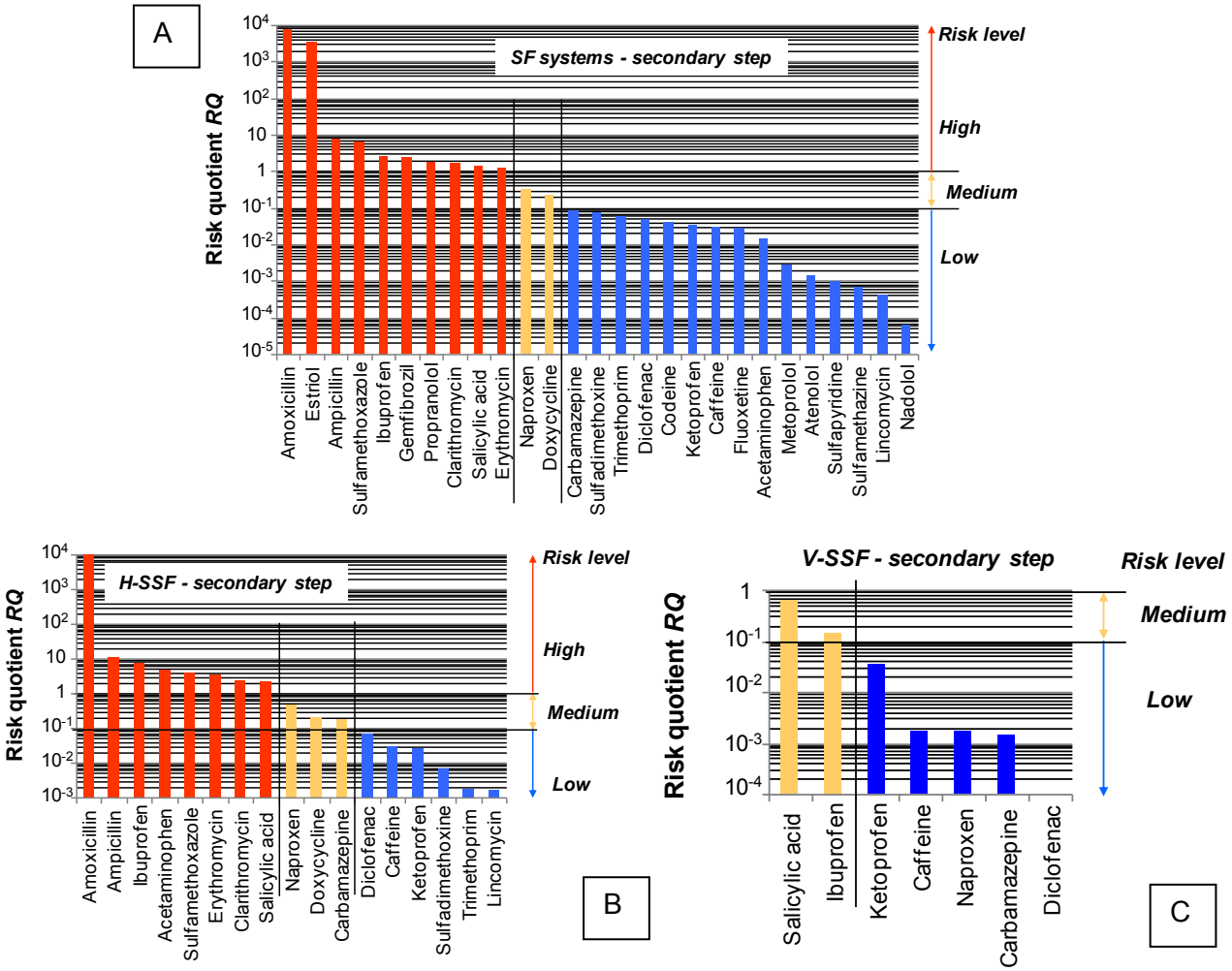


Fig. 4.17: Environmental risk posed by the occurrence of PhCs in the effluent of SF (A), H-SSF (B) and V-SSF (C) systems acting as a secondary step

In effluent from CW as a tertiary step:

- the risk remains high for amoxicillin, sulfamethoxazole and salicylic acid in SF systems and in H-SSF beds, which also feature a high risk for ibuprofen, ofloxacin, clarithromycin and erythromycin.
- There is a moderate risk for ibuprofen, clindamycin, naproxen, clarithromycin and metoprolol in SF systems; for fluoxetine, acetaminophen, enoxacin, naproxen and sulfadiazine in H-SSF beds; and for carbamazepine, ibuprofen, naproxen and diclofenac in V-SSF beds.

Antibiotics seem to pose the greatest risks, especially in the case of amoxicillin and sulfamethoxazole, whose RQs are also greater than 1 in the tertiary effluent from SF and H-SSF systems. In contrast, RQ values for diclofenac (that is the pharmaceutical candidate to be

monitored in the future in the aquatic environment) were found to be below 0.1 in all cases, with the exception of the tertiary effluent from V-SSF systems.

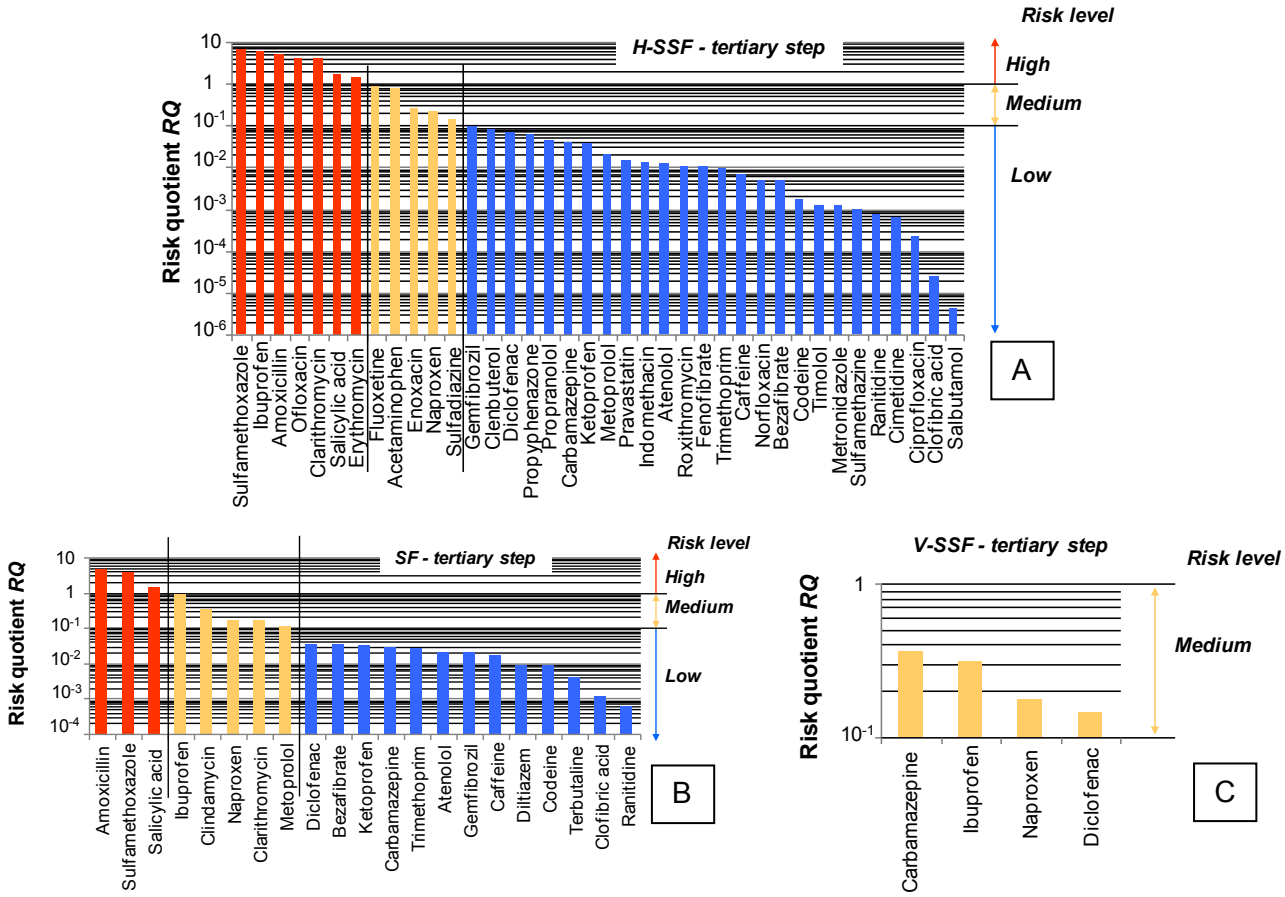


Fig. 4.18: Environmental risk posed by the occurrence of PhCs in the effluent of H-SSF (A), SF (B) and V-SSF (C) systems acting as a tertiary step

Table 4.8 reports a comparison of the environmental risk posed by common PhCs in CAS effluent (Verlicchi et al., 2012c) with that in the effluents from CWs acting as secondary or tertiary steps. This shows that:

- A high risk is posed by 12 compounds in CAS effluents, 7 in secondary H-SSF beds (II), 8 in secondary SF basins (II), 7 in tertiary H-SSF beds (III) and 3 in tertiary SF basins (III).
- A medium risk is posed by 13 compounds in CAS effluent, 3 compounds in H-SSF beds (II), 2 compounds in SF basins (II), 5 compounds in H-SSF beds (III), and 5 in SF basins (III).
- Amoxicillin, clarithromycin, erythromycin, sulfamethoxazole, gemfibrozil and ibuprofen are the most critical compounds for a CAS effluent, as well as for a CW acting as a secondary step.
- Amoxicillin, clarithromycin, erythromycin, sulfamethoxazole and ibuprofen still pose a high risk in the effluent of a CW acting as a tertiary step, as already discussed.

Table 4.8: Risk posed by PhCs in CAS effluent and those from CWs acting as secondary and tertiary steps.

Risk level	CAS	CWs (II)	CWs (III)
High (RQ > 1)	Amoxicillin	Amoxicillin (SF, H-SSF)	Amoxicillin (SF, H-SSF)
	Clarithromycin	Clarithromycin (SF, H-SSF)	Clarithromycin (H-SSF)
	Diazepam		
	Erythromycin	Erythromycin (SF, H-SSF)	Erythromycin (H-SSF)
		Estriol (SF)	
	Fenofibrate		
	Fenofibric acid		
	Fluoxetine		
	Gemfibrozil	Gemfibrozil (H-SSF)	
	Ibuprofen	Ibuprofen (SF, H-SSF)	Ibuprofen (H-SSF)
		Propranolol (SF)	
	Ofloxacin		Ofloxacin, (H-SSF)
		Salicylic acid (SF, HSSF)	Salicylic acid (SF, H-SSF)
	Sulfamethoxazole	Sulfamethoxazole (SF, HSSF)	Sulfamethoxazole (SF, H-SSF)
	Tetracycline		
Medium (0.1<RQ <1)	Acetaminophen		Acetaminophen (H-SSF)
	Atenolol		
	Bezafibrate		
		Carbamazepine (H-SSF)	
			Clarithromycin (SF)
			Clindamycin (SF)
	Codeine		
	Doxycycline	Doxycycline (SF, H-SSF)	
	Enoxacin		
			Fluoxetine (H-SSF)
		Naproxen (SF, H-SSF)	Ibuprofen (SF)
			Enoxacin (H-SSF)
	Metronidazole		Metoprolol (SF)
	Naproxen		Naproxen (SF, H-SSF)
	Propranolol		
	Roxithromycin		
	Salicylic acid		
	Sulfadiazine		Sulfadiazine (H-SSF)
	Trimethoprim		

Investigations are needed in order to evaluate if there are any operational and environmental conditions that could be modified to improve the removal of these compounds in CWs. It would also be important to investigate the risks posed by their transformation products, as it seems that the ecotoxicity of some PhC photoproducts (including diclofenac, naproxen, and the fibrates), could be greater than that of the parent compounds. Other future avenues of research could include the genotoxic and mutagenic effects of both.

4.14 Perspectives on CW use and Conclusions

The current survey shows that CWs have the potential to contribute to the removal of common PhCs that are usually present in urban wastewater. Their ability to remove a wide spectrum of PhCs can be ascribed to the coexistence of anoxic-aerobic-aerobic microenvironments within surface flow, as well as subsurface flow systems that favour the different mechanisms involved in their removal, mainly biodegradation, sorption, plant uptake and, for surface systems, also photodegradation. According to the articles reviewed, CWs provide comparable efficiencies to conventional WWTPs for the removal of many common PhCs, including naproxen, salicylic acid, ibuprofen and caffeine, all over-the counter compounds widely consumed for various purposes.

CWs frequently receive wastewater from small communities, and the drug load in the final effluents may therefore be lower than that found in urban WWTP effluents, although they remain detectable. As a consequence, if pharmaceutical contamination of surface water presents a risk for aquatic species, that risk is also present near smaller settlements, especially those that are landlocked and with no opportunity to discharge into a large surface water body and thus achieve effluent dilution. In effluent-receiving waters, photodegradation and biotransformation are typically one of the most important “auto-depurative” processes for the attenuation of organic micropollutants. As a consequence, it would be wise to optimize conditions for these processes (i.e., by using extended periods of aging in sewage lagoons, use of additional steps with bioreactors or oxidation ditches) to effectively minimize or prevent environmental exposure to biologically active concentrations of these chemicals. At the same time, the implementation of CWs would be a valid alternative for small and scattered (rural or urban) communities, also taking into consideration the investment and operational costs and the modest maintenance. Indeed, although CWs require a high footprint, with respect to conventional systems, these “natural” polishing treatments represent suitable solutions for small communities, or for the final step in treatment tailored to specific users, such as healthcare or hospital facilities (Verlicchi et al., 2010a). Such wastewaters require particular attention paid to the removal of pharmaceutical micropollutants and a final dedicated treatment by means of CWs would be a valuable aid.

However, further researches is needed, first and foremost to better evaluate CW design parameters, with a view to optimizing the removal of the most critical compounds (mainly antibiotics and some analgesics and anti-inflammatories), and to better assess the risk posed in the aquatic environment of residual PhCs and their photoproducts.

Reference

- Verlicchi P, Zambello E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. Sci Tot Enviro 2014;470-471;1281-1306.

Chapter 5

5 *Removal of PhCs by CAS followed by CW analysis of their respective contributions*

5.1 Introduction

Pharmaceutical compounds (PhCs) and other emerging contaminants, including personal care products, have provoked rising concern in recent years due to the growth in their use and the increasing awareness of the environmental impact of micropollutants discharged into surface water bodies. The primary and constant sources of PhCs to the aquatic environment are wastewater treatment plant (WWTP) discharges (Daughton and Ruhoy, 2009), as common treatments (based on conventional activated sludge processes, AS) are generally not able to efficiently remove such small particles (200–1000 Da) characterised by different chemical and physical properties (Verlicchi et al., 2012d). Observed removal efficiencies depend upon several factors, specifically the nature of themicro-pollutants, treatment train and operational conditions in question (Verlicchi et al., 2012d). As a consequence, varying concentrations of these compounds from treated effluents have been detected in surface, ground and coastal waters (Dougherty et al., 2010). Moreover, the high levels of several of these pollutants measured in river biota (Rimkus, 1999; Muñoz et al., 2009b) highlight the vulnerability of effluent-dominant rivers, which are very common worldwide (Al Aukidy et al., 2012). In fact, recent environmental risk assessments have shown that the concentrations of several pharmaceutical and personal care products in aquatic environments may exceed their predicted no-effect concentrations (PNEC) (Kleywegt et al., 2011; Rúa-Gómez and Püttmann, 2012).

However, the adverse effects of these compounds can be mitigated to some extent if the receiving surface water body features autodepurative processes, thanks, in particular, to its high flow rate, which results in a consistent dilution (Verlicchi et al., 2012b; Gros et al., 2010). Nevertheless, PhC contamination is still a largely unregulated area, and there is ongoing debate within the scientific community regarding which PhCs to include among the priority substances. Indeed, according to the European Draft (European Community, 2012), the anti-inflammatory diclofenac and the hormones 17β-estradiol and 17α-ethinylestradiol are prime candidates to be added to the European Priority List, while according to the US EPA, erythromycin, nitroglycerin, and 9 hormones (17α-ethinylestradiol, 17α-estradiol, 17β-estradiol, equilenin, equilin, estriol, estrone, mestranol and norethindrone), need to be considered a priority (Richardson and Ternes, 2011).

Various strategies may be employed to reduce the pharmaceutical load discharged into the environment, namely (i) source reduction, i.e., reducing the quantity of PhCs consumed (pharmacovigilance) and the use of more degradable compounds with comparable therapeutic effects (green pharmacy) (Ruhoy and Daughton, 2008); and (ii) the adoption of treatment types and trains more suited to the removal of PhCs by upgrading existingWWTPs or designing new ones. Unsurprisingly therefore, many experimental investigations have been carried out in recent years to test technologies for their ability to reduce the concentrations of these persistent organic micropollutants in the final effluent. In particular, the following advanced chemical and biological systems have been assessed: ozonation, O3/UV, O3/H2O2 (Hollender et al., 2009; Benitez et al., 2011), ultrafiltration, reverse osmosis, granular activated carbon contact (Acero et al., 2010; Michael et al., 2013), and membrane biological reactors (Radjenovic et al., 2009b; Lipp et al., 2012).

In contrast, less research has thus far been conducted to examine the efficacy and reliability of constructed wetlands (CWs) in removing PhCs, and the few studies that have been carried out have mainly focussed on pilot plants (Ávila et al., 2010, 2013; Hijosa-Valsero et al., 2010b), acting mainly as a secondary treatment (Ávila et al., 2010; Matamoros et al., 2005; Hijosa-Valsero et al., 2011a), and in only very few cases as a polishing step (Hijosa-Valsero et al., 2010a; Llorens et al., 2009).Most of the plants investigated included surface flowsystems (SFS, such as lagoons or anaerobic or facultative ponds) (Llorens et al., 2009; Matamoros et al., 2008b), and to a lesser extent horizontal subsurface flow systems (H-SSF, Hijosa-Valsero et al., 2011b). Very little work has yet been done on vertical subsurface flow systems (V-SSF) (Matamoros et al., 2007), but researchers have started looking into combinations of the different types of CWs, the so-called hybrid systems (Conkle et al., 2008; Matamoros et al., 2008b; Hijosa-Valsero et al., 2010a), and, more recently, the influence of the feeding mode (batch or continuous) and the hydraulic loading rate on the removal of selected PhCs in microcosm beds (Zhang et al., 2012).

In general, however, the occurrence and removal of only a very limited number of PhCs have been investigated in CWs. The most frequently included substances in such studies have been analgesic and anti-inflammatory drugs (mainly salicylic acid, ibuprofen, ketoprofen, naproxen and diclofenac), followed by psychiatric drugs (in particular carbamazepine) and lipid regulators (mainly clofibric acid and gemfibrozil) (Hijosa-Valsero et al., 2010a, 2010b, 2011b; Matamoros et al., 2008a, 2008b). Some analyses have also considered the beta-blockers atenolol, nadolol, propranolol, metoprolol and solatolol; the antibiotics sulfamethoxazole and sulfapyridine (Conkle et al., 2008; Park et al., 2009); and the diuretic furosemide (Hijosa-Valsero et al., 2010a, 2010b).

In order to conduct a wider-reaching investigation, we set out to analyse the occurrence and removal of 73 common PhCs from the liquid phase in a pilot H-SSF bed, fedwith a municipalWWTP biological effluent (also called secondary effluent orWWTP effluent in the following),situated in the Po Valley, Italy. The 73 investigated substances belong to 12 different therapeutic classes with distinct functions and biological activities: 12 were analgesics/anti-inflammatories, 25 antibiotics, 1 anti-diabetic, 3 antihypertensives, 3 barbiturates, 2 betaagonists, 9 beta-blockers, 1 diuretic, 7 lipid regulators, 5 psychiatric drugs, 4 receptor antagonists and 1 antineoplastic. The occurrence of the same compounds in the WWTP raw influent and effluent has already been presented and discussed in the literature (Verlicchi et al., 2012a).

In this study, the data collected was used to evaluate the contribution of the investigated bed to the overall removal efficiency(WWTP + H-SSF bed) of the treatment train for these compounds. The reduction in the PhC mass load in the raw WWTP influent and secondary effluent, as well as in the polished effluent, was then analysed and the mass loads discharged in the presence and absence of this polishing treatment were compared.

Compounds were only investigated in the liquid phase: analysis of sediments, soils and plant tissueswere not performed, as the aim of this study was to focus on the quality of the final effluent after a polishing treatment by means of CWs in terms of PhCs discharged into a surface body. Indeed, this is quite a common situation in the north of Italy, where a great number of small–medium WWTPs discharge their final effluent into a surface water network used for irrigation purposes from May to October. As the current Italian legislation (Decreto Legislativo 152/2006) suggests the adoption of CWs for the treatment of the wastewater from small urban settlements, the current study will provide useful information about the ability of such decentralised wastewater treatment plants to mitigate PhC discharge into surface water systems.

5.2 The study site

This study was performed on March 2010 at a large municipal WWTP in the Po Valley, northern Italy, where a pilot CW station, directly fed by the WWTP secondary effluent, has been in operation for 10 years.

The large WWTP — This was designed for a population equivalent (PE) of 120,000. Domestic wastewaters are conveyed to the WWTP through gravity-drained combined sewage,where they are accumulated in a basin, and then subjected to preliminary treatments (screening and grit removal) a biological treatment, and a final NaClO disinfection step (Fig. 1). The biological treatment consists of a conventional activated sludge system (AS), including denitrification (V = 4000 m^3) and nitrification (V = 6100 m^3) steps, followed by secondary sedimentation (V = 6000 m^3). The WWTP operates at a low-to-medium load, at an average hydraulic retention time of 6 h, a sludge age of 8 days and a mixed liquor concentration of approximately 3.5 kg/m^3. During the observation period (March 2010), the flow rate was, on average, 28 000 m^3/day. This WWTP had already been the subject of experimental investigation; in Verlicchi et al. (2012a) the PhC occurrence in its raw influent and secondary effluent are presented and discussed. That data refer to Fig. 5.1.

The pilot plant — The investigated H-SSF constructed wetland is a long, narrow bed (L × W: 28 m × 1 m, aspect ratio L/W = 28) filled with gravel (8–10 mm, porosity 33%). Its depth ranges from 0.7 m at the influent and to 1.75 m at the effluent (on average 1.2 m), and it is planted with Phragmites australis (see Fig. 1B). It has been operational since 2003 and it has been consistently fed by real wastewater from the nearby WWTP. As shown in Fig. 5.1, in this experimental campaign the feed

was the effluent from the secondary clarifier; it was pumped into a 10-m^3 tank placed 3 m above the ground and gravity-fed to the pilot plant. This tank acted as an equalisation tank. The influent flow rate was set by a valve, monitored regularly by a flow meter, and kept at a constant flow value of 8 m^3/day (assuming a water human daily consumption of 150 L/(day inh) this flow rate corresponds to a served population of 53 PE). The hydraulic retention time (HRT) was about 1 day. During the observation period (March), the external temperature ranged between −2 and 18 °C, being on average 10 °C; the maximum solar radiation ranged between 600 and 800 W m−2; and precipitation amounted to 150 mm, with 10 rainy days recorded (rain >2 mm/day). As evapotranspiration in the H-SSF bed under these environmental conditions corresponded to a daily water loss of 1–2%, as per a previous study (Verlicchi et al., 2009) it was not considered influential.

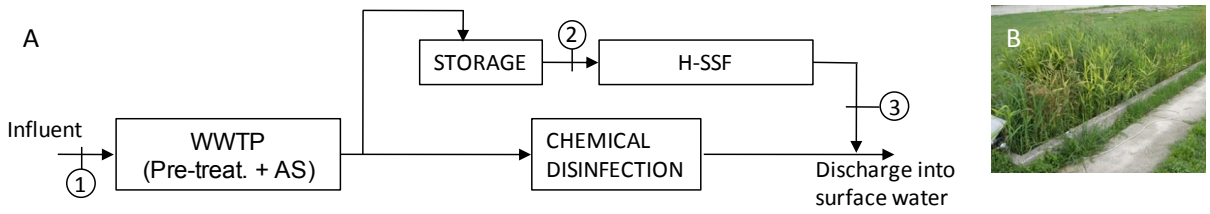


Fig. 5.1: Flow schematic of the investigated full-scale secondary and pilot polishing steps (A) and the H-SSF bed (B), showing sampling location.

5.3 Micropollutants investigated and their main characteristics

Table 5.1: List of selected compounds

Analgesics/anti-inflammatories (A)	Acetaminophen; Codeine; Diclofenac Ibuprofen; Indomethacine; Ketoprofen; Mefenamic acid; Naproxen; Phenazone; Phenylbutazone; Propyphenazone; Salicylic acid;
Antibiotics (B)	Azithromycin; Chloramphenicol; Chlortetracycline; Ciprofloxacin; Clarithromycin; Danofloxacin; Doxycycline; Enoxacin; Enrofloxacin; Erithromycin; Josamycin; Metronidazole; Nifuroxazide; Norfloxacin; Ofloxacin; Oxytetracycline; Roxithromycin; Spiramycin; Sulfadiazine; Sulfamethazine; Sulfamethoxazole; Tetracycline; Tilmicosin; Trimethoprim; Tylosin A
Antidiabetic (C)	Glibenclamide
Antihypertensive (D)	Enalapril; Hydrochlorothiazide; Lisinopril;
Barbiturates (E)	Butalbital; Pentobarbital; Phenobarbital
Beta-agonists (F)	Clenbuterol; Salbutamol;
Beta-blockers (G)	Atenolol; Betaxolol; Cerazolol; Metoprolol; Nadolol; Pindolol; Propranolol; Sotalol; Timolol
Diuretic (H)	Furosemide
Lipid regulators (I)	Atorvastatin; Bezafibrate; Clofibric acid; Fenofibrate; Gemfibrozil; Mevastatin; Pravastatin
Psychiatric drugs (J)	Carbamazepine; Diazepam; Fluoxetine; Lorazepam; Paroxetine
Receptor antagonists (K)	Cimetidine;Famotidine; Loratadine;Ranitidine
Antineoplastic (L)	Tamoxifen

Compounds analysed in this study were selected on the basis of several criteria: high consumption by the resident population (Gruppo di lavoro OsMed, 2011), pertinence for environmental and public health (De Voogt et al., 2009) and availability of detection techniques. No corresponding metabolites (molecules resulting from structural changes to the PhC within the human body or on the skin) or transformation products (molecules resulting from structural changes to the PhC structure after its excretion in the environment) of the investigated compounds were analysed. Table 5.1 reports the list of the 73 investigated contaminants, grouped according to their therapeutic class. The selected compounds cover a wide range of variability in terms of functional

groups (basic or acidic functional groups), even in the same molecules in some cases (e.g., ciprofloxacin), solubility in water (S_w) molecular charge, hydrophilicity (log K_{ow}), and tendency to sorb (log K_d and log K_{oc}), that influence their behaviour during treatment process. The main chemical and physical properties of these substances, including their molecular structures as well as their observed range of variability in secondary effluents are reported in Appendex A.

5.4 Sample preparation, sampling and chemical analysis

The sampling points under study are shown in the schematic in Fig. 5.1 and, in particular, were: 1) the inlet to the AS system; 2) the outlet from the secondary clarifier, before the chlorination tank — this point corresponds to the inlet to the H-SSF system; and 3) the final discharge from the H-SSF. Four-hour composite water samples were taken at each point during four dry days (n = 4), in order to avoid dilution effects due to precipitation. All the water samples were taken in the same observation period: March 2010 (see also Verlicchi et al., 2012a). The PhC concentrations observed in theWWTP influent and secondary effluent have already been published in a previous work (Verlicchi et al., 2012a), where details of the analytical methods are reported and discussed. Here, only details of polished effluent analyses and some considerations regarding the secondary effluent are reported. All water samples were transferred to amber polyethylene terephthalate (PET) bottles and immediately transported to the laboratory under cooled conditions (4 °C). Upon reception, samples were filtered through 0.45-μm nylon filters (Whatman, Maidstone, UK) to eliminate suspended solid matter, and then frozen (−20 °C) until analysis (less than a week later). As a consequence, the measured concentrations of pharmaceuticals correspond to their dissolved fractions. It is worth noting that PET bottles can adsorb micropollutants onto their surface, leading to an underestimation of the real concentrations in the water. Collected concentration measures did not consider this fact. Analyticalmethods—All the pharmaceuticals and the corresponding isotopically labelled internal standards were of high purity grade (>90%). Detailed information on the providers of the analytical standards, as well as about the preparation of the mixture solutions can be found elsewhere (Gros et al., 2009). The solvents, HPLC grade methanol, acetonitrile, water (LiChrosolv) and formic acid (98%) were supplied by Merck (Darmstadt, Germany).

The multiresidue analytical method developed by Gros et al. (2009) was used to quantify the selected pharmaceuticals in wastewaters. Briefly, after filtration, an appropriate volume of aqueous solution of 5% Na2EDTA was added to 200 mL of WWTP secondary and polished effluents to achieve a final Na2EDTA concentration of 0.1% in the samples. The measured volumes were subsequently pre-concentrated onto a lipophilic–hydrophilic balanced Oasis HLB cartridge (60 mg and 3 mL), at a flow rate of 5 mL/min, using a Baker vacuum system (J.T. Baker, Deventer, the Netherlands). The cartridges were conditioned with 5 mL of methanol followed by 5 mL of HPLC water at neutral pH. After sample preconcentration, cartridges were rinsed with 5 mL of HPLC grade water, and vacuum dried for 15–20 min to remove excess moisture. Elution of target compounds was performed with 2 × 4 mL pure methanol. Extracts were evaporated to dryness under a gentle nitrogen stream, and reconstituted with 1 mL of methanol– water (25:75, v/v). Prior to analysis, all samples were spiked with a standard mixture of isotopically labelled standards at a concentration of 20 ng/mL. Instrumental analysis of the samples was performed by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC–MS/MS). HPLC analysis was performed using Symbiosis™ Pico (SP104.002, Spark, Holland), equipped with an autosampler and connected in series to a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer equipped with a turbo ion spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved using a Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 μm), preceded by a C18 guard column (4 mm × 4 mm, particle size 5 μm), both supplied by Merck (Darmstadt, Germany).

To determine the recoveries, three samples were spiked for each matrix. Recoveries achieved for all selected compounds are reported in details in Table SD-2 in the Supplementary Data of Verlicchi et al., 2013a. They ranged from 50 and 130% for all target compounds, with few exceptions. Lower recoveries were found for josamycin (46%), butalbital (47%), phenobarbital (44%), paroxetine (45%) in the influent, josamycin (23%), metronidazole (45%), oxytetracycline (45%) and phenobarbital (26%) in the secondary effluent and for metronidazole (47%) and phenobarbital (41%) in the polished effluent. Higher recoveries were found for metronidazole

(136%), carbamazepine (145%) and tamoxifen (145%) in the influent, and for tylosin A (145%) and ranitidine (135%) in the secondary effluent. Extremely high and low recoveries have already been found by other studies (among them Gros et al., 2009). They can be correlated to the properties of the matrix itself and the complexity of the interactions with analytes, as well as possible errors in procedure. The limit of detection (LOD), calculated as three times signal-to-noise, were 1–16 ng/L for the influent, 1–18 ng/l for secondary effluent and 1–15 ng/L for H-SSF effluent.

5.5 Equations used

5.5.1 Evaluation of the average concentration of each therapeutic class

For each sampling point *p,* the average concentration of the therapeutic class *h* ($c_{av,\,h,\,p}$) is defined by eq. 5.1 as the sum of the average concentrations of each of the selected compound *i* $c_{av,\,i}$ belonging to the same group *h*:

$$c_{av,\,h,\,p} = \sum_{i \in h} c_{av,\,i}\Big|_{p} \qquad h = 1, 2, ...,12 \qquad p = 1, 2, 3 \qquad \text{(eq. 5.1)}$$

In eq. 5.1, *h* is the generic therapeutic class (in total 12), *p* represents the generic sampling points reported in Fig. 5.1 and *i* is the generic PhC belonging to the therapeutic class *h*.

5.5.2 Evaluation of the average removal efficiency of each treatment step for each selected PhC

The average removal efficiency (η_{av}) for each selected PhC *i* was evaluated for the following treatments *j*: secondary (between sections 1 and 2 in Fig. 5.1; *j* = 1), polishing (between sections 2 and 3 in Fig. 5.1, *j* = 2) and the entire treatment train (between sections 1 and 3 in Fig. 5.1, *j* = 3). $\eta_{av,\,i,\,j}$ was evaluated by means of eq. 5.2, based on the mean influent and effluent concentrations (*n* = 4) of each PhC at each sampling point in order to reduce the effect of the variability of this parameter on the evaluation of removal efficiency, as per Hijosa-Valsero et al. (2011b):

$$\eta_{av,\,i,\,j} = \frac{m_{i,\,removed}}{m_{i,\,influent}}\Big|_{j} \text{ x } 100 = \frac{c_{av,\,i,\,inf}\,Q_{inf} - c_{av,\,i,\,eff}\,Q_{eff}}{c_{av,\,i,\,inf}\,Q_{inf}}\Big|_{j} \text{ x } 100 \qquad j = 1, 2, 3 \qquad \text{(eq. 5.2)}$$

where:

$m_{i,\,removed}$ is the mass of PhC *i* removed during the treatment *j*, $m_{i,\,influent}$ is the mass of PhC *i* entering the treatment *j* (μg d^{-1}), Q_{inf} is the influent flow rate (m^3 d^{-1}), Q_{eff} is the effluent flow rate (m^3 d^{-1}), $c_{av,\,i,\,inf}$ is the mean influent concentration of PhC *i* (μg L^{-1}) at treatment step *j*, and $c_{av,\,i,\,eff}$ is the mean effluent concentration of the substance *i* (μg L^{-1}) after treatment step *j*.

Q_{eff} can vary with respect to Q_{inf} due to water losses caused by evaporation and evapotranspiration, but in this study, Q_{eff} was assumed to be equal to Q_{inf} for each treatment *j*, as for the activated sludge system evaporation is negligible with respect to the great volumes of water treated, and for the H-SSF bed, as reported in subparagraph 5.2, the average percentage of water losses amount to 1–2 % of the influent flow rate during the observation period (March), which was cold and humid, allowing evapotranspiration to be discounted. As a consequence, eq. 5.2 becomes:

$$\eta_{av,i,j} = \frac{c_{av,i,inf} - c_{av,i,eff}}{c_{av,i,inf}}\Big|_{j} \text{ x } 100 \qquad \text{(eq. 5.3)}$$

Evaluation of the average removal efficiency of each treatment step for each therapeutic class

The average removal efficiency for each therapeutic class *h* in each treatment step *j* was evaluated by means of eq. 5.4:

$$\eta_{av,h,j} = \left. \frac{\sum_{i \in h} c_{i,av,inf} - \sum_{i \in h} c_{i,av,eff}}{\sum_{i \in h} c_{i,av,inf}} \right|_j \text{ x } 100 \qquad h = 1, ...,12; \quad j = 1, 2, 3 \qquad \text{(eq. 5.4)}$$

where *i* represents each of the investigated PhCs belonging to the same therapeutic class *h*, $c_{i,av}$ is the average concentration of the generic compound *i* in the influent (subscript: *inf*) and in the effluent (subscript: *eff*) of the same treatment step *j.*

5.5.3 Evaluation of the contribution of the H-SSF bed to the overall average removal efficiency

The contribution of the H-SSF bed to the overall average removal efficiency for each compound was obtained as the difference between the average overall removal efficiency $\eta_{av,i,3}$ (*j* = 3 in eq. 5.3) and that found for the AS system $\eta_{av,i,1}$ (*j* = 1 in eq. 5.3) (eq. 5.5):

$$\eta_{H-SSF\,bed/overall,i} = \eta_{av,i,3} - \eta_{av,i,1} \qquad \text{(eq. 5.5)}$$

It is easy to show that the correlation between $\eta_{H\text{-}SSF\ bed/overall,\ i}$ and $\eta_{av,\ i,2}$ is given by eq. 5.6:

$$\eta_{H-SSF\,bed/overall,i} = \eta_{av,i,2}\ \frac{c_{av,i,2}}{c_{av,i,1}} \qquad \text{(eq. 5.6)}$$

1.5 Evaluation of the contribution of each treatment step to the overall average removal efficiency for each therapeutic class

The contribution of the H-SSF bed to the overall average removal efficiency for each therapeutic class *h* was obtained as the difference between the average overall removal efficiency $\eta_{av,h,3}$ (*j* = 3 in eq. 5.4) and that found for the AS system $\eta_{av,h,1}$ (*j* = 1 in eq. 5.4) (eq. 5.7):

$$\eta_{H-SSF\,bed/overall,h} = \eta_{av,h,3} - \eta_{av,h,1} \qquad h = 1, ...,12 \qquad \text{(eq. 5.7)}$$

5.6 Results and discussion

The occurrence of the 73 selected PhCs was investigated at the three sampling points shown in Fig. 5.1 in order to evaluate the removal efficiencies of the secondary treatment, the polishing step and the treatment train as a whole. Average concentrations of each PhC at sampling points 2 and 3, and their corresponding standard deviations (SD) are reported in Table 5.2. In addition, Table 5.2 compiles the average removal efficiencies of the two treatment steps (j = 1, 2) as well as that of the overall treatment train (j = 3), evaluated on the basis of the collected data. Moreover, an analysis of the average concentrations of each therapeutic class is also reported at the influent and effluent of each treatment step (j = 1, 2, 3). To complete the analysis, the average removal observed for each class is also evaluated for each j (Table 5.3), according to eq. 5.4.

Table 5.2: Average removal efficiencies of the investigated compounds at each step and in the treatment train as a whole, together with average values observed for each compound at the bed inlet and outlet with corresponding SDs. For each compound and at each sampling point n = 4. (n.e. = not evaluated, LOD = limit of detection).

Class	Compound	WWTP		H-SSF bed		WWTP+H-SSF
		Removal effic. (%)	Av. inf conc. (± SD) (ng/L)	Av. eff. conc. (± SD) (ng/L)	Removal effic. (%)	Removal effic. (%)
Analgesic/ Anti-inflammatory (A)	Acetaminophen	96	30 ± 20	16 ± 6	45	98
	Codeine	38	66 ± 13	28 ± 8	57	73
	Diclofenac	35	284 ± 49	271 ± 59	5	38
	Ibuprofen	92	81 ± 49	58 ± 14	28	94
	Indomethacine	39	98 ± 31	54 ± 17	45	66
	Ketoprofen	49	85 ± 21	69 ± 13	18	59
	Mefenamic acid	27	664 ± 209	533 ± 116	20	41
	Naproxen	79	178 ± 52	114 ± 64	36	86
	Phenazone	n.e.	<lod	<lod	n.e.	n.e.
	Phenylbutazone	51	52 ± 11	23 ± 8	55	78
	Propyphenazone	21	42 ± 20	50 ± 18	-19	7
	Salicylic acid	76	118 ± 8	110 ± 5	7	78
Antibiotics (B)	Azithromycin	-1	131 ± 46	19 ± 7	86	86
	Chloramphenicol	100	<lod	<lod	n.e.	100
	Chlortetracycline	n.e.	<lod	<lod	n.e.	n.e.
	Ciprofloxacin	71	638 ± 349	208 ± 105	67	91
	Clarithromycin	8	284 ± 24	265 ± 57	7	14
	Danofloxacin	n.e.	<lod	<lod	n.e.	n.e.
	Doxycycline	n.e.	<lod	<lod	n.e.	n.e.
	Enoxacin	41	61 ± 28	38 ± 26	38	63
	Enrofloxacin	n.e.	<lod	<lod	n.e.	n.e.
	Erithromycin	66	16 ± 14	28 ± 8	-78	39
	Josamycin	n.e.	<lod	<lod	n.e.	n.e.
	Metronidazole	34	28 ± 12	<lod	100	100
	Nifuroxazide	75	13 ± 8	<lod	100	100
	Norfloxacin	25	152 ± 13	74 ± 18	52	64
	Ofloxacin	61	394 ± 138	64 ± 17	84	94
	Oxytetracycline	n.e.	<lod	<lod	n.e.	n.e.
	Roxithromycin	54	29 ± 18	42 ± 34	-43	33
	Spiramycin	52	29 ± 14	12 ± 2	58	80
	Sulfadiazine	21	17 ± 5	20 ± 6	-17	7
	Sulfamethazine	41	11 ± 2	<lod	100	100
	Sulfamethoxazole	52	214 ± 35	180 ± 47	16	60
	Tetracycline	n.e.	<lod	<lod	n.e.	n.e.
	Tilmicosin	86	36 ± 29	18 ± 9	51	93
	Trimethoprim	31	40 ± 7	25 ± 8	38	58
	Tylosin A	n.e.	<lod	<lod	n.e.	n.e.
Antidiabetics (C)	Glibenclamide	37	55 ± 29	42 ± 14	23	52
Anti- hypertensive (D)	Enalapril	100	<lod	<lod	n.e.	100
	Hydrochlorothiazide	57	1165 ± 199	432 ± 174	63	84
	Lisinopril	n.e.	<lod	<lod	n.e.	n.e.
Barbiturates (E)	Butalbital	24	101 ± 16	59 ± 25	42	56
	Pentobarbital	41	18 ± 6	12 ± 1	32	60
	Phenobarbital	33	138 ± 27	114± 33	17	45
Beta-agonists (F)	Clenbuterol	29	182 ± 37	162 ± 24	11	37
	Salbutamol	11	12 ± 3	<lod	100	100
Beta-blockers (G)	Atenolol	65	734 ± 178	383 ± 151	48	82
	Betaxolol	n.e.	<lod	<lod	n.e.	n.e.
	Cerazolol	n.e.	<lod	<lod	n.e.	n.e.
	Metoprolol	29	182 ± 25	162 ± 22	11	37
	Nadolol	100	<lod	<lod	n.e.	100
	Pindolol	100	<lod	<lod	n.e.	100

	Propranolol	29	18 ± 6	11 ± 5	38	56
	Sotalol	40	323 ± 115	306 ± 114	5	43
	Timolol	25	10 ± 8	11 ± 5	-8	19
Diuretic (H)	Furosemide	35	274 ± 128	179 ± 85	35	58
Lipid regulators (I)	Atorvastatin	48	6 ± 4	<lod	100	100
	Bezafibrate	60	36 ± 17	26 ± 3	27	71
	Clofibric acid	80	2 ± 1	<lod	100	100
	Fenofibrate	50	3 ±2	<lod	100	100
	Gemfibrozil	46	108 ± 54	84 ± 46	22	58
	Mevastatin	52	83 ± 57	39 ± 16	53	77
	Pravastatin	53	54 ± 14	26 ± 12	52	77
Psychiatric drugs (J)	Carbamazepine	36	372 ± 69	387 ± 55	-4	33
	Diazepam	100	<lod	<lod	n.e.	100
	Fluoxetine	59	44 ± 9	44 ± 32	-1	58
	Lorazepam	45	120 ± 27	105 ± 25	12	52
	Paroxetine	67	13 ± 4	<lod	100	100
Receptor antagonists (K)	Cimetidine	35	31 ± 15	23 ± 11	24	51
	Famotidine	85	2 ± 1	<lod	100	100
	Loratadine	77	3 ± 2	2 ± 1	29	83
	Ranitidine	30	78 ± 26	46 ± 22	41	58
Anti-neoplastic (L)	Tamoxifen	n.e.	<lod	<lod	n.e.	n.e.

Table 5.3: Average concentrations measured during the observation period for the 12 therapeutic classes at the three sampling points, and average removal efficiency of the two steps for each class.

Therapeutic Class	WWTP Influent ng/L	H-SSF bed Influent ng/L	H-SSF bed Effluent ng/L	Removal in WWTP %	Removal in H-SSF bed %
Analgesics/anti-inflammatories	5107	1697	1326	66	22
Antibiotics	5034	2094	992	58	53
Anti-diabetics	87	55	42	37	24
Anti-hypertensives	2803	1165	432	58	63
Barbiturates	372	258	185	31	28
Beta-agonists	269	194	162	28	16
Beta-blockers	2924	1267	873	57	33
Diuretics	423	274	179	35	35
Lipid regulators	606	293	176	52	40
Psychiatric drugs	953	548	536	42	2
Receptor antagonists	185	114	72	38	36
Anti-neoplastics	<lod	<lod	<lod		

5.6.1 Removal efficiencies of the WWTP under study for the selected PhCs

The third column in Table 5.3 reports the average removal efficiency for the 73 compounds. As remarked in Section 0, average WWTP influent concentrations were already reported and discussed in Verlicchi et al. (2012a). It is also possible to obtain these values on the basis of data listed in Table 5.3, by dividing average secondary effluent concentrations with 100 — the corresponding average removal efficiency. Among the selected PhCs, 13 substances were not detected in either the WWTP influent or its effluent (Verlicchi et al., 2012a). As a consequence, it was not possible to evaluate their mean removal by the WWTP.

These substances were: phenazone, chlortetracycline, danofloxacin, doxycycline, enrofloxacin, josamycin, oxytetracycline, tetracycline, tylosin A, lisinopril, betaxolol, cerazolol and tamoxifen.

Table 5.3 reports “n.e.” (not evaluated) for these compounds. Among the remaining 60 substances, five (chloramphenicol, enalapril, nadolol, pindolol and diazepam) were detected in the WWTP influent, but not in the secondary effluent. Their removal during the passage through the secondary treatment was considered equal to 100% (see Table 5.2).

Fig. 2 shows the cumulative frequency curve of the average removal efficiencies evaluated on the basis of the collected WWTP influent and effluent data: the curve is quite smoothwithout any abrupt changes in slope. This would appear to confirm that the selected compounds exhibited quite different behaviours during their passage through the investigated AS system. 25–50% of most of the compounds were removed on average as shown in the bottom right panel of Fig. 5.2.

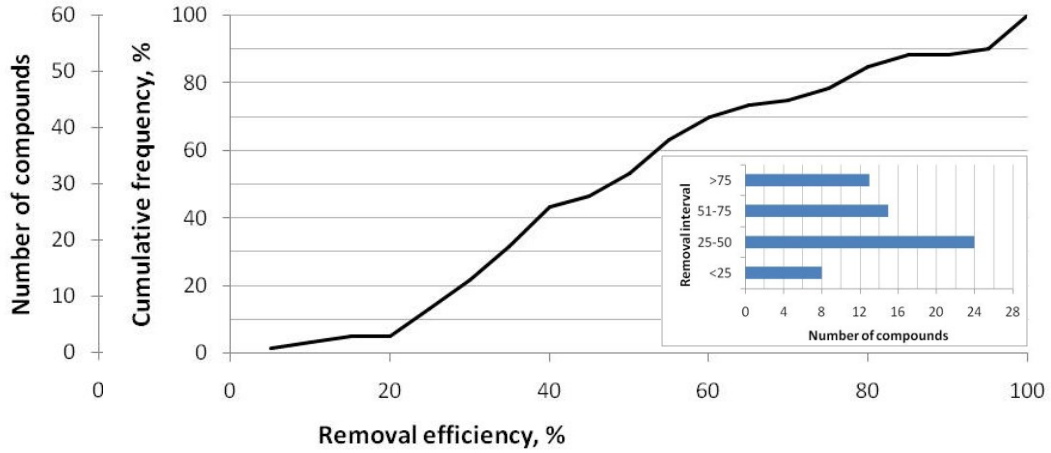


Fig. 5.2: Cumulative frequency curve for removal efficiency of the WWTP under investigation and number of compounds for selected removal intervals (in the lower right).

The mean WWTP removal efficiency of the entire group of compounds was equal to 52%, and the corresponding SD was 26.

Regarding analgesics and anti-inflammatory drugs, there is good agreement between these data and those reported by Radjenovic et al. (2007, 2009) in AS plants characterised by SRTs equal to 3 and 10 days respectively, and by Kimura et al. (2007) in an AS with an SRT equal to 7 days. Exceptions were, however, found for codeine, of which 82% was removed in a previous study (Wick et al., 2009, SRT = 18.5 days) (here only 38%) and indomethacin, whose corresponding removal efficiency was found to be lower (b10% and 23%) by Radjenovic et al. (2007, 2009).

Among the antibiotics, good agreement was found between our findings and previous works for ciprofloxacin (Zorita et al., 2009, with SRT = 8 days), clarithromycin and trimethoprim (Göbel et al., 2007, with SRT = 11 days), metronidazole and ofloxacin (Rosal et al., 2010), norfloxacin and roxithromycin (Li and Zhang, 2011 SRT = 12 days), and sulfamethazine and sulfamethoxazole (García-Galán et al., 2011). In contrast, discrepancies were found with respect to the literature, for azithromycin (Ghosh et al. (2009) found a removal of 39% in an AS characterised by a SRT = 18 days, whereas here a small release was found), erythromycin (Radjenovic et al. (2009) found a lower removal equal to 35%), spiramycin (Castiglioni et al. (2006) found no removal), and sulfadiazine (García-Galán et al. (2011) found a much higher removal equal to 78%). A similar comparison is not possible for the remaining compounds in the class due to lack of literature data.

Good agreement was also found with Radjenovic et al. (2007) (SRT = 3 days) for glibenclamide, with Rosal et al. (2010) for hydrochlorothiazide and ranitidine, with Vieno et al. (2007) for atenolol and sotalol, with Radjenovic et al. (2009) for metoprolol and pravastatin, with Alder et al. (2010) for propranolol, with Stumpf et al. (1999) for bezafibrate and gemfibrozil, with Bendz et al. (2005) for carbamazepine, with Zorita et al. (2009) for fluoxetine, and with Choi et al. (2008) for cimetidine.

However, considerable differences with respect to the literature were found for enalapril (Castiglioni et al. (2006) found a removal of 69%), phenobarbital (Yu et al. (2006) found a removal of 99% in an AS system with a SRT = 8–10 days), salbutamol (Jones et al. (2007) found a removal of 95% in an AS system with SRT = 13 days), furosemide and clofibric acid (Rosal et al. (2010) found 60% and 54% respectively), diazepam (Suárez et al. (2005) found a modest removal of 8%

with SRT = 60 days), paroxetine (Radjenovic et al. (2007) found a markedly lower removal of 8%), and famotidine and loratadine (Radjenovic et al. (2009) found lower removal efficiencies of 60% and 15%, respectively).

The average removal efficiencies of the WWTP observed for each therapeutic class h is reported in Table 5.3. These figures were obtained by means of eq. SD4, assuming j = 1, on the basis of average influent and effluent concentrations of each therapeutic class (second and third column of Table 5.3). The concentrations at the WWTP inlet are taken from previously published data (Verlicchi et al., 2012a). The best removal efficiencies found were for analgesics and antiinflammatories (66%), antibiotics and anti-hypertensives (58%), betablockers (57%) and lipid regulators (52%). The poorest removal was for beta-agonists (28%). Nevertheless, data reported in Table 5.3 should be interpreted with caution — the analysis refers only to the investigated compounds and not to all administered substances belonging to the same class. Moreover the numbers of compounds comprising each class in this study are different, ranging from 1 to 25 substances. Additionally, it is important to remark that for those classes represented by a small number of compounds, great differences may be observed between average removal efficiency for each single compound and average removal efficiency for the whole class, because the last parameter depends on all observed average concentrations of the compounds included in the class. As an example, for the class of beta-agonists only two compounds were investigated: clenbuterol and salbutamol. The sum of their average concentrations in the raw WWTP influent results is: 255 + 13 = 269 ng/L (see Verlicchi et al., 2012a). The sum of their average concentrations in the secondary effluent results is: 182 + 12 = 194 ng/L. On the basis of these values, the average WWTP removal efficiency for the beta agonists is equal to (194–162)/ 194 × 100 = 28%. The sum of their average concentrations in the polished effluent results is: 162 + 0.5 = 162.5 ng/L. On the basis of these values, the average H-SSF bed removal efficiency for the class of beta agonists is equal to (194–162.5)/194 × 100 = 16%. At the same time salbutamol was detected below its LOD value in the polished effluent.

5.6.2 PhC occurrence in H-SSF bed influent and effluent

Average concentrations and corresponding SDs for each of the 73 investigated compounds are reported in Table 5.2 for the bed influent and effluent (columns four and five). In addition, Figures Fig. 5.3 – Fig. 5.6 report the trend in the average influent and effluent concentrations, together with the corresponding observed average removal efficiencies for each selected compound, grouped according to its class, and reported in descending order with respect to the achieved removal efficiency.

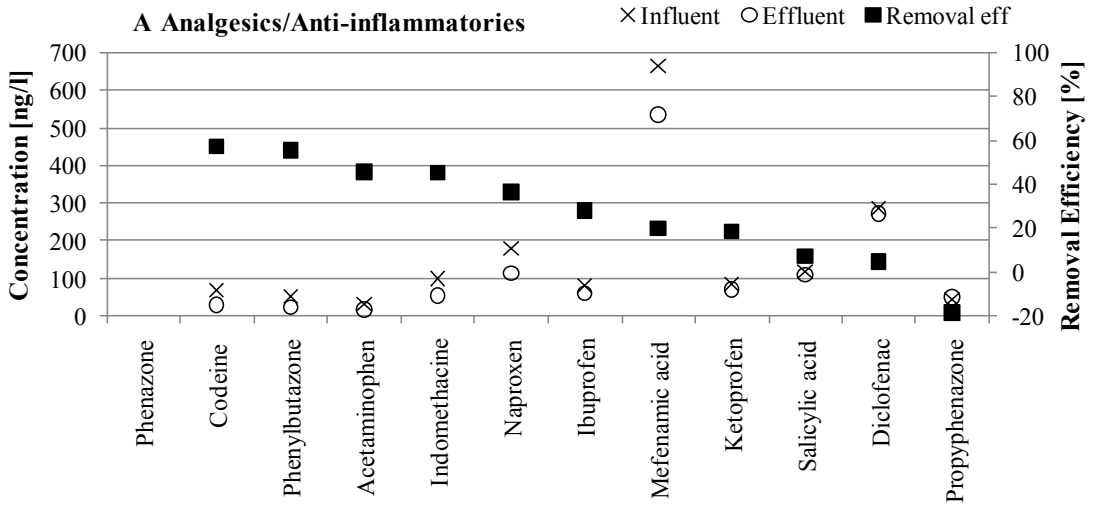


Fig. 5.3: Occurrence in the H-SSF bed influent and effluent for the selected analgesics and anti-inflammatories and observed average removal efficiency.

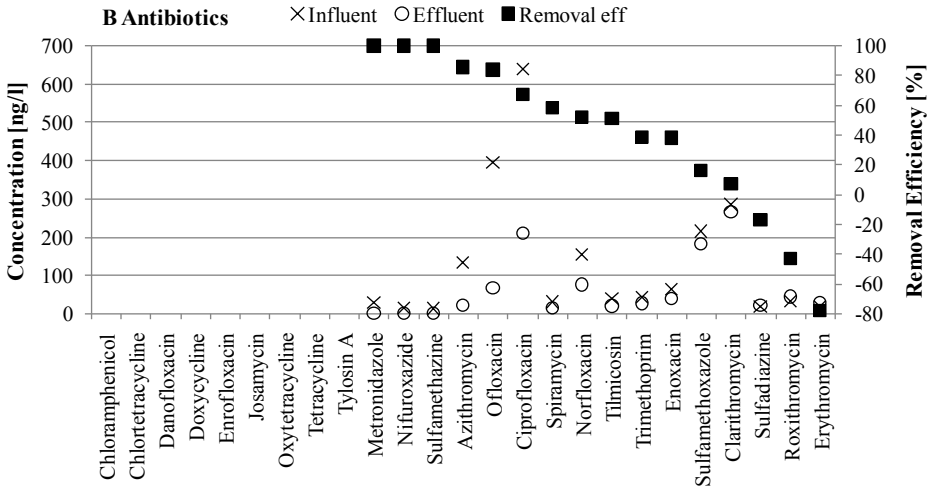


Fig. 5.4: Occurrence in the H-SSF bed influent and effluent for the selected antibiotics and observed average removal efficiency.

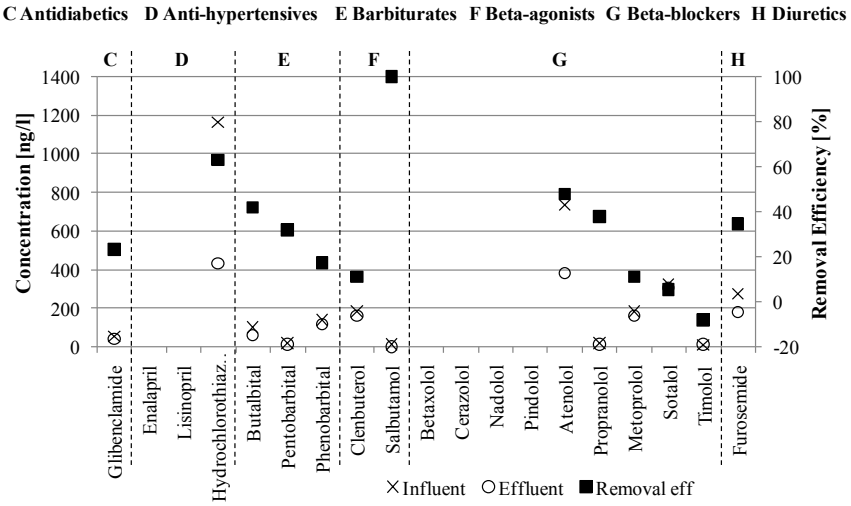


Fig. 5.5: Occurrence in the H-SSF bed influent and effluent for the selected PhCs belonging to therapeutic classes C-H and observed average removal efficiency.

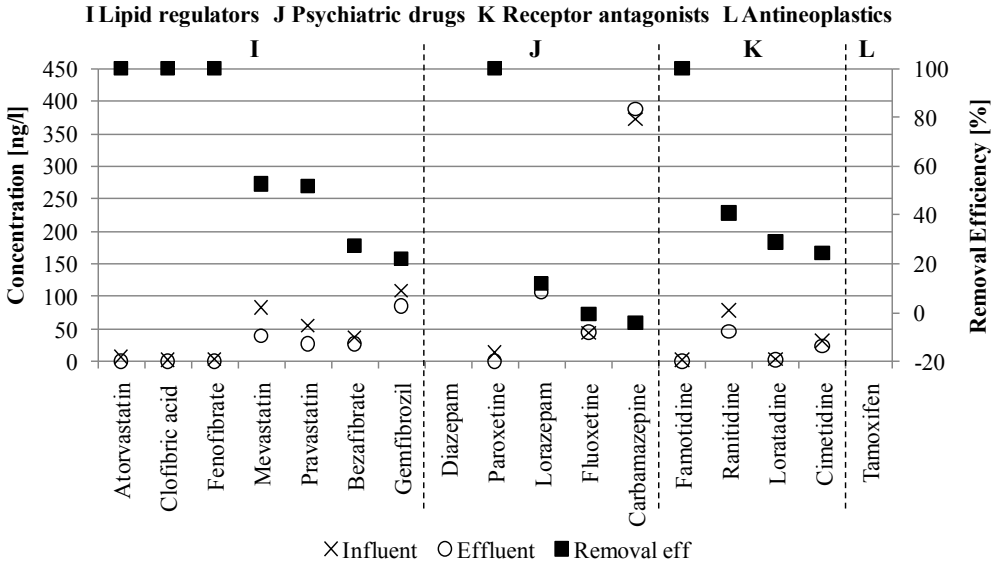


Fig. 5.6: Occurrence in the H-SSF bed influent and effluent for the selected PhCs belonging to therapeutic classe I-L and observed average removal efficiency.

Of the 73 investigated compounds, 55 were detected in the influent and only 46 in the polished effluent, all of which also occurred in the bed influent. A rapid glance at their SD values at the two sampling points reveals that SDs were higher in the influent than in the effluent for 35 of the 46 compounds detected at both sampling points. This leads us to suppose that the H-SSF bed has a "buffer capacity" with respect to most of the investigated pharmaceuticals, and tends to produce an effluent with smaller variability ranges of their concentrations with respect to the feed. This behaviour has already been noted inmany previous studies (among them Verlicchi et al. (2009)) for "macro-parameters" (namely conventional pollutants), including BOD_5, COD, SS, P compounds, and NH_4.

The highest average inlet concentrations were found for the antihypertensive hydrochlorothiazide (1165 ng/L), the beta-blocker tenolol (734 ng/L), the analgesic mefenamic acid (664 ng/L), and the antibiotic ciprofloxacin (638 ng/L), while the highest average values detected in the effluent were for mefenamic acid (533 ng/L) and hydrochlorothiazide (432 ng/L). All the other detected compounds showed average concentrations lower than 400 ng/L at both sampling points.

Further analysis of the average concentrations was performed with respect to two target values, set as 100 ng/L and 50 ng/L. The former value was chosen as it has been defined by the International Association of Waterworks in the Rhine catchment area (IAWR, 2008) and its members, and is included in the German Ministry environmental recommendations (UBA, 2008), as a micropollutant threshold value in water systems supplying drinking water. Accordingly, Beier et al. (2011) applied this target value to the effluent of a dedicated treatment plant receiving hospital wastewater in Germany. We chose to adopt the second target value of 50 ng/L to better investigate the behaviour of PhC concentrations at the half value proposed by IAWR (2008). The numbers of compounds with an average concentration >100 ng/L (Band A), between 50 and 100 ng/L (Band B) and b50 ng/L (Band C) in the influent and the effluent were evaluated, and the resulting data, reported in Table 5.2, evidenced that:

– Band A: out of the 21 compounds with a concentration >100 ng/L in the influent, only 16 occurred in the effluent at a concentration >100 ng/L, (those with a concentration >100 ng/L in the influent but b100 ng/L in the effluent were: the antibiotics azithromycin, norfloxacin and ofloxacin, the barbiturate butalbital and the lipid regulator gemfibrozil).

– Band B: in the influent, 10 compounds displayed a concentration of between 50 and 100 ng/L, while only 7 did so in the effluent. Among these 7, only 3 (ibuprofen, indomethacin and ketoprofen) were in the same B and C in the influent, the remaining 4 PhCs (norfloxacin and ofloxacin, butalbital and gemfibrozil) were in Band A in the influent. Codeine, phenylbutazone, enoxacin, glibenclamide, mevastatin, pravastatin and ranitidine were found in Band B in the influent and in Band C in the effluent.

– Band C: 24 compounds were detected at an average concentration <50 ng/L in the influent and 23 in the effluent. Of the latter, 15 compounds were found at a concentration b50 in the influent, one in Band A (azithromycin), 7 in Band B (codeine, phenylbutazone, enoxacin, glibenclamide, mevastatin, pravastatin and ranitidine). It is worth noting that these 23 do not include the 9 PhCs detected in the influent (in Band C) but not in the effluent.

– Finally, 18 compounds were not detected in the influent and 27 in the effluent. Out of these 27, 18 were the same as those undetected in the influent, and the other 9 were: metronidazole, nifuroxazide, sulphamethazine, salbutamol, atorvastatin, clofibric acid, fenofibrate, paroxetine, and famotidine.

This shows a reduction in the number of compounds belonging to each of the three groups, and the greatest reduction was found for the first band.

Out of the 46 compounds detected at both sampling points, 6 exhibited a higher average concentration in the effluent than in the influent, i.e., carbamazepine, roxithromycin, erythromycin, propyphenazone, sulfadiazine and timolol. The corresponding increments of average concentration in the effluent with respect to the influent were: 15, 13, 12, 8, 3 and 1 ng/L respectively. A more in-depth analysis will be conducted in the following paragraph to discuss the removal efficiencies observed for the different compounds.

A comparison between the influent and effluent PhC occurrence found here and corresponding data reported in the literature completes this analysis.While for the influent a great quantity of data is available, as it refers to an activated sludge plant effluent, very little has yet been published on the effluent of tertiary H-SSF bed.

Comparison with literature data: Secondary effluent — Fig. 5.7 – Fig. 5.11 compare the concentrations of PhCs found in the investigated H-SSF bed inlet with those reported in previous works on a considerable number of secondary AS effluents, taken from the recent review by Verlicchi et al. (2012c). In the graphs shown here, selected compounds are reported according to their therapeutic class. Squares (lightly shifted on the right with respect to reported literature data) refer to average concentrations found during this investigation and circles refer to those reported in the literature.

Fig. 5.7, which refers to analgesics/anti-inflammatories, shows good agreementwith the literature data, and figures obtained in this investigation fall within the literature intervals, with the exception of phenazone, whichwas not detected in this study butwas found by Rosal et al. (2010).

No literature data was found for phenylbutazone, and comparison is not therefore possible. Our figures are most similar to those reported by Gómez et al. (2007), Wick et al. (2009), Kasprzyk-Hordern et al. (2009), Ternes et al. (2003), Tauxe-Wuersch et al. (2005), and Nakada et al. (2006).

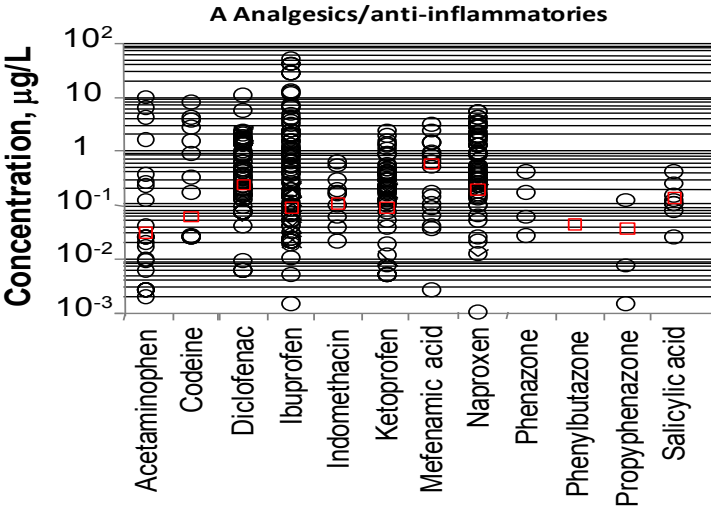


Fig. 5.7: Selected analgesics/anti-inflammatories: occurrence in secondary effluent (sampling point no. 2 in Fig. 5.1, full square) and comparison with literature data (empty circle). Literature data from: Andreozzi et al. (2005), Carballa et al. (2004), Choi et al. (2008), Clara et al. (2005a, 2005b), Coetsier et al. (2009), Foster (2007), Gómez et al. (2007), Kasprzyk-Hordern et al. (2009), Khan and Ongerth (2005), Kimet al. (2007), Kimura et al. (2005, 2007), Lishman et al. (2006), Muñoz et al. (2009a), Nakada et al. (2006), Paxéus (2004), Roberts and Thomas (2006), Rodriguez et al. (2003), Rosal et al. (2010), Santos et al. (2007, 2009), Stumpf et al. (1999), Tauxe-Wuersch et al. (2005), Ternes et al. (2003), Ternes (1998), Thomas and Foster (2005),Weigel et al. (2004), Wick et al. (2009), Yu et al. (2006), and Zorita et al. (2009)

A glance at the selected antibiotic trends reported in Fig. 5.8 shows good agreement with Göbel et al. (2005) for azithromycin, clarithromycin, roxithromycin and sulfamethoxazole, with Watkinson et al. (2007) for chlortetracycline, ciprofloxacin and tylosin, with Kasprzyk-Hordern et al. (2009) for erythromycin, and with Andreozzi et al. (2005) for ofloxacin and trimethoprim. Some antibiotics were not detected in this investigation, but were found in previous studies, namely chloramphenicol, reported by Kasprzyk-Hordern et al. (2009), doxycycline, detected by Lindberg et al. (2005), and enrofloxacin, oxytetracycline and tetracycline, found byWatkinson et al. (2007). Nifuroxazide, spyramicin, sulphamethazine and tilmicosin were detected in the secondary effluent in this investigation, but, as far as we know, no data for comparison is available in the literature. Finally danofloxacin was neither detected in this investigation nor has it been reported in other studies, to the best of our knowledge.

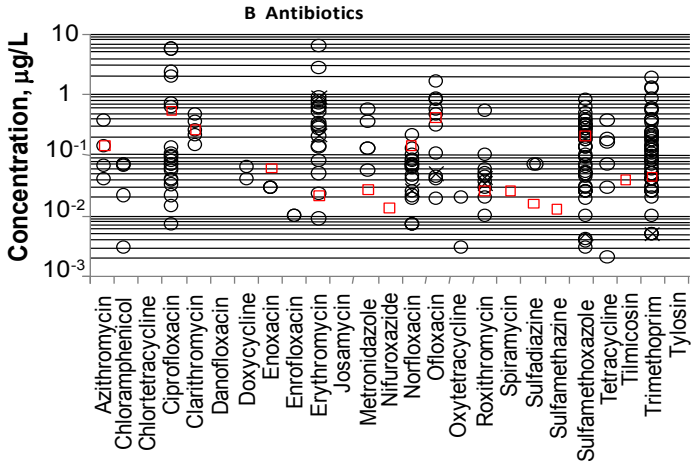


Fig. 5.8: Selected antibiotics: occurrence in secondary effluent (sampling point no. 2 in Fig. 5.1, full square) and comparison with literature data (empty circle). Literature data from: Andreozzi et al. (2005), Batt et al. (2006), Bendz et al. (2005), Brown et al. (2006), Carballa et al. (2004), Choi et al. (2008), Clara et al. (2005a, 2005b), Coetsier et al. (2009), Costanzo et al. (2005), Foster (2007),Golet et al. (2003), Gulkowska et al. (2008), Karthikeyan and Meyer (2006), Kasprzyk-Hordern et al. (2009), Kimet al. (2007), Lindberg et al. (2006), Lindqvist et al. (2005), Muñoz et al. (2009a), Paxéus (2004), Peng et al. (2006), Roberts and Thomas (2006), Rosal et al. (2010), Ruel et al. (2010), Ternes et al. (2003), Vieno et al. (2007), Watkinson et al. (2007), Xu et al. (2007), Yasojima et al. (2006), and Zorita et al. (2009).

The therapeutic classes reported in Fig. 5.9, good agreement was found with Rosal et al. (2010) for hydrochlorothiazide and atenolol, with Kasprzyk-Hordern et al. (2009) for salbutamol and furosemide, with Andreozzi et al. (2005) for metoprolol and propranolol, and with Alder et al. (2010) for sotalol. Clenbuterol was found at a slightly higher concentration than that reported by Ternes (1998). Glibenclamide, butalbital and pentobarbital were detected in the current study, but no literature data is available for comparison.

Phenobarbital was found in this investigation, while it was reported as bloq in the investigation by Yu et al. (2006).

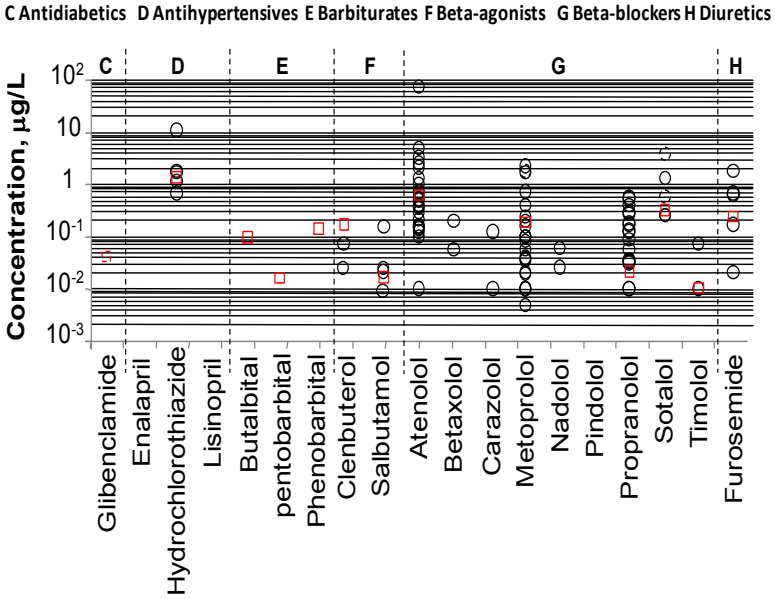


Fig. 5.9: Occurrence in secondary effluent (sampling point no. 2 in Fig. 5.1, full square) and comparison with literature data (empty circle) for selected antidiabetics, anti-hypertensives, barbiturates, beta-agonists, beta-blockers and diuretics. Literature data from: Andreozzi et al. (2005), Bendz et al. (2005), Choi et al. (2008), Clara et al. (2005a, 2005b), Coetsier et al. (2009), Foster (2007), Kasprzyk-Hordern et al. (2009), Muñoz et al. (2009a), Paxéus (2004), Roberts and Thomas (2006), Rosal et al. (2010), Ternes et al. (2003), Ternes (1998), Wick et al. (2009), and Yu et al. (2006).

With reference to Fig. 5.10, among the selected lipid regulators investigated there is good agreement between our findings and those reported by Rosal et al. (2010) for bezafibrate and gemfibrozil, and those by Kasprzyk-Hordern et al. (2009) for pravastatin. Fenofibrate was detected at one order of magnitude lower than that reported by Ternes (1998), clofibric acid was found to be lower than in the investigation by Rosal et al. (2010). For atorvastatin and mevastatin, literature data is not available. At a glance, all the reported literature data in Fig. 5.10 reveals that our average concentrations place in the lowest part of the published literature range for 4 compounds.

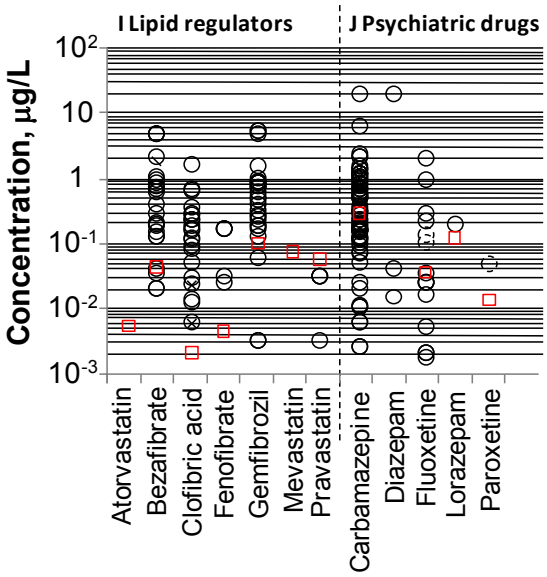


Fig. 5.10: Occurrence of selected lipid regulators and psychiatric drugs in secondary effluent (sampling point no. 2 in Fig. 1, full square) and comparison with literature data (empty circle). Literature data from: Andreozzi et al. (2005), Bendz et al. (2005), Choi et al. (2008), Clara et al. (2004, 2005a, 2005b), Coetsier et al. (2009), Foster (2007), Gómez et al. (2007), Kasprzyk-Hordern et al. (2009), Khan and Ongerth (2005), Kimet al. (2007), Kimura et al. (2005, 2007), Lindqvist et al. (2005), Lishman et al. (2006), Metcalfe et al. (2010), Muñoz et al. (2009a), Nakada et al. (2006), Paxéus (2004), Roberts and Thomas (2006), Rosal et al. (2010), Santos et al. (2007, 2009), Stumpf et al. (1999), Tauxe-Wuersch et al. (2005), Ternes et al. (2003), Ternes (1998), Vieno et al. (2005), Weigel et al. (2004), Wick et al. (2009), Yu et al. (2006), and Zorita et al. (2009).

A similar pattern is seen for the receptor antagonists cimetidine and ranitidine, for which data are in agreement with Choi et al. (2008) and Kasprzyk-Hordern et al. (2009), respectively (see Fig. 5.11).

Regarding famotidine and loratidine, no data are available for comparison.

The selected antineoplastic tamoxifen remained undetected in this investigation, while it was recorded by Coetsier et al. (2009). There are several different reasons for the lack of agreement between some of our findings and those previously published in literature.

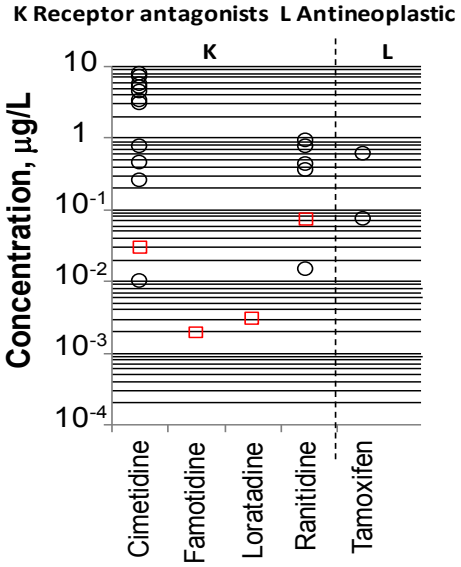


Fig. 5.11: Selected receptor antagonists and antineoplastics: occurrence in secondary effluent (sampling point no. 2 in Fig. 6.2, full square) and comparison with literature. data (empty circle). Literature data from: Choi et al. (2008), Coetsier et al. (2009), Kasprzyk-Hordern et al. (2009), Roberts and Thomas (2006), and Rosal et al. (2010).

In addition to the different sampling protocols adopted in the various investigations, some of these could be: different consumption patterns for the selected compounds, different biological reactor configurations, and different operational and environment conditions, which all influence PhC removal efficiency of biological treatments. An in-depth discussion is reported in Verlicchi et al. (2012c). Nevertheless, the above comparison of PhC occurrence does showthat the investigated H-SSF bed influent can be considered a typical effluent of an AS system, the most common treatment for domestic wastewaters around the world.

Comparison with literature data: H-SSF bed effluent — as mentioned, very little data is available in the literature for effluent from an H-SSF bed acting as a tertiary treatment. A comparison was, however, possible with data reported by Hijosa-Valsero et al. (2010a) for 5 analgesics/anti-inflammatories (ketoprofen, naproxen, ibuprofen, dicloenac, and salicylic acid) and one psychiatric drug (carbamazepine), detected in two different case studies (Cubillas de los Oteros and Bustillo

de Cea in Spain), in which the treatment trains include ponds/ surface flow constructed wetlands as a secondary step and H-SSF bed as a tertiary one. In that investigation, average PhC concentrations for both the influent and effluent were detected at one order of magnitude higher than those in the current investigation for almost all compounds.

Exceptions were diclofenac and naproxen in Bustillo de Cea and carbamazepine in both plants; for these substances average concentrations were of the same order of magnitude as those found in this study. To complete the discussion about the occurrence of PhCs in H-SSF bed influent and effluent, an analysis of the average concentrations $C_{av,h,p}$ of each therapeutic class was evaluated by means of eq. 5.1 (p = 2, 3) and reported in Table 5.2 (third and fourth columns). A reduction in the average values of each class at the third sampling point is evident, particularly for anti-hypertensives and antibiotics, while the lowest reduction was found for psychiatric drugs. As previously remarked, these figures should be considered cautiously due to the limited and different number of selected compounds in each class.

Nevertheless, these figures do highlight a reduction in the average concentrations per therapeutic class after the biological treatment and the polishing treatment by means of an H-SSF bed.

5.6.3 Removal efficiencies in the H-SSF bed

Table 1 reports the average removal efficiencies of each investigated compound (sixth column), evaluated by means of eq. 5.3 and data collected during this investigation. The graph in Fig. 5.12 shows the trend in the corresponding cumulative frequency curve, and Fig. 5.3 – Fig. 5.6 show removal efficiency as well as mean inlet and outlet concentrations of each of the selected compounds.

The analysis presented herein implies that removal efficiencies should be discussed together with influent and effluent concentrations, as high removal efficiencies not always mean low final concentrations of the PhC. 18 PhCs out of the selected 73 were never detected; hence, it was not possible to evaluate the removal efficiency of the polishing treatment in their case. In the histogram at the bottom right of Fig. 5.12, these compounds are indicated as n.e. (not evaluated). For 7 compounds, a release occurred after slow filtration through the bed ($\eta_2 < 0$). This was quite modest ($-10\% < \eta_2 < 0\%$) for fluoxetine, carbamazepine and timolol, modest ($-20\% < \eta_2 < -10\%$) for sulfadiazine and propyphenazone, high for roxithromycin ($\eta_2 = -43\%$) and very high for erythromycin ($\eta_2 = -78\%$). Unfortunately it was not possible to make a comparison with literature as no data regarding these substances is available. This release could be attributable to the presence of substances, e.g., human metabolites, in the inflow to the treatment step, being transformed into the investigated PhC during treatment. It is also possible that the PhC molecules were retained on the surface or in the volume of suspended materials in the bed influent, and later released into the water column during its passage through the filling medium due to local variation in redox conditions and pH (Imfeld et al., 2009).

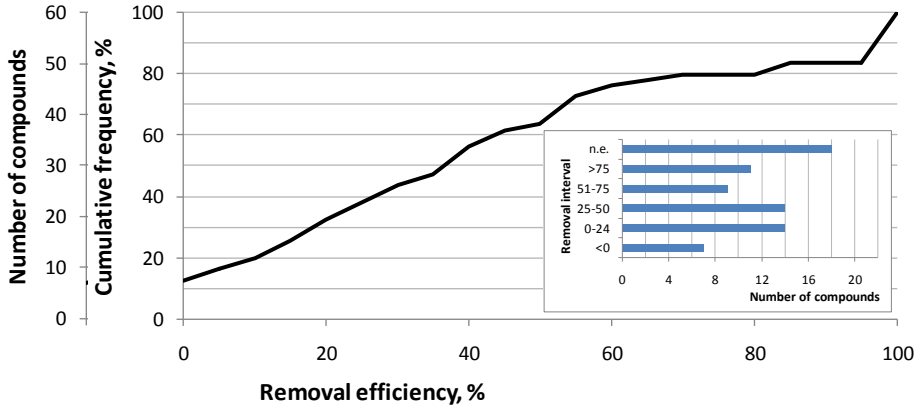


Fig. 5.12: Removal efficiency–cumulative frequency curve in the investigated H-SSF bed and number of compounds for the selected removal intervals (in the lower right).

Among the 11 investigated compounds exhibiting removal efficiencies higher than 75%, were ofloxacin and azithromycin, of which 84% and 86% were removed, respectively. For the remaining 9 compounds (metronidazole, nifuroxazide, sulfamethazine, salbutamol, atorvastatin, clofibric acid, fenofibrate, paroxetine and famotidine) the removal was assumed to be 100% as they were detected at a concentration lower than the corresponding detection limit in the polished effluent, but not in the influent.

As regards the 9 compounds with η_2 between 51 and 75%, namely codeine, phenylbutazone, mevastatin and pravastatin, 52–55% of these were removed on average, and their concentrations were quite similar at the two sampling points, i.e., in the range 50–83 ng/L in the influent and 23–39 ng/L in the effluent.

In contrast, the three antibiotics, norfloxacin, spiramycin and tilmicosin despite having different influent concentrations, which was higher for norfloxacin (152 ng/L) and lower for the remaining two (29 and 36 ng/L), showed similar removal (52, 58 and 51%,respectively). For ciprofloxacin and hydrochlorothiazide, although average removal efficiencies of 67 and 63% were observed, respectively, the influent concentrations were very high (638 and 1165 ng/L respectively), and consequently the two compounds still maintained high effluent concentrations (208 and 432 ng/L). Of the 14 compounds with removal efficiencies between 25 and 50%, the influent occurrence (98, 101, and 78 ng/L) and effluent concentrations (54, 59 and 46 ng/L) of indomethacin, butalbital and ranitidine were quite similar, as 41–45% of each were removed. In contrast, the four compounds atenolol, acetaminophen, trimethoprim and enoxacin had a similar average removal efficiency but far higher (the first) or lower (the other three compounds) influent concentrations.

In fact, 48% of atenolol was removed, but its influent concentration was on average 734 ng/L and its effluent one was accordingly still high (348 ng/L). Acetaminophen, trimethoprim and enoxacin showed average removal efficiencies of 45, 38 and 38% respectively, but their influent concentrations were 30, 40 and 61 ng/L, decreasing to 16, 25 and 38 ng/L in the effluent. Furosemide and naproxen also featured high average influent concentrations (274 and 178 ng/L), and, 35 and 36% being removed on average, respectively, they were still detected at high concentrations in the effluent (179 and 114 ng/L). Propranolol and pentobarbital had similar average removals, but their average occurrence was low, at 18 ng/L in the influent and around 11 ng/L in the effluent (for both). Bezafibrate, ibuprofen and loratadine had average removals of between 27 and 29%, but quite different average influent concentrations: 36, 81 and 3 ng/L. Of the remaining 14 PhCs, whose average removal efficiencies ranged between 0 and 24%, one (mefenamic acid) was detected at a very high influent concentration (664 ng/L), 10, were found, at a mean inlet concentration between 108 and 323 ng/L, and only three (ketoprofen, glibenclamide and cimetidine) at lower average concentrations, specifically in the range 35–85 ng/L. Due to the poor removal of these compounds, the average effluent concentrations remained greater than 100 ng/L for 10 compounds, with the exception of gemfibrozil and the three previously mentioned compounds.

A comparison of these figures with previous works is very difficult, as the latter generally refers to CWs acting as a secondary treatment or hybrid systems where H-SSF bed is the tertiary step, and in these cases the bed influent feed has far higher PhC concentrations than those measured in this investigation, as in Hijosa-Valsero et al. (2010a). Indeed, at the Cubillos de los Oteros plant, Hijosa-Valsero et al. (2010a) found average removal efficiencies of 37% for naproxen, 17% for ibuprofen, 30% for diclofenac, 31% for salicylic acid, and at Bustillo de Cea they detected a release of ibuprofen (−5.4%) and salicylic acid (−103%). The average removal efficiencies of each therapeutic class of the selected compounds were obtained by applying eq. SD4. Data reported in the last column of Table 2 show that the highest values were found for anti-hypertensives (63%) and antibiotics (53%), while the lowest was found for psychiatric drugs (2%).

5.6.4 Considerations about possible removal mechanisms within an H-SSF Bed

Despite this investigation only examining the water phase, and not considering sediments or filling material, an attempt to determine possible removal mechanisms for the investigated compounds during their passage through subsurface flow bed was carried out. This prompted a discussion about the removal mechanisms based on previous works on CWs and, in particular, H-SSF beds.

It is worth noting that experimental investigations aiming to explain the main removal mechanisms for PhCs in subsurface flow systems have been conducted on very few compounds: naproxen, ketoprofen, diclofenac, ibuprofen, salicylic acid and carbamazepine being the most studied (Matamoros et al., 2005; Matamoros and Bayona, 2006; Hijosa-Valsero et al., 2010a; Zhang et al., 2012). Moreover, as previously mentioned, they generally refer to H-SSF beds acting as a secondary treatment step, and in all cases the influent concentration to the bed was generally higher than that measured in the current study. In order to explain the removal mechanisms for the selected micro-pollutants, it is important to state that an H-SSF bed cannot be considered a homogeneous environment. Indeed, within the bed, several microenvironments coexist and allow both the thermodynamic feasibility of chemical reactions and the development of a great variety of microbiological communities able to guarantee the enzymatic capacity necessary to achieve the target biogeochemical reactions. This favours various metabolic pathways and therefore leads to PhC degradation via different mechanisms. This microenvironmental coexistence is due to the variation of physicochemical parameters on different gradients inside the CWs (Imfeld et al., 2009), some of which may be generated by the organisms inhabiting the CW or the presence of ramified roots within the medium. These tend to create aerobic zones near anoxic or anaerobic ones (Stottmeister et al., 2003; Imfeld et al., 2009), thereby establishing dynamic oxic/anoxic interfaces in wetlands as a result of water level fluctuations, oxygen diffusion/advection through the water column and filling medium, and active oxygen transport through the rhizosphere via plant tissues. In addition, the removal of PhCs is influenced by environmental temperature, redox potential (anaerobic, anoxic and oxic conditions) and pH conditions (Hijosa-Valsero et al., 2010a). The main removal pathways are biodegradation, sorption onto the filling medium, sedimentation, plant exudates and uptake (Imfeld et al., 2009).

Biodegradation — This is the most significant process for the removal of organic pollutants, and depends mainly on their intrinsic biodegradability, expressed in terms of a constant rate of biological reactions kbiol. This parameter is strictly correlated to the biological reactors where degradation occurs, specifically suspended biomass systems, including AS systems, membrane biological reactors and lagoons, and attached biomass systems, including trickling filters, and H-SSF beds (Joss et al., 2006; Radjenovic et al., 2009). It is likely that scarce, slow biodegradation can be ascribed to specific structural characteristics of the PhCs, for example two or more extended aromatic rings (see chemical structures of the investigated compounds in Table SD1 in the Supplementary Data). Previous studies have reported that:

– Ibuprofen ismainly removed by biodegradation: a shallow H-SSF bed is preferable to a deep one (Matamoros et al., 2005), and its removal is favoured by aerobic environments (Zwiener and Frimmel, 2003).
– Diclofenac is scarcely removed, and, based on the studies by Matamoros and Bayona (2006), it does not seem to be retained in the gravel matrix of the bed. According to Kimura et al. (2005) the presence of chlorine in its chemical structure makes it highly recalcitrant to biodegradation. In contrast, recent studies (Ávila et al., 2010) revealed that very high removal efficiencies can be reached by anaerobic degradation, confirming the results by Schwarzenbach et al. (2003) that polyhalogenated compounds, such as diclofenac, can be degradated by reductive dehalogenation.
– Naproxen is mainly biodegraded under aerobic conditions (Quintana et al., 2005) and in anoxic environments with respect to anaerobic conditions (Matamoros and Bayona, 2006).
– Salicylic acid is found to be easily degraded in all surface and subsurface flow systems (Hijosa-Valsero et al., 2010a), and its removal is favoured by high redox potential environments (Matamoros and Bayona, 2006).
– Acetaminophen is found to easily biodegrade in subsurface flow systems following a first order model (Ranieri et al., 2011).

Sorption and sedimentation — Sorption occurs when a pollutant creates interactions at the surface or in the volume of a particulate matter. Sedimentation, on the other hand, takes place when a pollutant becomes associated to a particle of organic material that settles, or it is mechanically retained within the grains of the filling bed.

Sorption may be due to: (i) hydrophobic interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms or the lipid fraction of the

suspended solids, or to (ii) electrostatic interactions between positively charged groups on the chemical and the negatively charged surfaces of microorganisms.
The phase distribution, sorption and hydrophobicity of a compound are strictly dependant on many factors, namely environmental pH, redox potential, stereochemical structure and chemical nature of both the sorbent (sediment, filling material, and particulate organic matter) and the sorbedmolecule,which determine its affinity for the particulate matter in question (Matamoros et al., 2005). In addition, some compounds contain planar aromatic structures, which tend to favour intercalation between the layers of solids. For these reasons, PhC sorption does not only depend on log Kow (measuring how lipophilic a compound is), and can be better expressed in terms of the organic carbon partition coefficient Koc (Imfeld et al., 2009), or, as many other authors suggest, the coefficient Kd, which is the quantity of the compound sorbed to the solid with respect to the amount remaining in solution at equilibrium. This latter coefficient depends heavily on the selected compound, the matrix, pH, and type of biological reactor, and for this reason it has to be experimentally evaluated. It has already been determined for ibuprofen by Matamoros and Bayona (2006) in an H-SSF bed. Sorption can be considered negligible (b10%) for compounds with Kd b 300 L/kg. Previous studies (Matamoros et al., 2005; Imfeld et al., 2009) investigated the behaviour of carbamazepine in an H-SSF bed. They found that carbamazepine is one of the most recalcitrant PhCs due to its high hydrophobicity (log Kow is 3.5) and scarcely able to sorb onto particles, and that its “main” removal pathway is by retention and adsorption onto the organic surfaces available in the H-SSF bed. It is also possible that when sorption–desorption equilibrium is reached, the contaminant will be “reversibly” retained and then released into the water column (Imfeld et al., 2009).

Plants — According to the investigation by Zhang et al. (2012), carried out on a pilot-scale H-SSF bed treating synthetic feed, removal efficiencies were found to be higher in planted beds than in unplanted ones for naproxen, diclofenac, ibuprofen, ketoprofen and clofibric acid. Only for carbamazepine and clofibric acid (among the most recalcitrant compounds) were the removal efficiencies not consistently different. The enhancement in planted beds could be attributed to the stimulatory effects of oxygen introduced into the subsurface by oxygen exudation into the rhizosphere by the higher aquatic plants (Hijosa-Valsero et al., 2010a). Root exudates released by the plant in the rhizosphere also result in intense local microbial activity (Brimecombe et al., 2001) and can therefore enhance overall bioavailability of PhCs.

Factors Affecting Removal Efficiencies

Hydraulic retention time HRT — This is an important parameter for the empirical design and operation of H-SSF beds. According to Zhang et al. (2012), the removal efficiencies for salicylic acid, ketoprofen, clofibric acid were linearly proportional to the influent mass loading rate at HRTs ranging between 2 and 6 days, making it possible to describe the removal of these substances by a constant first-order kinetic decay.

Temperature — High temperatures improve the removal of some compounds. Zhang et al. (2012) found that at tropical temperatures, ketoprofen was better removed than in temperate climates; Similarly, Hijosa-Valsero et al. (2010b) found higher removal efficiencies in summer than in winter.

Redox potential RP — It seems that anoxic (−100 mV b RP b 100 mV) and aerobic (RP > 100 mV) conditions favour the biodegradation of organic micropollutants through the promotion of biogeochemical reactions (Matamoros et al., 2008a).

Many attempts have been made to find a relationship between the physical and chemical characteristics of PhCs (for instance octanol water partition coefficient K_{ow}, Henry's constant H, water solubility S_w, vapour pressure P_v, organic carbon partition coefficient K_{oc}, acid dissociation constant K_a) and their behaviour in constructed wetland systems (Imfeld et al., 2009; Kümmerer, 2009; Park et al., 2009), but no clear correlation has yet been found due to the great variability of compounds and their respective behaviours. For example, the graph in Fig. 5.13, which reports average removal efficiencies and log K_{ow} for the investigated compounds, shows that data are dispersed in a large cloud, confirming that, as postulated by Park et al. (2009) and Zhang et al.

(2012), hydrophobicity (log K_{ow}) alone is not a useful parameter for predicting the behaviour of a PhC in an H-SSF bed.

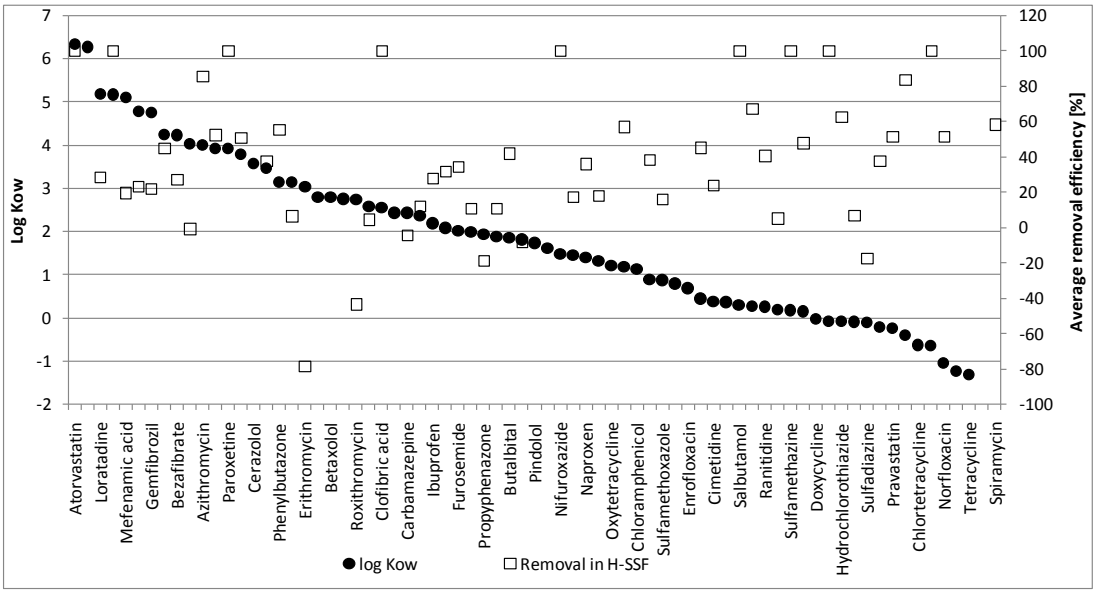


Fig. 5.13: Percentage average removal efficiency profiles and hydrophobicity (log Kow) of the selected compounds.

5.6.5 Relative Contributions of AS and H-SSF Bed to PhC Removal Efficiency

The specific contributions of the two treatment steps (AS and H-SSF bed) to the overall removal efficiency were assessed for each PhC i. For AS, eq. 5.3 was applied, assuming j = 1 (providing ηav,i, 1), while for the H-SSF bed, eq. 5.5 was used (providing η_{HSSF} bed/overall, i). Fig. 5.14 and Fig. 5.15 report their respective contributions to the overall percentage removal efficiencies for the investigated compounds. For those compounds not detected at any sampling point, no histogram is reported.

Results show that the contribution of the H-SSF bed varies between −27% and 89%, being on average 17% (SD = 22). In particular, it was:

– Excellent (η H-SSF bed/overall, i > 75%) for salbutamol (89%) and azithromycin (86%).

– Good (50 b η H-SSF bed/overall, i ≤ 75) for metronidazole (66%), sulfamethazine (59%) and atorvastatin (52%).

– Modest (26 b η H-SSF bed/overall, i ≤ 50) for twelve compounds.

– Poor (0 b η H-SSF bed/overall, i ≤ 25) for 25 compounds.

– Negative (i.e., a release occurred) for seven substances, namely fluoxetine (−0.24%), carbamazepine (−3%), timolol (−6%), sulfadiazine (−14%), propyphenazone (−15%), roxithromycin (−20%) and erythromycin (−27%).

As complete removal of 5 compounds, namely chloramphenicol, enalapril, nadolol, pindolol and diazepam, was achieved in the AS, the corresponding histograms in the Figures refer only to AS, and no considerations can be made with regard to the hypothetical contribution of the H-SSF bed to their removal.

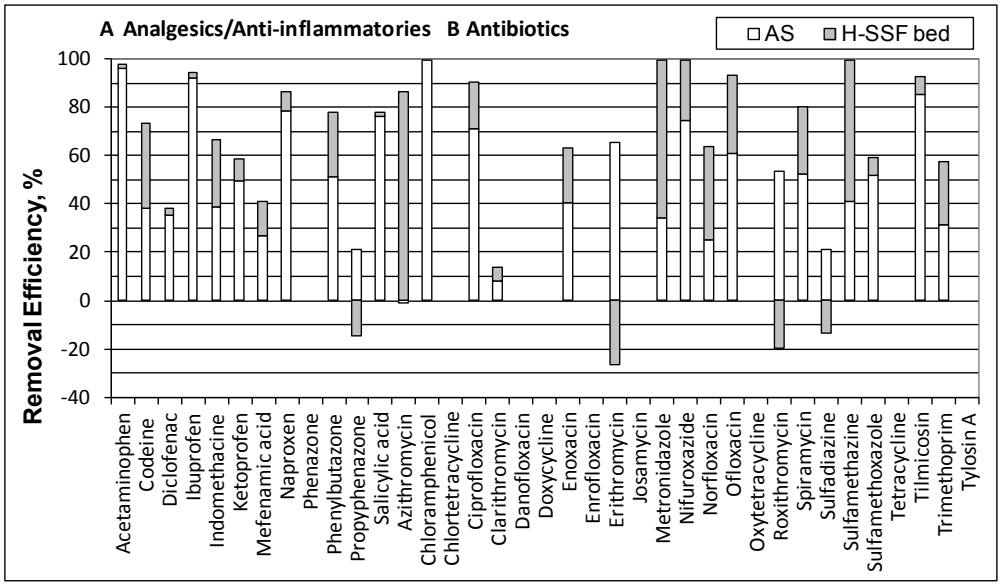


Fig. 5.14: Global average removal efficiencies for the selected analgesics/anti-inflammatories and antibiotics and relative contributions of each step.

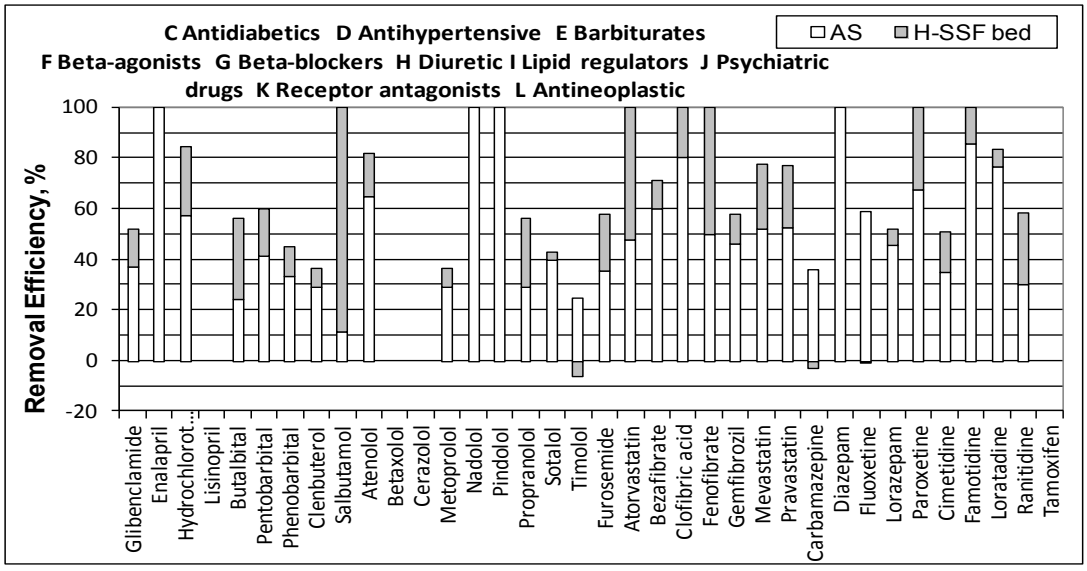


Fig. 5.15: Global average removal efficiencies for the remaining selected PhCs belonging to the other classes and the relative contributions of the two steps.

The ability of the investigated CW in removing the selected compounds is well documented by the graph shown in Fig. 5.16, which reports the mean overall removal efficiency, $\eta_{av,h,3}$, for each therapeutic class (obtained by means of eq. SD4), as well as the relative contributions of AS (obtained by applying eq. 5.4) and the H-SSF bed (calculates using eq. 5.7). This shows that the H-SSF bed manages to further reduce the overall concentration of each class, thereby improving the quality of the final polished effluent. Its contribution varies between 1% (psychiatric drugs) and 26% (anti-hypertensives), and is, on average, 16% (SD = 8). The bed showed a similar contribution (19–26%) in the removal of lipid regulators, anti-hypertensives, barbiturates, diuretics and antibiotics, all classes that include PhCs that are frequently administered for long periods or frequently consumed by the population.

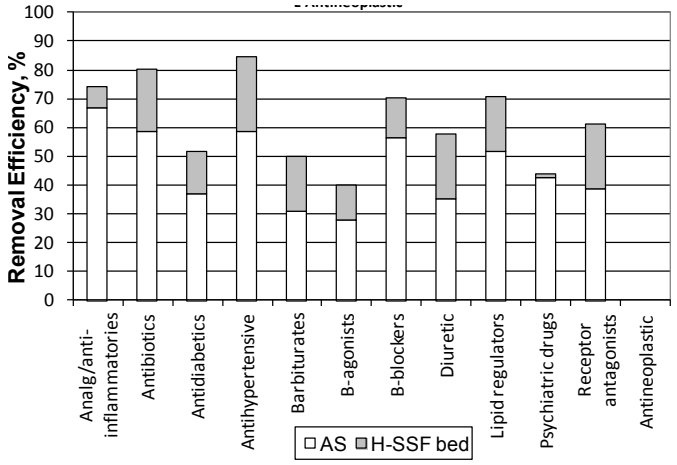


Fig. 5.16: Global average removal efficiencies for the therapeutic classes investigated and the relative contributions of the two steps.

In order to complete the evaluation of the contribution of the H-SSF bed as a polishing treatment for domestic wastewaters, an analysis and comparison of the mass loads of each of the therapeutic classes of interest at the three sampling points (raw wastewater, secondary and polished effluents) was carried out. Indeed, Table 5.4 reports the mass loads, referring to hypothetical small communities of 1000 inhabitants, on the basis of the observed average concentration discussed above (Table 5.2) and the average flow rate and PE served by the WWTP under investigation (respectively 28,000 m^3/day and 120,000 PE). This shows that the main contaminants are analgesics/anti-inflammatories, followed by antibiotics, in both the raw wastewater and the polished effluent, while in the secondary effluent the major pollutants are antibiotics, followed by analgesics/ anti-inflammatories.

In fact, the total mass load in raw domestic influent amounts to 4.38 g/(1000 PE day). After AS including nitrification–denitrification steps, this falls to 1.86 g/(10,000 PE day), and after a further

polishing horizontal subsurface flow treatment it is reduced to 1.16 g/(1000 PE day). These results highlight the fact that the internal and (often) persistent (micropollutant) mass loading of PhCs from a secondary effluent can be reduced by a polishing treatment able to favour different removal pathways, which are necessary due to the great variability of the contaminants in question.

If the receiving river is an effluent-dominant water body, it becomes necessary to improve the quality of the discharge in order to reduce its long-term environmental impact. Although CWs require a high surface/PE ratio, these “natural” polishing treatments represent suitable solutions for small communities, or for the last treatment step in treatment tailored to specific users, such as health care or hospital facilities, whose wastewaters need more attention paid to the removal of pharmaceutical micropollutants (Verlicchi et al., 2010b). In fact, Matamoros et al. (2008b) found that when these natural tertiary treatments are compared with advanced oxidation treatments like ozonation (Zwiener and Frimmel, 2000) or membrane bioreactors (Kimura et al., 2005), the PhC removal efficiencies are very similar.

Table 5.4: Specific mass loads in raw urban wastewaters produced by a hypothetical urban settlement of 1000 inhabitants and secondary and polished effluents treated by means of an H-SSF bed.

Therapeutic Class	Raw domestic wastewater g/(1000 PE d)	Secondary effluent g/(1000 PE d)	Polished effluent g/(1000 PE d)
Analgesics/anti-infl.	1.19	0.40	0.31
Antibiotics	1.17	0.49	0.23
Anti-diabetics	0.02	0.01	0.01
Antihypertensives	0.65	0.27	0.10
Barbiturates	0.09	0.06	0.04
Beta-agonists	0.06	0.05	0.04
Beta-blockers	0.68	0.30	0.20
Diuretics	0.10	0.06	0.04
Lipid regulators	0.14	0.07	0.04
Psychiatric drugs	0.22	0.13	0.13
Beta-antagonists	0.04	0.03	0.02
Total PhCs	4.38	1.86	1.16

5.7 Conclusions

The investigated constructed wetland did show a contribution in the removal of all therapeutic classes (from 1% for psychiatric drugs to 26% for antihypertensives, on average 16%, with an SD of 8), thereby suggesting that pharmaceutical residues can be dealt with by investment in this kind of tertiary treatment, especially for trains featuring a small flow rate. Evaluation of the mass loading in the raw wastewater, in the secondary effluent and effluent polished by H-SSF bed highlights the fact that suitable treatment can further reduce unavoidable (and persistent) micropollutant loading.

CWs are usually suggested to treat the effluent from medium–low urban settlements due to their large area requirement (extensive treatment), contributing in this way to mitigating the effect of modest flow rate of treated water discharged into surface water systems. Our results seem to confirm their ability to reduce the spread of PhC contamination in the environment. Due to the high surface/PE required H-SSF beds could be recommended for small communities, and also for specific users like hospitals and health care facilities, for which a tailored treatment system would be advisable. In larger communities, other treatments would be more suitable, but a system in which different microenvironmental conditions coexist, and hence provide different removal mechanisms, they could be extremely useful in limiting residual micropollutant concentrations.

That being said, due to the complex behaviour of these persistent compounds in conventional secondary treatments and after CW polishing (where very little data is available), it is difficult to determine the dominant removal mechanisms for the various kinds of investigated compounds, and further research is needed to elucidate the removal mechanisms in H-SSF beds, further to improving their abatement efficiencies.

Reference

- Verlicchi P, Galletti A, PetrovicM, Barceló D, Al Aukidy M, Zambello E. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed—analysis of their respective contributions. Sci Total Environ 2013b;454–455:411–25.

Part B

Hospital Wastewater

Occurrence, Treatment and Management

Chapter 6

6 Management and treatment of hospital wastewater

6.1 Introduction

In recent years, hospital effluent has been the object of study and research in various countries throughout the world facing different issues.

The specific driving and inspiring force has been to improve the knowledge of the chemical and physical characterization of such wastewater for conventional parameters, namely BOD_5, COD, TSS, N and P compounds, pH and T (Sarafraz et al., 2007; Verlicchi et al., 2012a); the microbiological load of hospital effluent and also the risk of the spread of antibiotic resistant bacteria (Boillot et al., 2008; Chitnis et al., 2004); differences in composition between hospital effluent and urbanwastewater (UWW) (Verlicchi et al., 2010a); seasonal variation of hospital effluent compositions (Verlicchi et al., 2012a, 2012c); strategies in their management (co-treatment or dedicated treatment with UWW) (Pauwels and Verstraete, 2006; Verlicchi et al., 2010a); evaluation of the adequacy of adopted treatment strategies with respect to the removal of specific contaminants (Mesdaghinia et al., 2009; Beier et al., 2010); technical and economic feasibility of dedicated treatment trains for hospitalwastewater (HWW) (PILLS Report, 2012); and contribution of hospital effluent to the influent of a municipal wastewater treatment plant (WWTP) (Verlicchi et al., 2012a; Santos et al., 2013). On occasion, the occurrence of disease outbreaks due to pathogens occurring in sewage, such as SARS (severe acute respiratory syndrome) in China in 2003, has led scientists to develop specific research projects to identify safety measures to rapidly adopt in existingWWTPs, in particular in plants receiving hospital effluent, not only to deal with the current emergency, but also to prevent further ones (Wang et al., 2005).

Quite rarely, national (or regional) legal regulations have been established to define how to manage and treat hospital effluent before its disposal (discharge in public sewage for treatment at a municipal WWTP or discharge into a surface water body) (Boillot et al., 2008; Verlicchi et al., 2010a). Indeed, hospital effluent was and (still) is generally considered of the same pollutant nature asUWWand thus it is commonly discharged in public sewage systems, conveyed to an urban WWTPwhere it is subjected to conventional treatment, often consisting in primary clarification, activated sludge process and sometimes disinfection. This practice is very common although recent studies (Verlicchi et al., 2010a; Santos et al., 2013; McArdell et al., 2011) highlighted that higher concentrations of pharmaceuticals (PhCs), disinfectants and X-ray contrast media occur in hospital effluent as well as a microbiological load exhibiting a higher resistance to treatment (Chitnis et al., 2004).

Municipal WWTPs were conceived and, in some cases, recently upgraded to guarantee a high removal efficiency of carbon, nitrogen and phosphorus compounds, as well as microorganisms (mainly bacteria): pollutants regularly arriving with and occurring in the WWTP influent at concentrations in the order of units (P compounds), tens (NH_4, TKN) and hundreds (COD, BOD_5) of mg/L and thousands of MPN/100 mL (Escherichia coli).

Commonly adopted treatments at municipal WWTPs include: preliminary treatments, (sometimes) primary clarification, secondary biological (usually consisting in a conventional activated sludge – CAS – process), and polishing treatments (chemical disinfection or sometimes rapid filtration followed by UV disinfection). Unfortunately, these WWTPs are not adequate enough to reach high removal efficiencies for the wide spectrum of micropollutants (PhCs, adsorbable organic compounds commonly known with the acronym AOXs) commonly present in hospital effluent.

They are also among the main sources of antibiotic release into the environment and thus they may promote the selection of antibiotic resistant genes (ARG) and antibiotic resistant bacteria (ARB), as deeply investigated in Rizzo et al. (2013). Moreover, in some circumstances, conventional treatments have been adopted for HWW, but they are not well managed and very low efficiencies are achieved even for common parameters, namely BOD_5, COD, TSS and total coliform (Mesdaghinia et al., 2009). Sometimes, a simple primary treatment is adopted for hospital effluent (primary clarification, prechlorination) but it is not efficient (Martins et al., 2008).

In other cases, no treatment is adopted at all and direct discharge of raw HWW into surface rivers is a common practice (Liu et al.,2010).

The main focus of this study is to present and discuss lessons learned from previous investigations and studies carried out on dedicated treatment of HWWin the different countries worldwide. It offers a critical analysis of data collected from lab, pilot and full scale treatment plants acting as primary, secondary and tertiary steps. Attention is paid to the removal efficiencies observed for contaminants, including conventional parameters but in particular emerging ones: mainly PhCs, detergents and disinfectants. The analysis also compares the assessment of investment and operational costs for each applied technology.

6.2 Object and framework of the survey

This study is based on 48 publications regarding investigations into the dedicated treatment of hospital effluent in lab, pilot and full scale plants acting as primary, secondary and tertiary steps. They were carried out in 24 different countries all over the world between 1995 and 2015 as shown in Fig. 6.1.

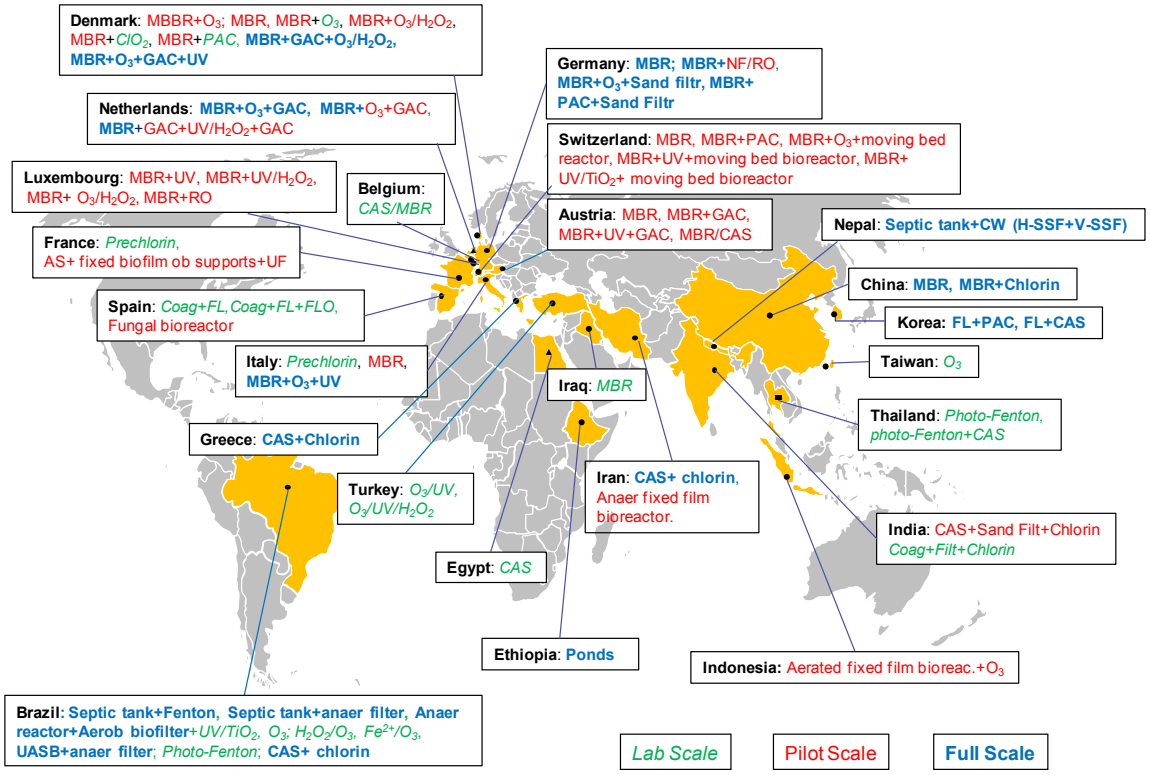


Fig. 6.1: World map of the investigations on dedicated treatment for hospital effluent between 1995-2015

Collected data that are presented and discussed herein mainly refer to observed removal efficiencies for 108 PhCs belonging to 17 different classes (see Table 6.1).

Table 6.1: Selected contaminants included in the review and corresponding class

Class	Compounds
Analgesics/anti-inflammatories (20)	4-Acetamidoantipyrine, 4-Aminoantipyrine, 4-Dimethylaminoantipyrine, 4-Formylaminoantipyrine, 4-Methylaminoantipyrine, Acetylsalicylic acid, Codeine, Dexamethasone, Diclofenac, Ibuprofen, Indomethacin, Ketoprofen, Mefenamic acid, Methylprednisolone, Morphine, Naproxen, Paracetamol, Phenazone, Salicylic acid, Tramadol
Anesthetics (1)	Lidocaine
Anthelmintics (5)	Albendazole, Fenbendazole, Flubendazole, Oxfendazole, Thiabendazole
Antibiotics (23)	Amoxicillin, Azithromycin, Cefuroxim, Chloramphenicol, Cilastatin, Ciprofloxacin, Clarithromycin, Clindamycin, Lindamycin, Erythromycin, Metronidazole, Moxifloxacin, N4-Acetylsulfamethoxazole, Norfloxacin, Ofloxacin, Roxithromycin, SMX + N4-AcSMX, Sulfadiazine, Sulfamethazine, Sulfamethizole, Sulfamethoxazole, Sulfapyridine, Trimethoprim
Antifungals (1)	Fluconazole
Antihypertensives (6)	D617 (metabolite of Verapamil), Furosemide, Hydrochlorothiazide, Telmisartan, Valsartan, Verapamil
Antineoplastics (6)	Capecitabine, Cyclophosphamide, CPC (cancerogenic platinum compounds), Gemcitabine, Ifosfamide, Tamoxifen
Antiseptics (1)	Triclosan
Antivirals (5)	4/5-methylbenzotriazole, Benzotriazole, Oseltamivir, Oseltamivir carboxylate, Ritonavir
Beta-blockers (6)	Atenolol, Atenolol acid, Bisoprolol, Metoprolol, Propranolol, Sotalol
Contrast media (9)	Amidotrizoe acid, Diatrizoate, Iodixanol, Iohexol, Iomeprol, Iopamidol, Iopromide, Ioversol, Ioxitalamic acid
Fragrances (3)	Celestolide, Galaxolide, Tonalide
Hormones (4)	17 α-ethinylestradiol, 17 α-estradiol, 17 β-estradiol, Estron
Lipid regulators (4)	Bezafibrate, Clofibric acid, Fenofibrate, Gemfibrozil
Psychiatric drugs (12)	Carbamazepine, Citalopram, Diazepam, Fluoxetine, Gabapentin, Levetiracetam, Oxazepam, Oxcarbazepine, Primidone, Ritalinic acid, Thiopental, Venlafaxine
Receptor antagonists (1)	Ranitidine
Stimulants (1)	Caffeine

Moreover, conventional pollutants (BOD_5, COD, SS, N and P compounds, microorganisms…) are also reported and discussed.

In discussing removal efficiencies of selected PhCs observed for the different treatment technologies and steps, particular attention is paid to the potential capacity of each technology in retaining/degrading specific compounds and, when possible, to the operational conditions which could maximize them. Data are presented in graphs in the manuscript and further details are provided in tables in the Supplementary data.

All removal values reported and discussed (in the following graphs and tables) must be considered with the necessary caution, bearing in mind their origin and that they may be affected by many factors, namely:

• influent characteristics (macro- and micro-pollutant concentrations),

• operational conditions (sludge concentration, sludge retention time (SRT), hydraulic retention time (HRT), pH, temperature (T), feeding mode, dosage of ozone, H_2O_2, UV irradiation, catalyst type and contact time),

• reactor types (conventional activated sludge system or membrane bioreactor (MBR); compartmentalization),

• environmental conditions (temperature, irradiation), and

• water sampling mode and frequency.

Before discussing the main results derived from these studies, a snapshot of the main chemical, physical and microbiological characteristics of HWW is provided in Table 6.2. References are also provided for each compiled parameter or class of compounds of PhCs.

To ease the reading of the manuscript, a brief presentation of each investigation is reported in Table 6.3 and the list of all the investigated treatment trains is provided in Table 6.4 with the corresponding references.

Table 6.2: Main chemical characteristics of hospital effluent in terms of conventional parameters and pharmaceuticals and other emerging compounds

Parameter	Range of concentrations	Reference
Conductivity, μS/cm	300-1000	Verlicchi et al., 2012d
pH	6-8	PILLS Report, 2012, Kosma et al., 2010
Redox potential, mV	100	Verlicchi et al., 2010a
Fat and oil, mg/L	50-100	Verlicchi et al., 2010a
Chlorides, mg/L	80-400	Verlicchi et al., 2012d
Total N, mg N/L	60-98	PILLS Report, 2012, Beyene and Redaie, 2011
NH_4, mgNH_4/L	10-68	McArdell et al., 2011, Verlicchi et al., 2012d Wen et al., 2004
Nitrite, mg NO_2/L	0.1	McArdell et al., 2011
Nitrate, mgNO_3/L	2	McArdell et al., 2011
Phosphate, mg P-PO_4/L	13	Verlicchi et al., 2012d
Suspended solids, mg/L	120-400	Verlicchi et al., 2012d
COD, mg/L	1350-2480	Kajitvichyanukul and Suntronvipart 2006; Berto et al., 2009
Dissolved COD, mg/L	380-700	McArdell et al., 2011
DOC, mg/L	120-130	McArdell et al., 2011
TOC, mg/L	31-180	Beier, 2012, Nardi et al., 1995
BOD_5/COD (biodegradability index)	0.3-0.4	Kajitvichyanukul and Suntronvipart 2006
AOX, μg/L	550-10000	Nardi et al., 1995
Microrganisms MPN/100 mL		
E. coli	10^3-10^6	Beier et al., 2012, Nielsen et al., 2013
Enterococci	10^3-10^6	Beier et al., 2012
Fecal Coliform	10^3-10^4	Beier et al., 2012
Total Coliform	10^5-10^7	Beyene and Redaie 2011
EC_{50} (*Daphnia*), TU	9.8-117	Emmanuel et al., 2004; Machado et al., 2007
Total surfactants, mg/L	4-8	Verlicchi et al., 2008, 2010a
Total disinfectants, mg/L	2-200	Verlicchi et al., 2012d
Specific disinfectants:		
BAC_C12-18, μg/L	49	Kovalova et al., 2012
BAC_C12, μg/L	34	Kovalova et al., 2012
DDAC-C10, μg/L	102	Kovalova et al., 2012
Antibiotics, μg/L	30-200	Verlicchi et al., 2012d
Antinflammatories, μg/L	5-1500	Verlicchi et al., 2012d
Lipid regulators, μg/L	1-10	Verlicchi et al., 2012d
Cytostatic agents, μg/L	5-50	Suarez et al., 2009
ICM, μg/L	0.2-2600	Verlicchi et al., 2012d
Beta-blockers, μg/L	0.4-25	Verlicchi et al., 2012d

6.3 Technologies and treatment trains for HWW under review

Table 6.3 reports the main characteristics of the studies included in this review referring to the dedicated treatment of hospital effluent and the rationale behind each one.

A rapid glance at Table 6.3 points out that hospital effluent was subjected to different treatment levels: just a preliminary/primary (potential or actual) dedicated treatment before its co-treatment with UWW at a municipal WWTP, sometimes conventional secondary biological treatments (CAS) or modified CAS processes that are systems combining attached and suspended biomass, but also MBRs, and advanced oxidation processes (AOPs). In some countries AOPs were investigated as preliminary–primary treatments in order to enhance biodegradation in the stream.

In order to help in the reading of this review, Table 6.4 lists all the types of investigated technologies and treatment trains with the corresponding references. Their distribution in the different countries in the world can be found in Fig. 6.1.

Most of the investigations referred to pilot/lab scale plants (69%) and the remaining 31% to full scale dedicated facilities (see Table SD-1 in the Supplementary data of Verlicchi et al., 2015). The latter include the following treatment trains:

septic tank followed by an anaerobic filter (Brazil, de Almeida et al., 2013; Martins et al., 2008); UASB + anaerobic filters (Brazil, Prado et al., 2011); series of maturation and facultative ponds (Ethiopia, Beyene and Redaie, 2011); septic tank + constructed wetlands (HSSF + V-SSF beds) (Nepal, Shrestha et al., 2001); MBR (in Germany, Beier et al., 2011, 2012; in China: Liu et al., 2010; Wen et al., 2004); CAS + chlorination (in Greece, Kosma et al., 2010; in Brazil, Prado et al., 2011; in Iran, Mahvi et al., 2009); MBR + chlorination (in China, Liu et al., 2010); flocculation + activated carbon or flocculation + CAS (Republic of Korea, Sim et al., 2013); MBR + O_3 +UV (Italy, Verlicchi et al., 2010a); MBR + O_3 or PAC and then sand filtration (in Germany, PILLS Report, 2012); MBR + O_3 + GAC (a full scale demo plant called Pharmafilter operating in the Netherlands, Pharmafilter report, 2013); and MBR + GAC + O_3/H_2O_2 and MBR + GAC + UV (in Denmark, Grundfos biobooster, 2012).

Moreover, 53% of the studieswere carried out in European countries (Austria, Belgium, Denmark, France, Germany, Greece, Italy, Luxembourg, Netherlands, Switzerland and Turkey), 27% in Asiatic countries (China, India, Indonesia, Iran, Iraq, Nepal, Republic of Korea, Thailand and Taiwan), 16% in South America (Brazil) and 4% in Africa (Egypt and Ethiopia). PhCs were detected and removal efficiencies were evaluated in 60% of the studies included, whereas the remaining ones only refer to conventional parameters. All the studies developed in Europe investigated PhCs with the only exception of Nardi et al. (1995) (referring to prechlorination of raw hospital effluent), and Arslan et al. (2014) regarding AOPs applied on a raw HWW.

It is worth noting that often in Asian countries, the main reason for investigating hospital effluent treatment is the need to guarantee “safe” treatment for this kind of wastewater and to evaluate the possibility of directly reusing the treated effluent due to water scarcity for various requirements, in particular for irrigation (Al-Hashimia et al., 2013). As discussed below, although it is highly appreciable that this problem has been tackled, their common conclusion, based on an analysis of conventional contaminants whereby a secondary biological treatment followed by chlorination may be considered adequate treatment even in case of direct reuse, is not backed up by comprehensive research into micropollutants or ecotoxicology.

In European countries, the main reason for research is generally an awareness of the potential risk posed by the occurrence of PhC residues in secondary effluent and the need to reduce the PhC load discharged into the environment via WWTP effluent. There is a lively debate on the need to adopt dedicated and proper treatments for hospital effluents (Ort et al., 2010a; Verlicchi et al., 2012a; Santos et al., 2013) based on the evaluation of the contribution of the health care structure and the corresponding catchment area in the discharge of PhCs.

Table 6.3: List of the studies included in the overview together with a brief description of the corresponding investigations and rationale

Reference	Main characteristics of experimental investigations and treatment plants	Rationale Investigated parameters
Abd El-Gawad and Aly, 2011	Investigation carried out at four hospitals in Egypt to assess hospital effluent quality and quantity, as well as the impact on the environment in terms of BOD_5, DO, TSS, total coliform, fecal coliform and trace elements (metals) when a CAS system is adopted as treatment prior to discharge into surface water.	Suitable HWW management based on standards set for conventional pollutants in UWW. Conventional parameters
Al Hashimia et al., 2013	Investigation carried out on real wastewater collected from a hospital located in Iraq to assess the performance of a lab-scale *sequencing* anoxic/anaerobic MBR for nutrient removal under different internal recycling time modes between anoxic and anaerobic conditions. Monitored parameters were COD, BOD_5, PO_4, NH_4, NO_3, NO_2, TSS, oil and grease, total and fecal coliforms.	Enhancement in nutrient removal in hospital effluent. Conventional parameters
Andersen et al., 2014	Investigation regarding to the treatment of the oncological ward effluent by means of a pilot plant consisting in a moving bed biofilm reactor followed by ozonation carried out in Denmark. System performances were provided for six pharmaceutical model substrates each representing different biological and chemical degradation: triclosan, mefenamic acid, diclofenac, naproxen, gemfibrozil, ketoprofen, ibuprofen, clofibric acid.	Optimization of the removal of selected compounds by means of a moving bed biofilm reactor and ozonation. PhCs
Arslan et al., 2014	Investigation carried out on raw hospital effluent in Turkey. Ozonation, O_3/UV, O_3/UV/H_2O_2 were tested as a *pretreatment* option in a batch reactor in order to evaluate the removal of COD and UV absorbance and the improvement in biodegradation.	Options in pretreatments Conventional parameters
Azar et al., 2010	Investigation carried out on real HWW collected from two hospitals located in Iran, by means of biological oxidation (aerobic/anaerobic) in an 80-litre pilot plant. Monitored parameters were COD, BOD_5, TSS, NO_2, NO_3, PO_4, Detergents, Oil and grease, total coliform, Escherichia coli, Ag, Hg and Ni.	Recommended treatment for hospital effluent in Iran, based on an analysis of conventional parameter removals.
Beier et al., 2010	Investigation carried out at Waldbrol hospital (Germany) by means of nanofiltration (NF) and revers osmosis (RO) membrane (pilot plant) for the treatment of a (full scale) MBR permeate. Monitored parameters were 11 pharmaceutical compounds.	Dedicated polishing treatment for HWWs to remove PhCs.
Beier et al., 2011	Investigation carried out at the full-scale MBR in operation at Waldbrol hospital in Germany to assess PhCs removal from hospital wastewater. The permeate is then sent to the municipal WWTP. Monitored parameters were 10 pharmaceutical compounds.	Separate treatment of HWWs will allow evaluation of the appropriateness of MBR for hospital effluent in high density urban areas, contributing to minimizing the operating and financial expenditure for municipal WWTP. PhCs
Beier et al., 2012	Investigation carried out at a hospital in Waldbrol (Germany) to assess the performance of a full-scale wastewater treatment plant equipped with a MBR. Monitored parameters were COD, TOC, AOX, NH_4, total phosphorus, *Escherichia coli* and Enterococci.	Evaluation of MBR as a dedicated treatment of HWWs to reduce the environmental input of PhCs in the environment. Conventional parameters
Berto et al., 2009	Investigation carried out at a hospital in Brazil to evaluate the effectiveness of “advanced” pretreatments consisting in a biological (full-scale septic tank) and a chemical stage (lab-scale Fenton reactor) to remove organic matter and pathogenic microbiota from HWW. Monitored parameters were COD, BOD_5, P and N	Adequate advanced (pre)treatments for hospital effluents to reduce their environmental impact.

Reference	Main characteristics of experimental investigations and treatment plants	Rationale Investigated parameters
	compounds, suspended solids, total coliform and thermotolerant coliforms.	Conventional parameters
Beyene and Redaie, 2011	Investigation carried out at Hawassa University Referral Hospital (Ethiopia) to examine the suitability of facultative and maturation ponds (full scale) for the treatment of HWW. Samplings were taken twice throughout the month of August 2010 to determine the overall efficiency of the whole treatment system. Monitored parameters were COD, BOD_5, P, PO_4, total Nitrogen, NH_3, NO_3, NO_2 TSS, TDS, Cl, S^2, total coliforms and fecal coliforms.	Evaluation of the risk posed by HWWs in terms of conventional pollutants and a proposal to upgrade existing WWTP in order to reduce it. Conventional parameters
Chiang et al., 2003	Investigation carried out in Taiwan on the disinfection by continuous ozonation of hospital effluent and in particular of the effluent from the kidney dialysis unit and on the increment of hospital effluent biodegradability.	Disinfection effect and improvement in biodegradability of hospital effluent by ozonation. Conventional parameters
Chitnis et al., 2004	Investigation carried out in India in a pilot plant consisting in preliminary and primary treatments, a conventional activated sludge system, sand filtration and chlorination.	Investigation into the microbiological community and evaluation of the risk of multidrug resistant bacteria spread
Cruz-Morato et al., 2014	Investigation carried out in Spain in a batch fluidized bed bioreactor (lab scale) under sterile and non-sterile conditions with *Trametes versicolor* pellets to examine the removal of 99 pharmaceutical compounds from HWW. Samples were collected from the main sewer of Girona University Hospital (Spain).	High concentration of PhCs in HWWs, separate treatment of HWWs allows the specific degradation of PhCs
de Almeida et al., 2013	Investigation carried out at the University hospital of Santa Maria (Brazil) by means of a septic tank and anaerobic filter (full scale). Monitored parameters were 5 anti-anxiety and anti-epileptic compounds.	Environmental risks of PhCs and adequateness of treatment trains. PhCs
Emmanuel et al., 2004	Toxicity evaluation after prechlorination (NaClO addition) of the effluent from the infectious and tropical disease department in Lyon, France.	Toxicity evaluation due to prechlorination Conventional pollutants.
Gautam et al., 2007	Investigation carried out at the hospital located in Vellore, Tamil Nadu (India), by means of a lab-scale plant consisting of coagulation (by adding $FeCl_3$), rapid filtration and disinfection (by adding a bleaching powder solution) steps. Monitored pollutants were the conventional COD, BOD_5, SS and P.	Options for hospital effluent pretreatment before discharge in public sewage. Conventional pollutants.
Grundfos Biobooster, 2012	Report from an on-going project in Denmark to evaluate the best available technologies (BATs) for the separated treatment of hospital effluent. Two sequences are being tested: MBR followed by O_3, GAC and/or H_2O_2 and UV, MBR followed by GAC and UV.	Evaluation of the BAT for hospital treatment.
Kajitvichyanukul and Suntronvipart, 2006	Investigation carried out in Bangkok, Thailand, on the pretreament of hospital effluent by using a lab-scale photo-Fenton process. Monitored parameters were the conventional COD, BOD_5, TOC, turbidity, TSS, conductivity and toxicity.	Improvement in biodegradability of hospital effluent by using the photo-Fenton process as a pretreatment. Conventional parameters
Kist et al., 2008	Investigation carried out on the treatment of wastewater produced in a hospital laundry in the Rio Pardo Valley (Brazil), by means of a (lab scale) ramp type reactor for catalytic photoozonation ($UV/TiO_2/O_3$). Monitored parameters were COD, BOD_5, turbidity, surfactants, *Escherichia Coli* and thermotolerant Coliforms.	Reduction of the risk posed by hazardous substances occurring in HWWs due to adequate pretreatments Conventional parameters

PART B

Reference	Main characteristics of experimental investigations and treatment plants	Rationale Investigated parameters
Kohler et al., 2012	Investigation carried out at the "Hospitalier Emil Mayrisch" (Luxembourg) by means of a pilot plant (MBR+UV; MBR+H_2O_2+UV) to assess the removal of 13 PhCs.	Technical and economical feasibility for hospital effluent treatment. PhCs
Kosma et al., 2010	Investigation carried out on the occurrence and removal of PhCs at the hospital (full scale) WWTP (CAS) in Ioannina (Greece). Monitored parameters were COD, BOD_5, NO_3, PO_4, TSS and 11 PhCs.	Impact of pharmaceuticals on the environment. PhCs
Kovalova et al., 2012	Investigation carried out in Switzerland, on a pilot-scale MBR installed and operated for one year at Cantonal Hospital in Baden. The aims were an in-depth analysis of the removal efficiency of 56 micropollutants in hospital effluent and potential biomass inhibition of disinfectants	Analysis of performance and removal in MBR of many PhCs. Reduction of the spread of multi resistant or pathogenic bacteria, virus, parasite eggs and PhCs.
Kovalova et al., 2013	Investigation carried out at the Cantonal Hospital in Baden (Switzerland) in a pilot plant consisting in a primary clarifier, MBR, and five post-treatment technologies: O_3, O_3/H_2O_2, powdered activated carbon (PAC), and low pressure UV light with and without TiO_2. Monitored parameters were 56 pharmaceutical compounds.	Removal of typical pollutants in hospital effluent (disinfectants, pathogens and antibiotic resistant bacteria) by advanced treatments. PhCs
Lenz et al., 2007a	Investigation carried out at a hospital in Vienna (Austria), by means of a pilot membrane bioreactor system (MBR) installed and fed with oncologic in-patient treatment ward effluent. Monitored pollutants were the cancerostatic platinum compounds.	Risk of cancerostatic platinum compounds to humans. PhCs
Lenz et al., 2007b	Investigation carried out at the oncological ward in a hospital in Vienna (Austria), by means of a pilot MBR followed by granular activated carbon (GAC) and UV. Samples were taken from the influent and the effluent of the MBR-system as well as behind the advanced wastewater treatment processes. The monitored pollutants were cancerostatic platinum compounds.	Environmental risk of cytostatic. PhCs
Liu et al., 2010	Investigation carried out in China on operating conditions, MBR efficiency in treating hospital effluent. Monitored parameters were COD, BOD_5, NH_3, TSS, Bacteria and fecal coliform.	To avoid the spread of pathogenic microorganisms and viruses, especially following the outbreak of SARS in 2003. Conventional parameters
Machado et al., 2007	Investigation carried out in Brazil, on a lab-scale advanced oxidation process ($UV/TiO_2/O_3$) operating as a tertiary treatment, fed with secondary HWW. Monitored parameters were COD, BOD_5, turbidity, total nitrogen, total phosphorus, surfactants, thermotolerant coliforms and toxicity	Proposal of a (sustainable) treatment schematic to reduce microorganisms and toxicity from hospital effluent. Conventional contaminants and AOX
Mahnik et al., 2007	Occurrence and treatability of 4 cytostatics (5-fluorouracil, doxorubicin, epirubicin and daunorubicin) in the effluent from the oncologic in-patient treatment ward of the Vienna University Hospital was investigated as well as their removal by an MBR (pilot scale)	Pollution level of the effluent from particular hospital wards. PhCs
Mahvi et al., 2009	Analysis of the performance of seven WWTPs (CAS + chlorination) in Kerman Province (Iran) receiving hospital effluent in terms of removal of main conventional parameters (COD, BOD_5, DO, TSS, pH, NO_2, NO_3, PO_4, Cl and SO_4^{2-})and malfunctions.	Malfunctions in WWTPs receiving hospital effluents. Conventional parameters
Martins et al., 2008	Investigation carried out in Brazil into the pretreatment of hospital effluent by using a septic tank and an anaerobic filter. Analysis was referred to occurrence, removal of ciprofloxacin and the resulting risk due to its	Evaluation of the adequateness of specific pretreatment in Brazil. PhC

Reference	Main characteristics of experimental investigations and treatment plants	Rationale Investigated parameters
	residue in the treated effluent	
McArdell et al., 2011	Report including all the details of the investigations described in Kovalova et al. (2012, 2013) and in PILLS Report 2012 referring to the Swiss investigations on MBR and MBR+ AOPs applied to a hospital effluent	Testing and comparing the removal of PhCs from HWW by different technologies
Mousaab et al., 2015	Investigation into the removal ability of PhCs and conventional pollutants in an upgraded ultrafiltration membrane system coupled with an activated sludge (AS) reactor by the addition of biofilm support media in the aeration tank in case of hospital effluent treatment.	Improvement in PhC removal from hospital effluent and in membrane functioning resulting in a reduction of operation costs. PhCs
Nardi et al., 1995	Investigation into disinfection of the effluent of an Italian infectious disease ward by means of different doses of ClO_2 and evaluation of AOX production.	Disinfection performance of ClO_2 with respect to NaClO in case of hospital effluent and evaluation of AOX production. Conventional parameters
Nielsen et al., 2013	Investigation carried out in Denmark with pilot and lab scale plants into the ability of different technologies acting as a secondary (MBR) or a tertiary (O_3, O_3/H_2O_2, ClO_2, PAC) treatment in removing common PhCs from hospital effluent. Monitored parameters were 93 pharmaceutical compounds.	Risk to human health posed by Hwws during combined sewers overflow. PhCs
Pauwels et al., 2006	Investigation carried out in Ghent (Belgium) to compare the performance of two lab-scale plants (CAS and MBR) in treating hospital effluent. Monitored parameters were COD, total ammonium nitrogen, ethinylestradiol, total coliforms, fecal coliforms, total aerobic bacteria, total anaerobic bacteria and Enterococci.	Potential risk of HWWs-correlation between PhC and conventional parameters removal. PhCs and conventional parameters
Pharmafilter Report, 2013	Report on the characteristics and the performance of a full-scale system (Pharmafilter) installed and tested in the Reinier de Graaf Gasthuis in Delft (Netherlands) in the period 2010-2012. The system is an integral concept for the optimization of care, processing waste and purifying wastewater in hospitals. It consists in: pretreatment (sieve), biological process (UF MBR), ozonation, GAC filtration. The sludge discharged from the MBR is fed back into the digester and any excess sludge water from the digestate formed in the digester can be transported to the MBR. The fate and removal of about 100 PhCs was observed.	Potential health risk posed by HWWs PhCs
PILLS Report, 2012	Report of the main results achieved within the European PILLS project developed in 2010-2012 involving four research units in different countries that investigated the removal of PhCs from HWW by means of MBR+PAC, MBR+O_3+moving bed bioreactor, MBR+UV+moving bed bioreactor in Switzerland, MBR+RO, MBR+UV, MBR+O_3/H_2O_2 in Luxembourg, MBR+O_3+sand filtration, MBR+ PAC+sand filtration in Germany, MBR+O_3+GAC, MBR+GAC+UV/H_2O_2+GAC in the Netherlands. Monitored parameters were PhCs and toxicity. See also Kovalova et al. (2012, 2013), Koeler et al. (2011); McArdell et al. (2011)	Effects of pharmaceuticals on environment water and potential measures to reduce their occurrence. PhCs
Prado et al., 2011	Investigation carried out in Brazil involving detection of some enteric viruses and hepatitis A in hospital effluent and in the effluent from two different full scale treatment plants. The removal efficiencies observed in the two sequences: upflow anaerobic sludge blanket (UASB) +three serial anaerobic filters and CAS system followed by a chlorination tank were investigated and compared.	Quantification of eneteric viruses and hepatitis A in the effluent of different hospital WWTPs. PhCs
Prayitno et al., 2014	Investigation on a pilot scale plant consisting in an Aerated Fixed Film Biofilter (AF2B reactor) coupled with an ozonation reactor fed by the effluent from Malang City hospital in Indonesia.	Pollution and health problems for humans being caused by the discharge of HWWs.

PART B

Reference	Main characteristics of experimental investigations and treatment plants	Rationale Investigated parameters
	Sampling was done every 2 hours for 120 hours at the outlet AF2B reactor and every 5 minutes for 60 minutes on the outlet O_3 reactor. Monitored parameters were BOD_5, phenols, fecal coliform and Pb.	Conventional pollutants
Rezaee et al. 2005	Investigation carried out in Iran on a pilot-scale system consisting in an integrated anaerobic-aerobic fixed film reactor fed with hospital effluent before co-treatment with urban wastewater. Samples were collected twice a day (10 a.m. and 2 p.m.) during the study period. Monitored parameters were COD, BOD_5, NH_4, Turbidity, Bacteria and *Escherchia coli*.	Potential reduction of the organic load in hospital effluent by biological pretreatment before its cotreatment. Conventional parameters
Shrestha et al., 2001	Analysis of the removal performance in a full scale two stage constructed wetland (CWs) designed and constructed in Nepal to treat hospital effluent (20 m^3/d). The system consists in a three chambered septic tank, a horizontal flow bed (140 m^2), with 0.65 to 0.75 m depth and a vertical flow bed (120 m^2) with 1 m depth. The beds were planted with local reeds (*Phragmites karka*).	Transfer CW technology to developing countries to reduce pollution in aquatic environments. Conventional parameters
Sim et al., 2013	Investigation carried out at two hospital WWTPs located in Korea to assess the occurrence and removal of selected pharmaceutical and personal care products using 24 h composite sampling. The wastewater treatment plants consist of (i) flocculation (FL)+ activated carbon filtration (AC); (ii) flocculation + CAS.	Potential risks of anthelmintics on non-target organisms in the environment and their resistance to biodegradation. PhCs
Suarez et al., 2009	Investigation carried out in Spain into the pretreatment of hospital effluent. The efficacy of coagulation-flocculation (Coag-FL) and flotation (FLO) processes in removing 10 PhCs was investigated in case of two kinds of hospital effluent: one from radiotherapy and outpatient consultation wards and one from hospitalized patients, surgery, laboratories, radiology and general services. Coagulation-flocculation assays were performed in a jar-test device and in a continuous pilot-scale plant. Ferric chloride ($FeCl_3$) and aluminium sulphate ($Al_2(SO_4)_3$) were added.	Potential risk of hospital wastewater to the environment. PhCs
Vasconcelos et al., 2009	Investigation carried out in Brazil into the potential pretreatment of hospital effluent to degrade persistent compounds.In particular the study investigated the performance of a lab-scale photo-induced oxidation, heterogeneous photocatalysis, ozonation and peroxone in degrading the antimicrobial ciprofloxacin.	Environmental impact of Ciprofloxacin and analysis of its degradation by ozone and photoprocesses.
Venditti et al., 2011	Investigation carried out in Luxembourg on the removal of conventional pollutants and selected PhCs by means of MBR fed with hospital effluent	Adequateness of MBR as a pretreatment for hospital effluent. PhCs
Verlicchi et al., 2010a	Investigation carried out at an Italian hospital by means of a pilot-scale MBR equipped with UF membranes. Twenty wastewater samples were taken from the influent and the effluent of the MBR taking into consideration the HRT. Monitored parameters were COD, BOD_5, SS, NH_4, Total P and *Escherichia coli*.	Hospitals are the main source of PhCs. Guidelines for a full scale plant for hospital effluent
Wen et al., 2004	Investigation carried out at Haidian community hospital (China), where a full-scale submerged hollow fiber MBR was installed. Monitored pollutants were COD, BOD_5, NH_4, turbidity and *Escherchia coli*.	Efficiency and operation stability of MBR equipped with microfiltration membranes in treating HWWs.
Wilde et al., 2014	Investigation carried out in Brazil into the degradation of a mixture of beta-blockers (atenolol, propranolol, metoprolol) in hospital effluent by ozonation and Fenton reaction	Optimization of the operational condition in the degradation of a mixture of PhCs in hospital effluent

Table 6.4: Dedicated treatment trains for hospital effluent included in the review

Investigated Treatment/treatment train*	**Reference**
(pre)Disinfection with ozone[1]	Chiang et al., 2003
(pre)Disinfection with chlorine[1]	Emmanuel et al., 2004; Nardi et al., 1995; Liu et al., 2010
(pre)Photo-Fenton[1]	Katjitvichyanukul and Suntronvipart 2006
Coagulation-flocculation; Coagulation-flocculation+flotation	Suarez et al., 2009
Coagulation+filtration + disinfection	Gautam et al., 2007
Screening + O_3/UV or O_3/UV/H_2O_2 (+ biological step)[2]	Arslan et al., 2014
Septic tank+ anaerobic filter	de Almeida et al., 2013; Martins et al., 2008
Septic tank+HSF+VSF	Shrestha et al., 2001
Septic tank + Fenton	Berto et al., 2009
Flocculation + CA	Sim et al., 2013
Flocculation+ CAS	Sim et al., 2013
Anaerobic-aerobic fixed film reactor	Rezaee et al., 2005
Facultative and polishing ponds (II + III)[2]	Beyene and Redaie 2011
Aerated Fixed Film Biofilter+O_3	Prayitno et al., 2014
CAS	Abd El Gawad and Aly, 2011; Azar et al., 2010
CAS + support media + UF	Mousaab et al., 2015
CAS + chlorination	Kosma et al., 2010; Mahvi et al., 2009; Prado et al., 2011
Fungal bioreactor	Cruz-Morato et al., 2014
UASB+ anaerobic filter	Prado et al., 2011
MBBR + ozonation	Andersen et al., 2014
MBR	Al Hashmia et al., 2013; Beier et al., 2012; Kovalova et al., 2012; Lenz et al., 2007a; Liu et al., 2010; Mahnik et al., 2007; Nielsen et al., 2013; Venditti et al., 2011; Weng et al., 2004
MBR + chlorination	Liu et al., 2010, Nielsen et al., 2013
MBR + GAC	Lenz et al., 2007b
MBR + GAC + O_3 and or H_2O_2 + UV	Grundfos Biobooster 2012,
MBR + GAC + UV	Lenz et al., 2007b
MBR + H_2O_2+UV	Koheler et al., 2011,;Kovalova et al., 2013
MBR + O_3 + GAC	Pharmafilter, 2013
MBR + O_3 + GAC+ UV	Grundfos Biobooster 2012,
MBR + public sewage+ cotreatment	Beier et al., 2011
MBR + UV	Lenz et al., 2007b
MBR+ H_2O_2	Koheler et al., 2011
(MBR+) PAC[3]	Kovalova et al., 2013; Nielsen et al., 2013
(MBR+) O_3 [3]	Kovalova et al., 2013; Nielsen et al., 2013
(MBR+) O_3/H_2O_2 [3]	Nielsen et al., 2013

(MBR+) UV with/without TiO_2 [3]	Kovalova et al., 2013
UV/O_3/ TiO_2	Kist et al., 2008
(Septic tank+ anaerobic filter+) O_3, H_2O_2/O_3 [3]	Vasconcelos et al., 2009
(Septic tank+ anaerobic filter+) O_3, Fe^{+2}/O_3 [3]	Wilde et al., 2014
(Septic tank+ anaerobic filter+) UV [3]	Vasconcelos et al., 2009
(Septic tank+ anaerobic filter+)TiO_2/UV [3]	Vasconcelos et al., 2009
NF/RO (polishing) [4]	Beier et al., 2010

[1] (pre): means preliminary treatment
[2] (biological treatment) means that the investigated treatment is upstream of a biological step
[3] Upstream treatments reported in brackets have to better define the step of the treatment considered and reported data on the removal efficiencies of PhCs do not include their contribution in the cited investigations.
[4] (II+III) means a series of secondary and tertiary ponds

6.4 Results and discussion

The following sections present and discuss collected data on the removal efficiencies of selected PhCs as well as conventional parameters from HWW by different systems acting as primary, secondary and tertiary steps. A specific section is devoted to the removal ability of microorganisms observed in the different technologies and on measures suggested to reduce the spread of pathogens and also of antibiotic resistant bacteria. Supplementary data provides a brief overview on the main reactions taking place during AOPs and might help in reading the following discussion.

6.4.1 Preliminary and primary treatments — pharmaceutical removal

Preliminary treatments are generally adopted and tested with the aim of removing rough and coarse material from raw wastewater, thus protecting mechanical and electrical parts in the downstream treatment steps. Specific treatments have also been tested in lab and pilot plants to reduce the toxicity of chemical mixtures occurring in hospital effluent and to enhance biodegradability (namely to increase the BOD_5/COD ratio) and to improve downstream biological processes.
Coagulation–flocculation and flotation are processes that satisfy the first objective as they promote the removal of suspended solids and colloids from wastewater which do not settle spontaneously (Gautam et al., 2007; Suarez et al., 2009), whereas ozonation (Chiang et al., 2003) and AOPs (Kajitvichyanukul and Suntronvipart, 2006) satisfy the second objective. COD removal was found greater than 70% when 200 mg/L of ferric chloride was added to raw hospital effluent and removal increased to over 98% if the coagulant was added to settled HWW.
A following step of disinfection by calcium hydrochloride not only reduces microorganisms, but also COD. It was found that with a contact time of 30 min, the $Ca(ClO)_2$ break point dose is 20 mg/L (Gautam et al., 2007). A few studies have been carried out on the effectiveness of coagulation, flocculation and flotation in removing PhCs from hospital effluent (Suarez et al., 2009; Martins et al., 2008). Fig. 1 shows the main results when common coagulants $Al_2(SO_4)_3$ and $FeCl_3$ at a dosage of 25 mg/L are added to the raw wastewater,with and without flotation. These processes are not particularly efficient in removing PhCs, confirming the considerations reported in Verlicchi et al. (2012b). In fact, only diclofenac and some fragrances are removed by more than 60%. Fig. 1 also reports the somewhat modest removal efficiency (17%) observed for ciprofloxacin using a septic tank followed by an anaerobic filter fed with raw effluent from a hospital in Brazil (Martins et al., 2008). Attempts to improve COD removal and increase biodegradability in raw hospital effluent were made by applying ozonation, O_3/UV and O_3/UV/H_2O_2 as a pretreatment (Arslan et al., 2014). Based on lab scale tests on effluent from a diagnostic centre, nuclear medicine, oncology, radiology and medical genetics departments, it was found that the highest COD removal (47.5%)was obtained in a systemO_3/UV/H_2O_2 operating at pH 6.0, O_3 concentration 10 mg/L, monochromatic UV lamp (254 nm) and dosage of H_2O_2 1.8 mL within 60 min. As for absorbance removal, the best AOP is O_3/UV: in fact the addition of H_2O_2 led to a scavenger effect on hydroxyl radicals resulting in a lower removal efficiency (see Supplementary data for more details).

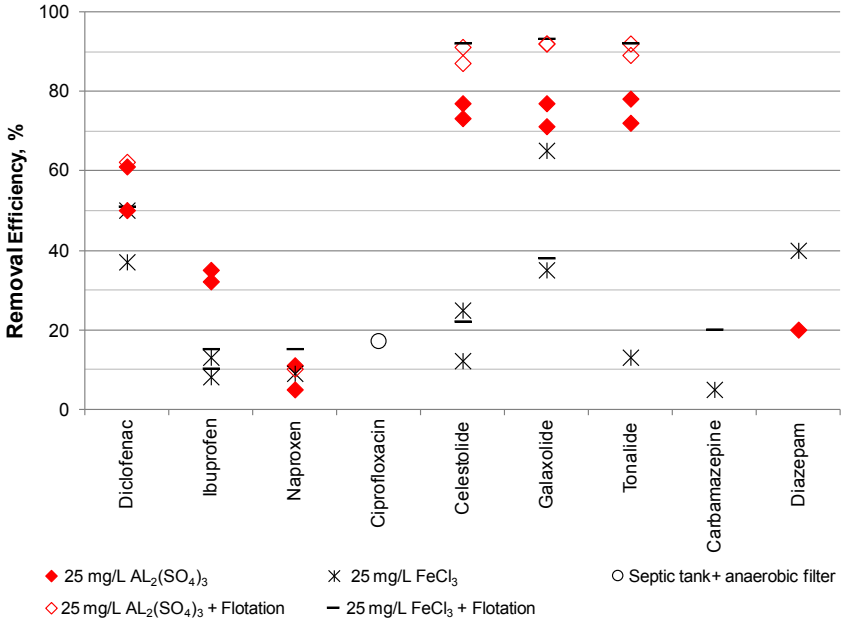


Fig. 6.2: Observed removal efficiencies from HWWfor selected PhCs in different primary treatments. Data from: Suarez et al., 2009; Martins et al., 2008.

The results achieved from the ozonation of effluent from a kidney dialysis unit are quite interesting: at a dose of 25 mg/L of ozone and a contact time of 20 min, COD was reduced from132 mg/L to 97mg/L and the ratio BOD_5/COD increased from 0.15 to 0.26 confirming a consistent increment in the biodegradability of the stream (Chiang et al., 2003).

Another option to improve biodegradability is achieved using photo-Fenton processes (see Supplementary data for the main reactions involved).

It was found that in hospital effluent of average pollutant strength (COD 1350–2250 mg/L, BOD_5/COD 0.30) with a dosage ratio COD:H_2O_2:Fe^{+2} equal to 1:4:0.1, a reaction pH of 3 and a reaction time of 2 h, the removal efficiencies for BOD_5, COD and TOC were: 61%, 77% and 52% and the BOD_5/COD ratio increased from 0.30 to 0.52.

It was also found that for higher COD values, optimum reaction conditions have to be tested to guarantee good mineralization of organic compounds and to enhance biodegradability (Kajitvichyanukul and Suntronvipart, 2006). The increased biodegradability of the wastewater was also confirmed by batch experiments on raw and pretreated effluent subjected to a biological process using activated sludge. It was found that in the case of pretreated wastewater, the removal of COD amounted to 90% after a 72 h treatment time, whereas it was only 30% in the case of raw hospital effluent (Kajitvichyanukul and Suntronvipart, 2006).

A Fenton process may also act as a disinfectant step: in fact it greatly removes total coliforms and thermotolerant coliforms as documented by Berto et al. (2009). The cases of complete removal observed in their investigation were ascribed to acidic conditions and the occurrence of hydroxyl radicals. Low pH values would cause bacteria death and HO• would assure DNA denaturation.

These studies led to suggest ozonation, Fenton as well as photo-Fenton processes as suitable solutions for the preliminary treatment of hospital wastewater from a technical viewpoint. An economic analysis would be necessary to assess investment, operational and maintenance costs. Moreover, the adequateness of adopting these advanced technologies as “pretreatment” also needs to be confirmed from a toxicological view point, but unfortunately, there is no available research to investigate.

6.4.2 Secondary treatments — pharmaceutical removal

Most of the studies investigated the capacity of MBRs as a biological stage for the treatment of HWW. Other systems analyzed include: CAS systems in Iran (Mahvi et al., 2009), Greece (Kosma et al., 2010), Egypt (Abd El-Gawad and Aly, 2011) and Belgium (Pauwels et al., 2006), an

anaerobic–aerobic fixed film bioreactor in Iran (Rezaee et al., 2005), an aerated fixed film biofilter in Indonesia (Prayitno et al., 2014), a moving bed biofilm reactor in Denmark (Andersen et al., 2014), ultrafiltrationmembranes coupledwith amodified CAS reactor by the addition of biofilm supports in France (Mousaab et al., 2015), maturation and polishing ponds in Ethiopia (Beyene and Redaie, 2011), horizontal and vertical subsurface flow systems in Nepal (Shrestha et al., 2001), and a fungal bioreactor in Spain (Cruz-Morato et al., 2014). In the first part of this section MBRs and CAS are critically analyzed and compared, the remaining systems are analyzed and compared in the second part.

MBR

Lessons learned from the reviewed studies, carried out all over the world, regarding the efficacy of MBRs applied to UWW in the removal of macro- and micro-pollutants (Verlicchi et al., 2012c) are certainly useful in an analysis of the performance of an MBR fed with hospital effluent.

As regards this type of wastewater, special attention must be paid to evaluate the potential inhibition effect on the biological activities of PhCs, heavy metals, disinfectants, detergents that occur at higher concentrations in HWW rather than UWW thus, the risk that they could negatively affect the degradation processes of micro contaminants has to be assessed.

In the studies included herein, hospital effluent is generally subjected to a coarse screening (2 mm), sometimes through a fine screen or a sieve (0.5–1 mm), whereas a primary clarifier is only rarely adopted (HRT 2–10 h). Adequate pretreatments are extremely useful in guaranteeing continuous operation of MBRs. As reported in the investigation by Verlicchi et al. (2008), the raw HWW may contain rags, filaments, pieces of cardboard that can adversely interfere with moving parts within the WWTPs or clog membranes and thus they have to be efficiently removed at the start of the treatment train. This is in agreement with suggestions by Gabarron et al. (2013)which investigated different pretreatment processes to find the most adequate technology that would consistently contribute in minimizing the ragging impact over MBR performance.

A storage/equalization tank before an MBR guarantees homogeneous feeding avoids damage to the membrane units and may also promote sorption removal mechanisms due to the contact between solid particles and micropollutants. This is the case of cancerogenic platinum compounds (CPCs), such as cisplatin, that show a high affinity for suspended solids (Lenz et al., 2007a). In this study, the feed from the oncological ward was first collected in a tank (24 h residence time), then processed through a sieve (1 m, to separate suspended solids from the liquid phase) and finally sent to an MBR treatment. The CPC concentration was significantly reduced after passing through the sieve and the membranes due to particle and biomass sorption onto the surface.

A biological reactor usually consists in an anoxic/oxic compartments to promote complete nitrification and denitrification. P removal, when necessary, is achieved by a co-precipitation with $FeCl_2$. Biomass concentration in the aerated compartment varied between 2 and 20 g/L, the sludge retention time ranged between 20 and 100 d with the only exception of an MBR operating in parallel with a CAS system whose SRTs were 12–15 d in each (Pauwels et al., 2006).

Ultrafiltration membranes (tubular or flat sheet, 0.03–0.06 μm)were more frequently investigated (Nielsen et al., 2013; Lenz et al., 2007a; PILLS Report, 2012 — at the Swiss, German and Dutch units within the project) than microfiltration membranes (sheet, 0.4 μm; Pauwels et al., 2006; Beier et al., 2011; Luxembourg unit within the PILLS project — PILLS Report, 2012). Submerged membrane modules integrated in the bioreactor were the most commonly adopted configuration; side stream modules were equipped only in the Dutch unit within the PILLS project and in the Austrian investigation where the MBR was fed by the oncological ward effluent (Lenz et al., 2007a). A rapid glance at the macro-pollutant removal observed in the different MBRs shows that notably high values were found (94% for DOC, 99% for COD, 93–99% for NH_{4+}, around 85% for nitrates) resulting in a high quality permeate, with reduced variability intervals for the different pollutants: DOC 6–11 mg/L, COD 20–30 mg/L and total N 3–17 mg/L with a few exceptions (McArdell et al., 2011;Wen et al., 2004).

Good biological activity was in general guaranteed and maintained throughout each observation period in the different investigations.

Chemical or physical parameter shocks could occasionally occur resulting in disturbances at the biological reactors and, from a macroscopic point of view, reduced removal of macro-pollutants, namely COD, SS and N compounds, from a microscopic point of view changes, modification or disintegration of the activated sludge flocks (Pauwels et al., 2006; McArdell et al., 2011).

In this context, quaternary ammonia disinfectants are potential critical parameters, as their consumption may greatly vary fromone hospital to another as remarked by Kovalova et al. (2012). As for the common quaternary ammonia disinfectant BAC C12, tolerable concentrations may reach up to 150 μg/L without inducing negative effects on the biomass (Kovalova et al., 2012; McArdell et al., 2011). Moreover, hospital laundrette effluent represents a hotspot for certain pollutants (Kist et al., 2008). A sudden increase in formic acid concentrations may occur as reported by Pauwels et al. (2006), leading to a pH shock (2.5) in the bioreactor. This results in a process performance ecrease due to the disintegration of the sludge and consequently in a dramatic decrease in COD removal.

Fig. 6.3 and Fig. 6.4 report all collected data on removal of PhCs in hospital effluent by an MBR operating at different SRT values.

As underlined by different studies (Clara et al., 2005; Verlicchi et al., 2012a, 2012b; Monteiro and Boxall, 2010), SRT greatly affects the removal performance ofmany PhCs. Long SRT values promote adaptation of different kinds of microorganisms and the presence of slower growing species which could have a greater capacity for removing more recalcitrant compounds while simultaneously improving suspended solid separation (Kreuzinger et al., 2004). Based on data shown in Fig. 6.3 and Fig. 6.4 involving removal efficiencies of compounds observed at different sludge ages, it emerges that an SRT equal to 20–25 d promotes the removal of atenolol and clarithromycin, slightly higher values (around 30 d) enhance diclofenac and erythromycin removal and around 50 d a larger number of compounds are better removed: naproxen, lidocaine, ciprofloxacin, sulfamethoxazole and cyclophosphamide.

Very good removal efficiencies of over 90% were in general observed at a SRT greater than 30 d for many of the selected compounds. Modest removal efficiencies (<50%) were observed for metoprolol, iopamidol, carbamazepine, gabapentin and ritalinic acid.

Unfortunately, removal efficiencywas always scarce (<25%) for various PhCs, namely: indomethacin, phenazone, roxithromycin, D617 (Ndealkylverapamil, a metabolite of Verapamil), cyclophosphamide, oseltamivir carboxylate, propranolol, sotalol, iodixanol, iohexol, iomeprol, ioversol and oxazepam.

The antineoplastic agents included in the CPC group show a higher removal efficiency with respect to cyclophosphamide, due to their higher affinity to sorbing onto particles and activated sludge flocks within the MBR (Lenz et al., 2007a,b).

Releases sometimes occur for diclofenac, phenazone, ciprofloxacin, clarithromycin, sulfadiazine, sulfamethoxazole, propranolol, iopamidol and carbamazepine, probably due to deconjugation during biological treatment (Kovalova et al., 2012; Nielsen et al., 2013). These are not reported in the graph in Fig. 6.3 and Fig. 6.4. An in-depth discussion of the potential release of many PhCs is reported in Verlicchi et al. (2012b) as well as in Monteiro and Boxall (2010).

Based on the Swiss research carried out within the PILLS project involving 56 compounds of different therapeutic classes, it emerged that an MBR (SRT equal to 30–50 d) is able to remove up to 90% of pharmaceuticals and metabolite load (X-ray contrast media excluded), although removal of some of the selected compounds was very poor (in particular, clindamycin, diclofenac and furosemide). Only 2% of the influent contrast media load was removed in the investigated MBR. An MBR is not a satisfactory treatment process for the removal of AOX: in the permeate, AOXs occur in the range of 0.56–0.85 mg/L (Beier et al., 2011; McArdell et al., 2011) and further advanced treatment is necessary to reduce their content in the final effluent (Machado et al., 2007).

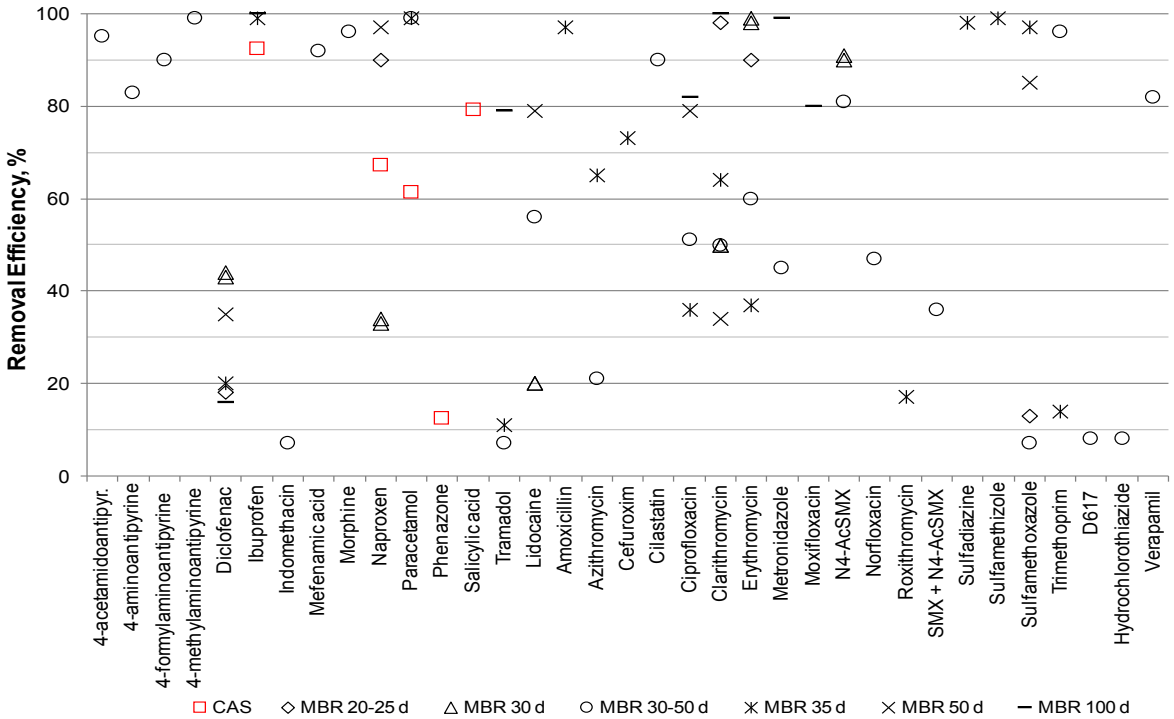


Fig. 6.3: Observed removal efficiencies for a group of selected compounds in MBRs and CAS operating at different SRTs. Data from: Kosma et al., 2010; Kovalova et al., 2012; PILLS Report, 2012, Nielsen et al., 2013; Beier et al., 2011; Kohler et al, 2012.

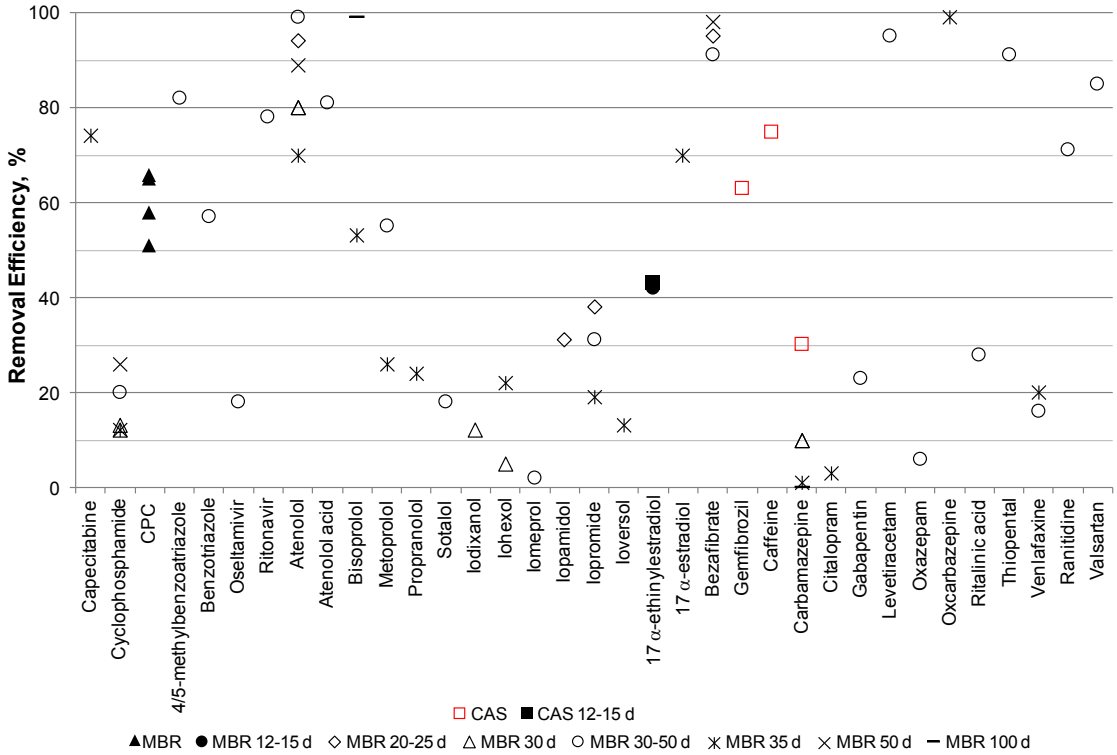


Fig. 6.4: Observed removal efficiencies for a group of selected compounds in MBRs and CAS operating at different SRTs. Data from: Kosma et al., 2010; Pauwels et al., 2006; Lenz et al., 2007a, 2007b; Kovalova et al., 2012; PILLS Report, 2012; Nielsen et al., 2013; Beier et al., 2011; Kohler et al., 2012.

The absence of suspended solids in the MBR effluent represents a strength as it is the most important condition required by many advanced technologies in the removal of trace contaminants, as suspended solids may negatively interfere with the removal performance of said technologies.

An MBR appears to be an adequate secondary treatment for hospital effluent as it produces very good quality and stable effluent throughout the running time, and is thus suitable for advanced technologies (Venditti et al., 2011; Beier et al., 2011), including NF/RO and AOPs.

Full scale MBRs have been adopted for the treatment of HWW in Italy (Verlicchi et al., 2010a), Germany (PILLS Report, 2012) and China (Liu et al., 2010).

CAS

Only two research projects were found dealing with the removal of PhCs from hospital effluent involving "dedicated" CAS systems: one lab scale (Pauwels et al., 2006) and one full scale (Kosma et al., 2010).

Pretreatment was only reported in the second case, consisting in a grit removal and mixing tank. Biological reactors had anoxic/aerobic compartments in the first case and only aerobic in the second. In the research by Kosma et al., 2010 removal efficiencies were provided for PhCs after CAS (HRT 6 h) + chlorination.

Only 10 PhCs were monitored in these dedicated CAS systems. High removal efficiencies were observed for ibuprofen (92%), salicylic acid (79%) and caffeine (75%), naproxen, gemfibrozil, paracetamol and ethynylestradiol (EE2) were moderately removed (67%, 63%, 61% and 43% respectively), whereas scant removal was found for carbamazepine and phenazone (30% and 13% respectively). A modest release (−17%) was observed for diclofenac.

6.4.2.1 Comparison between CAS and MBR

In the research by Pauwels et al. (2006), CAS and an MBR were operating in parallel, fed with the same hospital effluent (spiked with EE2 up to 1 mg/L). With respect to the MBR, the CAS system exhibited a slower start up and was more prone to bulking. Moreover, COD removal was worse in the CAS system (88% in CAS vs. 93% in an MBR) as was the removal of various bacterial groups: total coliforms, faecal coliforms and total anaerobic bacteria (about 2 log units less) and total aerobic bacteria (1.4 log units less). No differences were found in the removal of EE2 between CAS and MBR.

The higher removal efficiencies observed for some bacterial groups in the MBR permeate are due to membrane retention. Their occurrence in the MBR effluent may instead be explained by unavoidable bacteria regrowth from the effluent vessel into the permeate collecting tube and also by the absence of proper membrane cleaning while the system was running, as disinfection was not applied (Pauwels et al., 2006).

Lessons learned from previous studies on removal of PhCs by means of CAS and an MBR fed with UWW (Verlicchi et al., 2012a,b) highlighted that in the MBR, the combination of higher biomass concentration in the aerated basin, development of different bacterial species within the biomass, smaller sludge flocks that may enhance sorption on the surface of different contaminants, higher SRTs and higher removal of suspended solids, greatly contributes to the removal of PhCs from the stream.

Moreover, as discussed below, passage through ultrafiltration membranes guarantees disinfection of the wastewater, thus reducing the risk of spread of pathogenic bacteria and of multi-drug resistant bacteria.

6.4.2.2 MBR upgrade

Recently, an upgrade of the MBR system was researched by Mousaab et al. (2015) with the aim of improving PhC removal efficiencies and membrane function. The system consisted in an activated sludge basin coupled with an external ultrafiltration membrane module (0.2 μm), operating at a SRT 20 d, HRT 22 h, T 18–20 °C and pH 6.8–7.9. In the first 75 d, it worked under "usual" conditions. Then, HDPE support media were added to the biological reactor (specific area: 600 m_2/m_3; diameter: 12.2 mm; length: 12mm, density: 0.95–0.98 kg/m^3) promoting the development of a hybrid (attached and suspended) biomass and a longer SRT of fixed organisms. In the modified bioreactor, higher removal efficiencies were observed for soluble COD (91.8% vs. 86.9%), TSS (100% vs. 99.6%) and VSS (93.2% vs. 87.9%) and removal efficiencies greater than 95% for

codeine, pravastatin, ketoprofen, diclofenac, roxithromycin, gemfibrozil and iohexol, whereas in the unmodified MBR their removal was either absent or very low. The presence of biofilm supports also enhanced particle sorption and improved effluent quality, thus offering better protection of the membranes against fouling and reducing cleaning operations. Enhanced removal of P compounds from hospital effluent could be obtained by sequencing anoxic/anaerobic MBRs. Al-Hashimia et al. (2013) found that the optimal phase for this type of systemis operating with an internal recycling mode of 2 h anoxic followed by 2 h anaerobic. These conditions provide an optimal simultaneous removal efficiency of 93% for N compounds and 83% for P compounds (expressed as $P\text{-}PO^{4-}$).

6.4.2.3 Other investigated biological systems

In Nepal, in 1997 a dedicated treatment plant was built for hospital effluent. It consists of a three chambered septic tank (16.7 m^3) providing pretreatment, followed by CW systems: a horizontal subsurface low bed (140 m^2, 0.65 m deep and 0.75 m high, filled with 5 mm crushed gravel) and a vertical flow bed (120 m^2, 1 m deep, filled with clean sand) as a secondary step. Very good removal efficiencies were observed for TSS and BOD_5 (97–99%), COD (94–97%), $N\text{-}NH^4$ (80–99%), total coliform(99.87–99.999%), E. coli (99.98–99.999%) and Streptococcus (99.3–99.99%) (Shrestha et al., 2001).

In Ethiopia, a series of waste stabilisation ponds (2 facultative ponds, 2 maturation ponds and 1 fish pond covering an area of about 3000 m^2 with a total retention time of 43 d) was found to be reasonably efficient in the removal of BOD_5, COD, sulphide, suspended solids and N compounds from hospital effluent (Beyene and Redaie, 2011). Despite the satisfactory removal of total and faecal coliforms (99.7 and 99.4% respectively), their final concentrations do not fulfil WHO recommendations for restricted and unrestricted irrigation. Options to improve the quality of the final effluent were considered: for instance adoption of (i) constructed wetlands; (ii) two successive lagoons followed by infiltration into the land; (iii) MBR advanced oxidation treatment to better remove all the parameters as well as pharmaceuticals; and (iv) photo-Fenton process to reduce toxicity. Only the first option was considered feasible, whereas the second could lead to groundwater contamination and the applicability of the remaining options was found difficult in terms of cost, installation, operation and maintenance.

In Iran, hospital effluents are generally discharged into a public sewage system and then co-treated with urban effluents. Usually they are subjected to a secondary treatment; disinfection is mandatory in case of disease outbreaks and in critical periods (in the summer and autumn due to reduced river water flow) (Mahvi et al., 2009). The most common malfunctions are due to operator inexperience at the WWTP and negligent WWTP management by the authorities. Investigations were carried out on pilot plants with the aim of evaluating (i) proper pretreatment of hospital effluent before discharge into a public sewage system followed by co-treatment (Rezaee et al., 2005) and (ii) a (co)- treatment train able to respect Iranian legal requirements for physical, chemical and microbiological parameters for direct discharge into the surface body, disposal to wells and reuse in agriculture (Azar et al., 2010). These investigations found that an integrated anaerobic/aerobic fixed film bioreactor can greatly remove organic and nitrogen compounds from raw hospital wastewater and when followed by cotreatment consisting in primary treatment, an aerobic/anaerobic activated sludge reactor fulfils the legal requirements for conventional parameters.

These conclusions however do not consider any kind of more recalcitrant compounds (pharmaceuticals, contrast agents, disinfectants) whose removal is poor in the investigated biological systems.

Another treatment train was investigated in Indonesia consisting in an aerated fixed film biofilter followed by an ozone reactor. Satisfactory removal efficiencies were observed for BOD_5 (97.5%), faecal coliform (99.23%), Pb and phenol (100%), but there was no chemical analysis involving pharmaceuticals, disinfectants or detergents (Prayitno et al., 2014).

As for preliminary treatments, in addition to what has already been reported in Section 6.4.1, chemical flocculation followed by a CAS process represents an efficient barrier for anthelmintic drugs (albendazole and flubendazole) considering that overall removal is in the range of 67– 75% (Sim et al., 2013).

Modifications to biological reactors to enhance micropollutant removal have undergone in-depth analysis during the last years. This is the case of Andersen et al. (2014) where on a pilot scale, the combination of a moving bed biofilm reactor followed by an ozonation stage was investigated. A biological system was developed (called a staged

MBBR) to attempt to improve the creation of fixed biofilms where slow-growing bacteria would stand a better chance of development(these bacteria are very efficient in removing pharmaceuticals) compared to biomass developed in CAS systems. Higher removal efficiencies were observed for ketoprofen and gemfibrozil and occasionally for diclofenac and clofibric acid.

Interesting and promising results were observed for many PhCs in a batch fluidized bed bioreactor under sterile and non-sterile conditions with Trametes versicolor pellets (Cruz-Morato et al., 2014) fedwith hospital effluent, operating at pH 4.5, T 25 °C, 1.4 g dry weight biomass per litre and with a continuous addition of glucose and ammonium tartrate as a nutrient source for the biomass. Sterile conditions showed that T. versicolor is responsible for the removal of the detected compounds.

Very good removal efficiencies were observed for analgesics and anti-inflammatory drugs after 1 d and complete removal of most was observed after 8 d,with the only exception of salicylic acid and dexamethasone. Although antibioticswere partially removed and required longer times (5 d against 1 d for analgesics), the fungal treatment achieved better results than conventional activated sludge (CAS) processes(Verlicchi et al., 2012a,b) for themost part. This is the case of ciprofloxacin (69% and 99% in sterile and non-sterile conditions respectively, vs. 58–78% in CAS) and clarithromycin (80% in non-sterile conditions vs. 46–62% in CAS). Higher removal efficiencies were also observed for the anti-hypertensives: valsartan (90 and 95% after 8 d in sterile and non-sterile conditions), irbesartan (73 and 98% in sterile and nonsterile conditions) and diuretic furosemide (100% and 80% in sterile and non-sterile conditions vs. 33–54% in CAS).

As for diclofenac, complete removal was observed. This is an important result as it is one of the most persistent compounds in CAS and also a potential candidate for regulation by European legislation. On the other hand, a disadvantage of this process is that after treatment, pH neutralization is necessary as secretion of organic acids by the fungus lowers the overall pH. As concerns the investigations carried out in Iran, Iraq and Indonesia, it is important to underline that final effluent from treatment trains including CAS or ponds generally should not be directly reused for irrigation purposes due to the occurrence of residues of PhCs and other emerging contaminants. AOPs should be included in the treatment trains and in any case, further research into the ecotoxicological characteristics of the final effluent should be carried out.

6.4.3 Tertiary treatments — pharmaceutical removal

6.4.3.1 Filtration through powdered or granular activated carbon (PAC and GAC)

Filtration through PAC and GAC has undergone in-depth investigation by different European research groups. Fig. 6.5 and Fig. 6.6 report all the collected data. In all cases included in this study, PAC/GAC treatment followed an MBR fed only with hospital effluent. In the permeate DOC was in the range of 6–8 mg/L and TOC around 20 mg/L (McArdell et al., 2011; Nielsen et al., 2013). The adsorbent used in the Swiss research was PAC (McArdell et al., 2011) with a surface area of 1300 m_2/g, a particle size d_{50} 15 μm and a zero surface charge point pH_{PZC} equal to 8.8 (this last value represents the pH at which on the carbon surface there are as many positively as negatively charged functional groups; below this value the carbon surface is positively charged). In the PAC reactor, good mixing guaranteed a constant concentration of the adsorbent, its retention time was 2 d as a few differences were found with longer times. Good separation between loaded PAC and treated effluent was achieved by filtration through UF membrane flat sheets (pore size 0.04 μm) in the PILLS project plants (McArdell et al., 2011; PILLS Report, 2012) and through a 1 μm glass fibre filter in the Dutch research (Nielsen et al., 2013). Nanofiltration opposed to ultrafiltration would certainly be convenient from a technical view point (improved PhC removal), but not from an economic one, as nanofiltration concentrate would require dedicated treatment due to the high concentrations of micropollutants. Another option could be pumping the loaded activated carbon from the PAC reactor to the MBR for recycling: a consistent improvement in the removal of contaminants could result. But neither of these processes were researched.

The investigated doses of PAC ranged between 8 and 23 mg/L in the Swiss and German research studies (PILLS Report, 2012) and between 150 and 450 mg/L in Dutch studies (Nielsen et al., 2013). The former range, which is absolutely more sustainable from an economic view point, was defined on the basis of costs and reasonable removal rates for a wide spectrum of micropollutants (56 compounds), the latter was based on a Swedish study on the removal of micropollutants in aquatic environments (Walhberg et al., 2010).

In the PAC filter effluent, DOC occurred at about 4–4.5 mg/L (PAC dose 8 mg/L), 2.7–3.7 (PAC dose 23 mg/L) and about 2 mg/L (PAC dose 43 mg/L).

Within the Swiss campaigns, at the applied PAC dose of 8 mg/L, 25 out of the 56 investigated pharmaceuticals were subjected to high removal efficiencies (>80%) whereas 10 compounds exhibited removal efficiencies below 20%; at the intermediate value of 23 mg/L a removal efficiency greater than 80% was observed for 36 compounds and less than 20% for only two contrast media (diatrizoate and ioxitalamic acid).When 43mg/L of PACwas dosed, 38 compounds had high removal efficiencies (>80%) and the same two contrast agents still had scant removal efficiencies (<20%). A rapid glance at the results achieved within the Dutch research (Nielsen et al., 2013) shows that no significant differences were observed in the removal of the 30 selected pharmaceuticals by applying 150 mg/L or 450 mg/L of PAC.

A comparison between the Dutch campaign and the PILLS project, referring only to the 24 compounds monitored in all the cited studies, highlights that only for 5 PhCs a higher removal efficiency was achieved with the (extremely high) Dutch dosages. This occurred for the antibiotics sulfadiazine (40% vs. 78% at both high doses), sulfamethoxazole (62% vs. 71% and 99% at the two doses) and trimethoprim (83% vs. 99.9% at both doses), the contrast agent ifosfamide (60 vs. 96%), and the beta blocker atenolol (88 vs. 99%).

Attempts to correlate the observed removal efficiency of PhCs by using PAC and their sorption potential expressed in terms of Kow or Dow (also accounting for acid–base speciation) were done by the Swiss research group (Kovalova et al., 2013; McArdell et al., 2011). As regards neutral (i.e., not charged) compounds at pH 8.8 (namely carbamazepine, oxazepam, 4-acetamidoantipyrine, cyclophosphamide, iomeprol, iopamidol, iopromide, metronidazole, phenazone and primidone), it was found that the higher the Dow value, the higher the observed removal by sorption. On the contrary there is no agreement between experimental data and prediction from Log D_{ow} of sorption removal for charged compounds.

These results confirm that removal mechanisms consist in nonspecific dispersive interactions and electrostatic interactions as well as between the charged adsorbent surface and ionic adsorbate. Moreover, not only Log D_{ow} influences the behaviour of a pharmaceutical, but also its pKa, molecular size and aromaticity/aliphaticity potential as well as the presence of functional groups. As regards PAC, effective removal mechanisms depend on surface area, pore size and texture, surface chemistry (in particular functional groups and point of zero charge) and mineral matter content. As a rule of thumb, adsorption is most effective for compounds which are uncharged and apolar. An interesting analysis and discussion of the behaviour of many compounds is reported in Kovalova et al. (2013) and McArdell et al. (2011).

A consistent improvement in the removal of contrast media may be achieved by recycling PAC to biological treatment as documented in the MicroPoll projects (Zwickenpflug et al., 2010).

GAC filtration was investigated at the Netherlands research unit within the PILLS project (PILLS Report, 2012) and also in Austria where the oncological ward effluent in a hospital was subjected first to an MBR then to GAC treatment (Lenz et al., 2007b). In the first case, the filter bed had a height of 3.0 m and an empty bed contact time of 51 min. It was fed by MBR permeate (TOC equal to 8.7 mg/L). After GAC filtration, all investigated pharmaceuticals were found below their detection limits. Also sulfamethoxazole, reluctant to PAC sorption, was removed by more than 96%. Unfortunately data referring to contrast agents were not collected. In the second case, the GAC filter had a height of 36.7 cm, a cross surface of 19.6 cm^2 and a flow rate of 7.6 L/h. Antineoplastic compounds (the cancerostatic platinum compounds CPC cisplatin, carboplatin, oxaliplatin and 5-fluorouracil) were monitored in the GAC influent (corresponding to an MBR permeate) and effluent.

Referring to total Pt content, it was observed that GAC contributed to a removal rate of about 50%. As discussed below, a combination of UV with GAC leads to a lesser removal rate of total Pt. This may be due to the fact that the photodegradation products of CPCs exhibit lower affinity to activated carbon than the parent compounds.

It is intereting to observe that with PAC and GAC no byproducts occur, with respect to all oxidation processes (ozonation and AOPs in general) where oxidation and photodegradation compounds are unavoidable and often they have ecotoxicological effects.

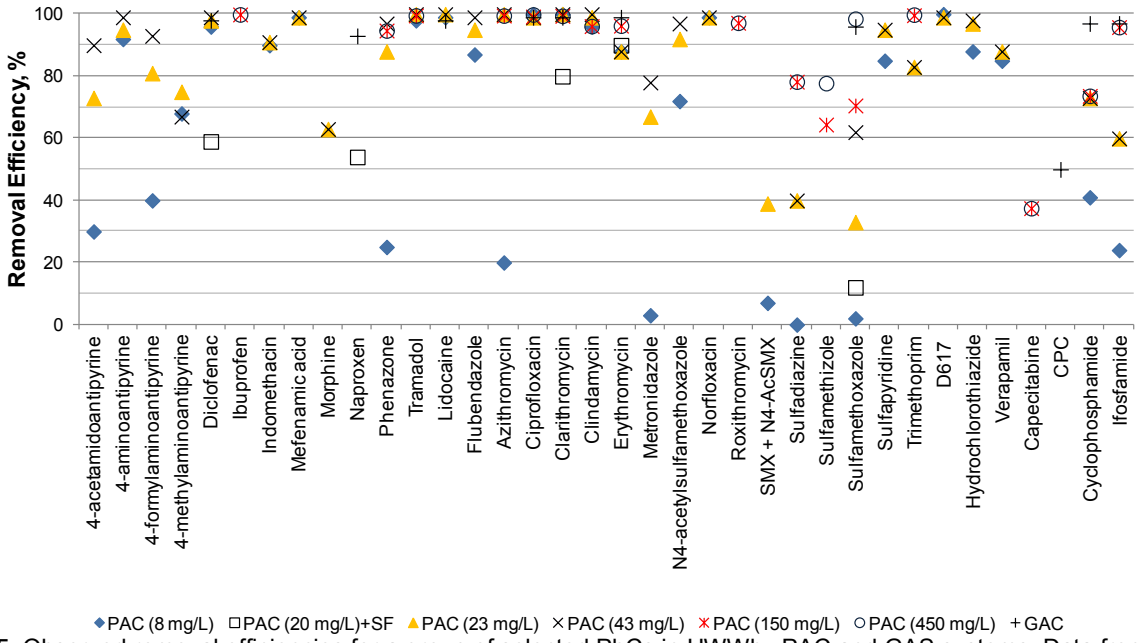


Fig. 6.5: Observed removal efficiencies for a group of selected PhCs in HWWby PAC and GAS systems. Data from: Kovalova et al., 2013; PILLS Report, 2012; Nielsen et al., 2013; Lenz et al., 2007b.

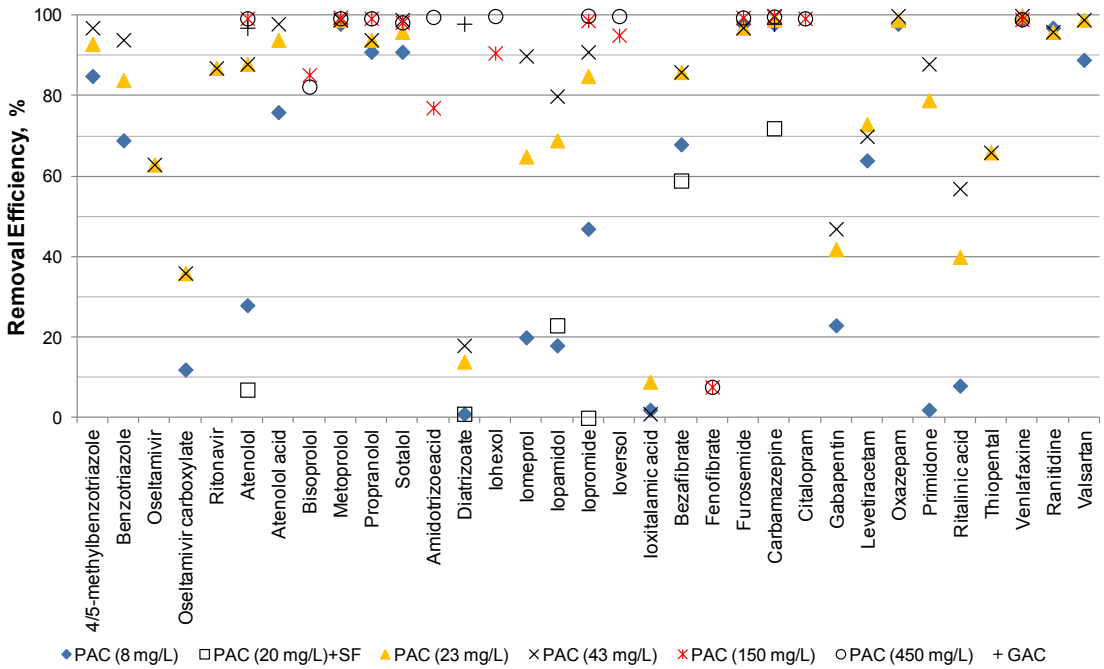


Fig. 6.6: Observed removal efficiencies for a group of selected PhCs in HWWby PAC and GAC systems. Data from: Kovalova et al., 2013; PILLS Report, 2012; Nielsen et al., 2013.

6.4.3.2 Ozonation

In ozonation investigations, the influent to each ozone reactor was always an MBR permeate (McArdell et al., 2011; Nielsen et al., 2013), with a COD ranging from 12 to 30 mg/L, a DOC ranging from 6 to 11 mg/L, pH 8–8.5 and T 20–22 °C (Kovalova et al., 2012). Contact time within the ozone reactor was between 12 and 23 min and the applied dose of ozonewas between 0.45 and 2 g O_3/g DOC (PILLS project) and between 4.1 and 7.8 g O_3/g TOC in the study byNielsen et al. (2013).

Higher concentrations of ozone were not tested as they would lead to the formation of potentially toxic bromates, according to literature (von Gunten, 2003).

As is clearly shown in Figs. 6.7 and 6.8 the higher the applied ozone dose, the greater the number of compounds with a removal efficiency >90%. At the lowest tested value of 0.45 g O_3/g DOC (German unit within the PILLS project, PILLS Report, 2012), 3 out of the 11 investigated compounds were efficiently removed (namely diclofenac, sulfamethoxazole and erythromycin), the number increases to 26 out of the 48 selected compounds at 0.64 g O_3/g DOC (Kovalova et al., 2013), to 28 out of 49 at 0.89 and 29 out of 49 at 1.08 g O_3/g DOC (Kovalova et al., 2013).

The classes of cytostatics and contrast agents were quite reluctant to removal by ozonation: the average removal efficiencies observed were always lower than those observed for other classes. At medium-high ozone doses, only some compounds of these two classes were removed by about 50–60%. This occurred to cyclophosphamide, ifosfamide, iopamidol and iopromide at doses of about 1.1 g O_3/g DOC and 4.1–7.8 g O_3/g TOC (Nielsen et al., 2013). The most reluctant compounds to be removed by ozone were the contrast agents diatrizoate and ioxitalamic acid, the antibiotic metronidazole and the anthelmintic flubendazole whose average observed removal efficiencies were between 13 and 27%.

This treatment did not consistently decrease COD and DOC as ozonation does not eliminate (that is, mineralize) organic matter and micropollutants but rather transformstheminto other more degradable compounds also measured as COD and DOC.

It is quite interesting to point out that ozonation seems to be a quite promising treatment for the abatement of most of the micropollutant load in hospital effluent. It is important to bear in mind one of the lessons learned by the PILLS project: based on a Swiss research referring to the top 100 administered pharmaceuticals in the investigated large hospital (McArdell et al., 2011), a removal efficiency of 90% was observed for all the PhCs and metabolite load (ICM excluded) by ozone (1.08 g O_3/g DOC, pH 8.5, T=22 °C). This removal reduces to 50% if contrast agents are included.

This could lead to the consideration that sewage conveying radiological ward effluent could be separated and treated by a dedicated WWTP, so it could also be possible to recover iodium. The main disadvantage in adopting ozonation, and more in general AOPs, is the formation of oxidation byproducts (like bromates) due to the matrix compounds (for instance bromides). As these products could have ecotoxicological effects, it is advisable to adopt a biological step (namely a sand filter or an MBBR) that will act as a barrier. In the Swiss research, the concentration of bromide in the permeate was 30–40 μg/L and after the addition of the highest dose of ozone (1.08 g O_3/g DOC, corresponding to 7 mg O_3/L), bromate was found at a concentration of 1 μg/L, well belowthe Swiss drinking water standard set at 10 μg/L.

Ozonation reactions were due to the very selective attack of ozone to specific functional moieties of organic substances and to the less selective attacks of hydroxyl radicals ($HO^{\cdot}$), formed during ozone decomposition, to a wider spectrum of functional groups within the molecules.

Ozone decomposition is favoured by the presence of hydroxyl ions (OH^-) at alkaline pH (pH > 9).

The following rules of thumb could lead to a rough prediction of the efficacy of ozonation in removing different types of micropollutants resulting from studies on the kinetics of ozonation reactions and on the potential correlation between molecular structure (presence of moieties within the molecule) of a compound and its reactivity with ozone (Lee and Von Gunten, 2010):

(i) olefin, phenol, aniline, thiophenol, thiol and tertiary amine exhibit a high reactivity with ozone,

(ii) secondary amines, thioester and anisol an intermediate reactivity,

(iii) primary amines and nitro group a slow reactivity and

(iv) amides do not react with ozone.

Compounds with a high reactivity to ozone are already removed to a high extent at the lowest dose of 0.64 g O_3/g DOC. For compounds with intermediate reactivity, such as benzotriazole and ritalinic acid, higher removal efficiencies were observed with higher ozone doses. Lowest removal efficiency was found in contrast agents without moieties.

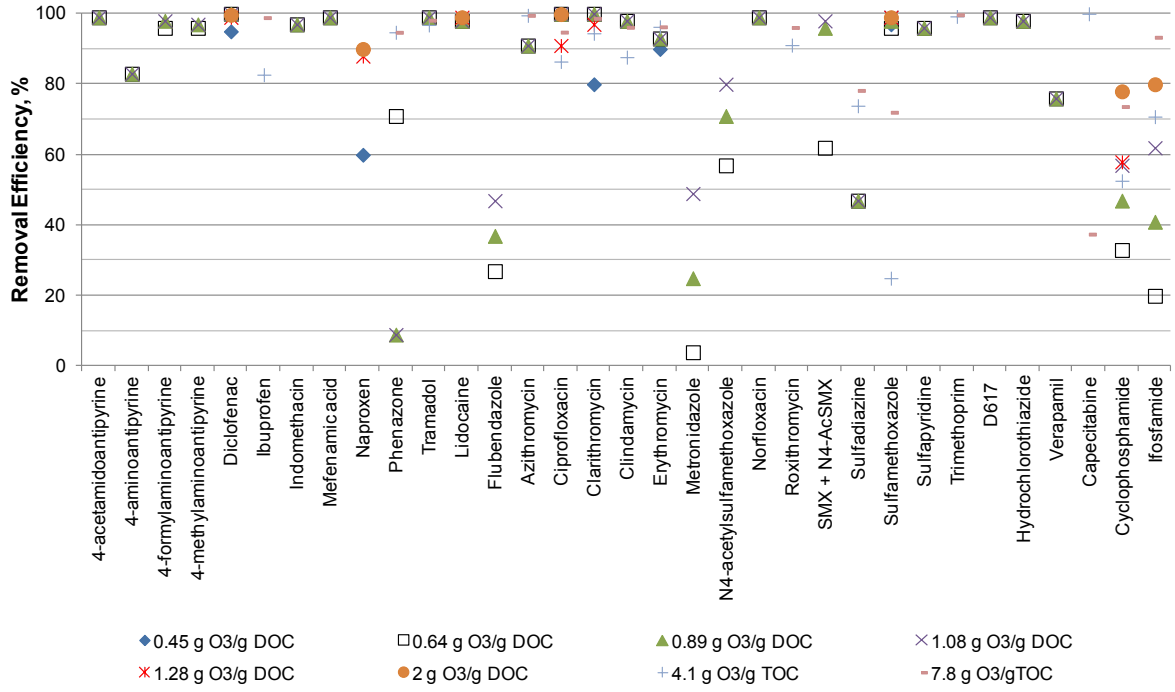


Fig. 6.7: Observed removal efficiencies for a group of selected PhCs in HWWby ozonation. Data from: PILLS Report, 2012; Kovalova et al., 2013; Nielsen et al., 2013; Lenz et al., 2007b.

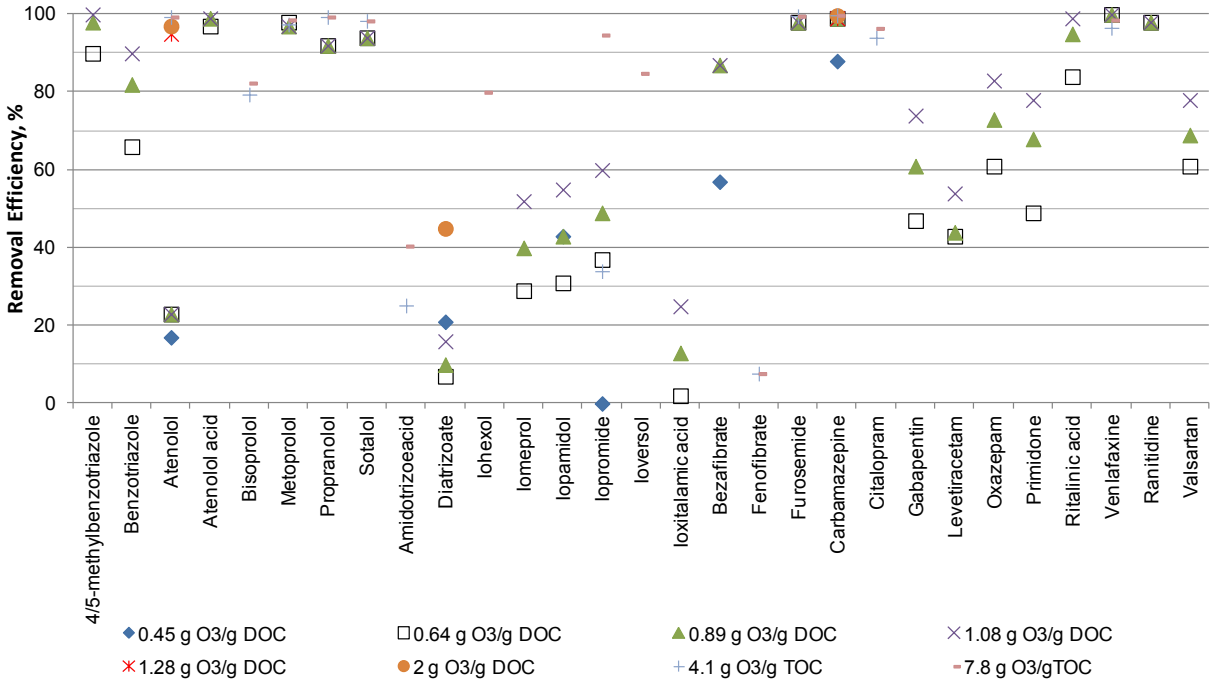


Fig. 6.8: Observed removal efficiencies for a group of selected PhCs in HWW by ozonation. Data from: PILLS Report, 2012; Kovalova et al., 2013; Nielsen et al., 2013; Lenz et al., 2007b.

6.4.3.3 UV radiation

Only a few investigations (within the PILLS project (PILLS Report, 2012) and at the oncologic ward in a hospital in Vienna (Lenz et al., 2007b)) dealt with the ability and the contribution of an UV irradiation process in the removal of PhCs from (pretreated) hospital effluent: in each one, the UV reactor was always fed by an MBR permeate (DOC = 6–8 mg/L). The main characteristics of the tested equipment are reported in Table 6.5 (PILLS Report, 2012, McArdell et al., 2011; Lenz et al., 2007b): in particular different fluence values were tested and, in the Luxembourg unit, low and medium pressure (LP, MP) UV lamps were used and for some runs, a polychromatic light was applied to the water stream. The collected data are reported in Fig. 6.9 and Fig. 6.10 referring to the lamp type and the applied fluence. Observed removal efficiencies for the investigated compounds were always less than 50% when the UV fluence of 800 J/m^2 was applied. At 2400 J/m^2, 12 out of 31 PhCs were removed at more than 50% and with 7200 J/m^2, 18 out of 31 compounds exceeded the 50% removal threshold. If the UV is irradiated at higher fluence values, removal increases (for instance at 29700 J/m^2 or 47250 J/m^2). When MP lamps were used, a polychromatic lightwas produced and all the seven investigated compounds were successfully removed. Fig. 6.9 and Fig. 6.10 clearly show, with the exception of cyclophosphamide (η = 58%), that the removal efficiency of the other compounds ranged between 81 and 98%, on average 83%.

Compounds with the highest removal efficiencies were: 4- acetamidoantipyrine (99% with LP and 7200 J/m$_2$), diclofenac (99% with LP lamp and 29,700 and 47,250 J/m$_2$), diclofenac and 4-formylaminoantipyrine (98%,with LP and 7200 J/m$_2$), sulfamethoxazole (98% with LP lamp and 47,250 J/m$_2$), diatrizoate (97% with LP and 7200 J/m$_2$), sotalol (95% with LP and 7200 J/m+) and the remaining X ray contrast media (iomeprol 90%, iopamidol, iopromide and ioxitalamic acid 92% with LP and 7200 J/m^2). This last result is quite interesting, as the UV process seems to be the most effective treatment to remove these from the wastewater.

The contribution of an UV process in the removal of antineoplastic compounds was found to be negligible. This was concluded by Lenz et al. (2007b) who monitored the cancerostatic platinum compounds (CPCs) cisplatin, carboplatin, oxaliplatin and 5-fluorouracil in the effluent of a hospital oncological ward. They found that oxidation of CPC by UV leads to a marginal reduction of total Pt as, even if the substances are transformed by oxidation, the total amount of Pt remains the same. As for cyclophosphamide, removal efficiency was found higher in the case of medium pressure UV lamps than in the case of LP lamps (58% vs. 3%).

It was observed that UV irradiation is a promising technology in the removal of X-ray contrast media. Very appreciable results were observed when a fluence of 7200 J/cm^2 was applied. At higher values the removal of different analgesics, antibiotics and beta-blockers increased (Kovalova et al., 2013).

Transmission of UV in water is strictly correlated to water turbidity. Very low turbidity is recommended in order to greatly reduce potential interferences with the water matrix. Excessive dosages of chemical oxidisers may act as a scavenger thus inhibiting contaminant destruction efficiency.

UV transmission is subject to decrease due to lamp fouling. To reduce lamp fouling, adequate pretreatments are necessary, insoluble oil and grease concentrations should be minimized and heavy metal ion concentration should be maintained at a concentration less than 10 mg/L.

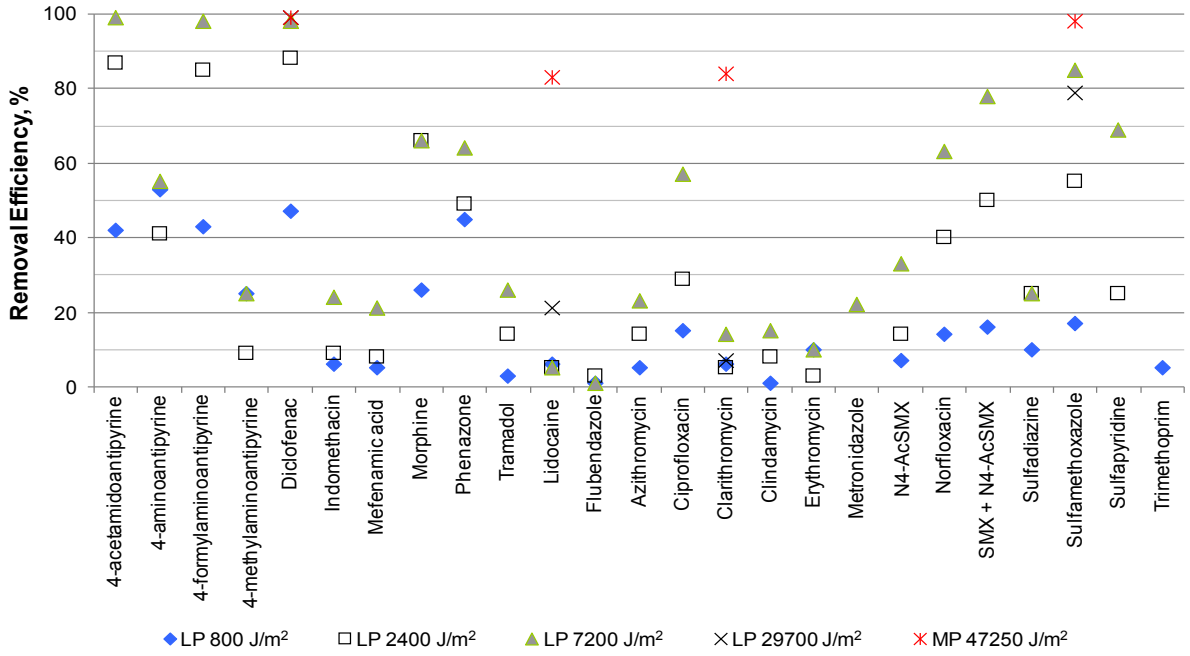


Fig. 6.9: Observed removal efficiency for a group of selected PhCs in HWWby UV treatment. Data from: Kovalova et al., 2013; PILLS Report, 2012; Kohler et al., 2012.

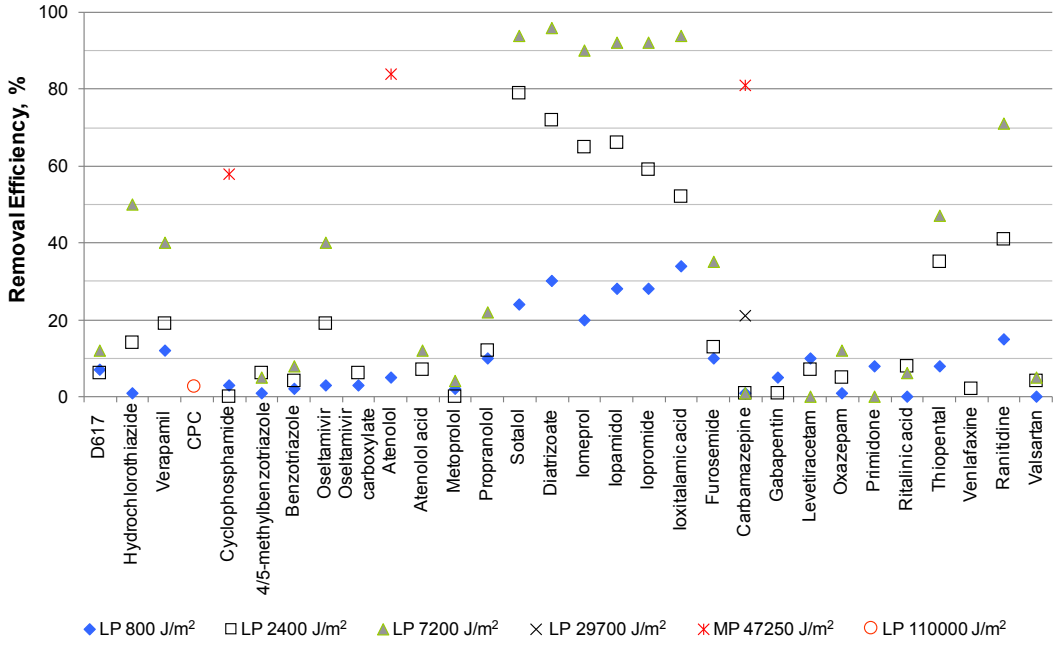


Fig. 6.10: Observed removal efficiency for a group of selected PhCs in HWWby UV treatment. Data from: Lenz et al., 2007b; Kovalova et al., 2013; PILLS Report, 2012; Kohler et al., 2012.

Table 6.5: Main operational parameter in the UV reactors included in this study

Parameter	Austria	Switzerland	Luxembourg
Plant type	Pilot	pilot	Pilot
Lamp	LP	LP	LP and MP
Actual Fluence, J/m^2	110000	800, 2400, 7200	7400-29700 (LP) 10125-506250 (MP), λ=200-280 nm 5400-270000 (MP), λ =280-315 nm 4725-236250 (MP), λ =200-280 nm and 315-400 nm
Residence time, s	120	18, 54,162	18-71 (LP), 1.3-64 (MP)

6.4.3.4 Advanced oxidation processes (AOPs)

Removal of pharmaceuticals. - Advanced oxidation processes include different technologies aiming to completely oxidize and/or destroydifferent kinds of organic pollutants in water and wastewater streams into H_2O, CO_2 and mineral salts.

Each one is characterized by a variety of radical reactions due to highly reactive species (mainly hydroxyl radical HO•, but also superoxide radical anion O_2^{-}•, hydroperoxyl radicals HO_2•, ROO^{-}), generated on site in different ways, involving combinations of chemical agents (namely ozone, hydrogen peroxide, transition metals, metal oxides) and auxiliary energy sources (namely UV irradiation, electronic current, y-radiation and ultrasound). This study includes combinations between O_3 and H_2O_2 as chemical agents and UV irradiation as an energy source.

HO• is the primary oxidant in AOPs and unlike many other radicals it is non-selective, it readily reactswithmany organic pollutants occurring in the water, converting them into more hydrophilic compounds than the original ones.

A brief presentatin of each, including the main reactions occurring during AOPs is reported in the Supplementary data, whereas below, the results obtained in the different investigations into AOPs applied to hospital effluents as polishing treatments are presented (Fig. 6.11) and discussed.

In the experimental setup tested in Switzerland within the PILLS project (McArdell et al., 2011), the photocatalysis process UV/TiO_2 was compared to the UV process alone. This setup includes a reaction column containing four conical cartridges, consisting in a photocatalytic fibre (titanium-dispersed silica-based fibrewith a sintered anatase-TiO_2 layer on the surface), around a low pressure UV lamp (254 nm, 220 V, 100–400 W overall energy consumption, 10 mW/cm_2 nominal fluence rate). To protect the fibre from particle contamination, two pre-filters with a mesh width of 25 and 5 m were installed. The elimination rate was evaluated after 1, 3 and 9 cycles with the photocatalytic chamber (UV/TiO_2) and with UV only. Removal obtained with 1 cycle was marginal.

Another interesting investigation was carried out by Vasconcelos et al. (2009), aiming to compare the degradation of just ciprofloxacin in hospital effluent by ozonation, UV irradiation, UV/TiO_2 and O_3/H_2O_2. As to TiO_2/UV lab scale equipment was used and TiO_2 was added as a uspension (400 mg TiO_2/700 mL) to the hospital effluent set at pH = 3 to enhance photocatalyst activity (see Supplementary data for process details). After the treatment, the samples were filtered through a 0.22 μm membrane to separate TiO_2 particles from the solution. Complete removal of ciprofloxacin was observed after 60 min within the photocatalytic reactor. The same result was obtained after 300 min in an UV reactor (equipped with a 125 W medium pressure mercury lamp).

UV/TiO_2 exhibited a better removal than UV only for a few compounds, in particular for 4-aminoantipyrine, 4-methylaminoantipyrine and sulfapyridine. In general the removal efficiencies increased by a factor of two for most of the compounds without a photocatalyst.

An increment in the cycles slightly improved the removal of contaminants. Only X-ray contrast agents achieved higher removal efficiencies than in the other post-treatments (20–70%). These results led to the consideration that direct phototransformation with UV dominated the micropollutant removal and indirect phototransformation due to the presence of the embedded TiO_2 did not occur. Generally the removal efficiencies observed with TiO_2/UV in 9 cycles were observed in only 3 cycles when using UV alone. The lower removal efficiency observed by UV/TiO_2 might also be due to the fact that photocatalytic fibre could have adsorbed UV light and shaded part of the reaction chamber, thus the water could have been exposed to less UV irradiation.

An improvement in the removal of PhCs was observed when H_2O_2 was added to the UV reactor. No consistent differences were found between a dosage of 0.56 g/L and 1.11 g/L (Kohler et al., 2012). It was also found that the optimum light wavelength for the UV/H_2O_2 system is 254 nm as it guarantees the lowest background absorbance of the investigated water and high H_2O_2 absorbance resulting in an efficient generation of hydroxyl radicals. As a consequence, LP lamps are recommended as about 90% of their irradiated light is emitted at 254 nm, whereas MP lamps emit 254 nm light for 5–10% of the total emission. The good results obtained with LP UV irradiation in

AOPs lead to the consideration that for many PhCs, degradation processes are mainly due to chemical oxidation (between the molecule and the generated radicals)rather than to direct photolysis (Kohler et al., 2012).

Wilde et al. (2014) achieved promising results thanks to the degradation of a mixture of beta-blockers (atenolol, propranolol and metoprolol) in hospital effluent (pretreated in a septic tank followed by an anaerobic filter) by O_3 and Fe^{+2}/O_3: they showed that, in 120min, complete degradation of the parent compounds was observed but not their complete elimination. The degradation process was found strictly correlated to pH. Alkaline pH values promote the removal of metoprolol and propranolol, whereas acidic values enhance the removal of organic load (expressed as COD). The investigation also highlighted the risk of undesired byproducts due to ozonolysis with a more intense degree of recalcitrance with respect to their parent compounds. This lead to better investigated ecotoxicological characteristics of the polished effluent. A slight increment in the removal of micropollutants was observed by adding H_2O_2 into the system. H_2O_2 accelerates the decomposition of ozone and partially increases the amount of hydroxyl radicals. Two different application modes were tested within the PILLS project (McArdell et al., 2011):

– addition of H_2O_2 into the ozone reactor influent;

– pre-ozonation of the MBR permeate with 1.2 g O_3/g DOC, addition of 2.5mg/L H_2O_2 to half of the treated wastewater and both parts again treated with 0.7 g O_3/g DOC.

Differences were observed of about ± 20% which were not considered significant because within experimental error, in agreement with data already published confirming that little improvement was found especially in water with relatively high DOC (Acero and von Gunten, 2001) and that hydroxyl radicals attack is less effective than O_3 attack.

A significant removal efficiency is observed if very high doses of ozone and H_2O_2 are applied to the permeate as tested by Nielsen et al. (2013) (130 mg O_3/L and 60 mg H_2O_2/L 5 min; 450 mg O_3/L and 200 mg H_2O_2/L 15 min): in these operational conditions with few exceptions (sulfamethoxazole) all the selected micropollutants were removed below their PNEC/EQS (environmental quality standard) value.

In order to guarantee a clear, polished effluent, sometimes a “trap” step follows the AOP reactor. In this context, the effluent of a PAC reactor was filtered through UF membrane flat sheets (pore size 0.04 μm) (Switzerland, McArdell et al., 2011). Moreover within the PILLS project units, a moving bed bioreactor (HRT = 0.3–1 d) was used following PAC, O_3 or TiO_2/UV and a sand filter (filtration velocity v_f b 12 m/h) was equipped after ozone or the PAC unit.

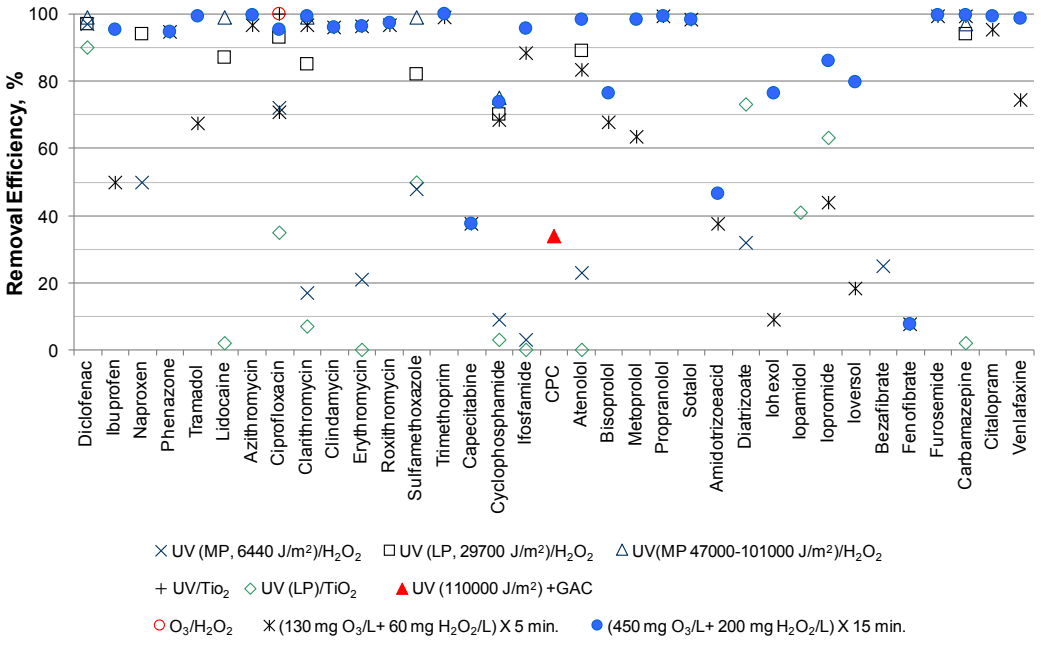


Fig. 6.11: Observed removal efficiencies for a group of selected PhCs in HWWby AOPs. Data from: Lenz et al., 2007b; Vasconcelos et al., 2009; PILLS Report, 2012; Nielsen et al., 2013.

Removal of microorganisms

Disinfection efficiency is strictly correlated to the applied technologies. Table 6.6 reports the efficacy of 7 different treatments applied to a secondary hospital effluent (Machado et al., 2007) or a secondary hospital laundry effluent (Kist et al., 2008) carried out in Brazil: Themain influent characteristics to the disinfection stepwere: 25 °C, pH=9.5, upstream treatments: septic tank +anaerobic/aerobic treatment fed with hospital/laundry effluent. A dose of 12 mg O_3/L was applied and equipped with a UV lamp with an emission at 254 and 365 nm, radiating an energy of 31.9 J/cm_2. Catalyst fixation was obtained by preparing a suspension of TiO_2 in $CHCl_3$ (10% m/v) and by spreading it on a plate (2.96 mg TiO_2/cm_2). The contact time was 60 min for each.

The best disinfection efficiency was observed for the combination UV/TiO_2/O_3, that also provides very good turbidity removal (from 234 to 36.5 NTU), surfactants (8.0 10^6mg/L to detection limit) and toxicity (EC_{50} Daphnia magna from 65 to 100). A contact time of 10 min will result in a concentration of 330 MPN/100 mL and of 30 min of about 70 MPN/100 mL.

The disinfection performance is due to damage of the microorganism's cell wall and cytoplasmatic membrane. Thus cell permeability increases allowing intracellular content to flow through the membrane leading to cell death.

Table 6.6: Disinfection performance by means of AOPs

Method	Secondary effluent thermotolerant Coliforms Machado et al., 2007	Laundry effluent thermotolerant Coliforms Kist et al., 2008
Secondary effluent	1.1 10^6	9 10^6
UV/O_3	17 000	110
UV	9000	
TiO_2	170	
O_3	170	
O_3/TiO_2	120	1700
UV/TiO_2	40	20
UV/TiO_2/O_3	< 2	< 20

6.4.3.5 Nanofiltration and reverse osmosis

Nanofiltration (NF) and reverse osmosis (RO) processes are considered potential polishing treatments for hospital effluent, pretreated in an MBR from a technical view point. Residues of PhCs, still present in the permeate, may be retained due to molecularweight and size, sorption onto the membrane and also charge. Each membrane is characterized by a molecular weight cut off (MWCO) that represents the weight of those substances retained between 60 and 90%. Sorption is a potential removal mechanism for poorly soluble non-polar compounds, negatively charged compounds are rejected by NF/ROmembranes due to electrostatic repulsion between the compounds and the negatively charged membrane surface (Kimura et al., 2004). Moreover, water characteristics such as pH, ionic strength, hardness, organic matter and membrane biofouling also have an influence on solute rejection.

In the study by Beier et al. (2010) the permeate of an MBR (COD < 30 mg/L, 5–10 mg N/L) equipped with microfiltration membranes was then subjected to NF and RO processes, characterized by a MWCO of 300–400 Da and 100–150 Da, respectively. It was found that RO exhibited a higher removal for all selected PhCs with respect to NF. However, RO presents major disadvantages due to the limited yield and the retentates that have to be properly disposed of. However, no suitable prediction model has been developed up to now as the rejection of the different micropollutants in NF/RO processes is specific for each membrane (Siegrest and Joss, 2012).

6.4.3.6 Chlorination

Only a few data are available regarding the removal efficiency of PhCs observed after a final chlorination. These are reported in Fig. 6.12 and refer to the investigation carried out by Nielsen et al. (2013). The added amount of ClO_2 was 60 mg/L in each run, and two different contact times were adopted: 15 min and 60 min. Ciprofloxacin showed higher concentrations in the effluent rather than in the influent to the treatment. In addition, chlorination seems to be able to remove diclofenac: in the study by Nielsen et al. (2013), its concentration in the influent (MBR permeate) was quite low (<5 ng/L) and in the effluent it was 1 ng/L (15min s contact time). But it was found that under lab scale controlled chlorination with surface water, diclofenac exhibited a large degree of reactivity and its final concentration was below detection limit (Westerhoff et al., 2005).

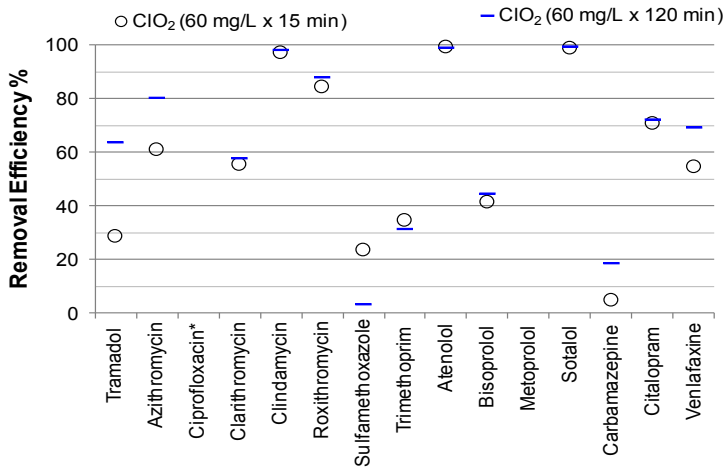


Fig. 6.12: Removal of PhCs by final chlorination. Data from: Nielsen et al., 2013.

6.4.4 Disinfection performance

In some countries disinfection is mandatory for the effluent generated in infectious disease wards or in health care specialized in infectious diseases (Nardi et al., 1995; Emmanuel et al., 2004). Faecal and total coliforms were found in the ranges 10_2–10_4 MPN/100 mL and 10_4– 10_6 MPN/100 mL respectively (Table 6.2). These values are lower than those usually found in raw urban wastewater (Verlicchi et al., 2012a), probably due to the antimicrobial activity of antibiotic and disinfectant residues present in the infectious disease ward effluent.

At a dosage of 10 mg/L of ClO_2 and a contact time of 30 min faecal and total coliforms drop to less than 12,000 and 20,000 MPN/100 mL and a complete removal of viruses was always observed (Nardi et al., 1995). Predisinfection of raw hospital effluent is still an issue of great concern: based on a theoretical hypothesis, Korzeniewska et al. (2013) recommend a preliminary disinfection of the hospital effluent before its immission into public sewage in order to minimize the spread of antibiotic resistant bacteria, on the other hand, research by Emmanuel et al. (2004) found that disinfection by means of NaOCl of the effluent from infectious and tropical disease departments can reduce the content of microorganisms, but at the same time it has toxic effects on aquatic organisms.

In many countries, including China, direct chlorination or primary treatment followed by chlorination represents the most widely used methods to treat and, in particular, disinfect hospital effluent in order to prevent the spread of pathogenic microorganisms (Liu et al., 2010).

Despite the fact that chlorine disinfection has a broad spectrumof activities against bacteria, virus and fungi and it is simple to use, it may produce toxic byproducts, its performance depends on the water quality and only a low removal efficiency is achieved for viruses as they have a greater tolerability against chlorine compounds than bacteria. As a consequence, a high excess of disinfectant is generally applied to guarantee a (rough) disinfection of the hospital effluent, but inevitably extremely high concentrations of residual chloride (as high as 100– 130 mg/L) will occur, resulting in serious pollution problems to the receiving aquatic environment, as remarked by Emmanuel et al. (2004).

who investigated the effect of the addition of NaClO to hospital effluent: it can greatly reduce bacteria population, but it has toxic effects on aquatic organisms.

In China, to avoid an excessive use of chlorine, the removal of different types of microorganisms from hospital effluent is dealt with by means of an MBR, mostly employing submerged membranes (pore size about 0.2–0.4 μm), followed by a chlorination step with a dosage of NaClO of 1–2 mg/L as free chlorine with a contact time of 1.5 min. Since 2000, many plants based on membrane technologies have been built for the treatment of hospital effluent, with a capacity ranging between 20 and 2000 m_3/d, in compliance with the severe limits of 50 PFU/100 mL such as E. coli (Liu et al., 2010).

While a (UF) MBR followed by a specific disinfection step may be considered a viable option for the removal of a wide group of bacteria occurring in hospital effluent, studies into their performance in reducing pathogenic viruses are still scarce. The removal of viruses in an MBR is substantially due to three mechanisms: virus rejection depending on the cake generating on the membrane surface, viral inactivation of the biomass, and adsorption onto the surface of suspended solids which makes these microorganisms more stable.

In a Brazilian investigation (Prado et al., 2011) the removal of some enteric viruses (rotavirus A, human adenovirus, norovirus genogroups I and II and hepatitis A viruses)was compared in two different treatment trains: an anaerobic one including a UASB followed by three anaerobic filters and an aerobic one consisting of a conventional activated sludge process followed by chlorination. It was found that both systems are not suited to their removal. Their frequencies of detection and quantification results varied according to the virus type and effluents coming from different health care structures. An MBR, equipped with ultrafiltration membranes, is able to remove groups of bacteria as reported above mainly due to membrane retention, reducing the spread of multiple antibiotic resistant strains, usually occurring in hospital effluent. But specific disinfection is advisable, in order to avoid regrowth of (survival) bacteria as discussed in Pauwels et al. (2006). For inactivation of pathogens and possible removal of antibiotic resistant bacteria, UV and ozonation are more efficient with respect to PAC and GAC.

In wastewater disinfection, the fluence to apply depends on the required microorganism limits (Verlicchi et al., 2011). For instance 100 J/m^2 is applied if the aim is to guarantee 1000 MPN/100 mL of total coliforms, 750–850 J/m^2 if a concentration of 23 MPN/100 mL of total coliform has to be guaranteed and finally a fluence greater than 1000 J/m^2 if the residual concentration of total coliform is <2.2 MPN/100 mL, thus allowing an unrestricted irrigation of the disinfected effluent (Crites and Tchobanoglous, 1998).

To inactivate specific microorganisms, oocysts or viruses, the requested fluence could be higher. To inactivate 3 log of Adenovirus type 40, a fluence of 1670 J/m^2 is required, whereas to inactivate up to 3 log of Cryptosporidium and Giardiasis, a fluence of 120 J/mis required (Hijnen et al., 2006).

These considerations lead to the consideration that when ozonation, UV, AOPs in general are applied to hospital effluent to remove recalcitrant compounds, at the same time it is disinfected to a very high degree. But in order to guarantee safe reuse of the disinfected effluent for unrestricted irrigation, a higher fluence is required (as well as further studies into the ecotoxicologic characteristics of the water).

6.4.5 Comparison between the different treatments

A comparison of the performance of the different analyzed secondary and tertiary dedicated treatments for HWW is depicted in Fig. 6.13 in terms of number of investigated compounds and the number of compounds exhibiting a removal efficiency greater than 80%. It is based on all the data collected about PhCs in the peer reviewed papers included in this manuscript. What clearly emerges is that the most investigated technologies are MBR, PAC, ozonation and UV. The best results were performed by MBR (secondary step) and PAC (tertiary step). Moreover Table SD-3 in the Supplementary data of Verlicchi et al., (2015) compiles compounds that exhibited a removal efficiency greater than 80% during secondaryand tertiary treatments, with the corresponding references.

An in-depth analysis of the comparison of pairs of treatment is performed in Kovalova et al. (2013) with respect to the different classes of PhCs. They found that iodinated contrast media were better removed by MBR+ UV (66% of the total influent load), all the selected PhCs except iodinated contrast media by MBR + PAC or MBR + UV (99%). Lessons learned from these campaigns led to consider 1.08 g O_3/g

DOC, 23 mg/L PAC and 2400 J/m_2 UV the values that best satisfy the two following choice criteria: relatively good abatement for most micropollutants and reasonable running costs (Kovalova et al., 2013).

Table 6.7 reports a rough estimation of the global removal of the different kinds of classes with respect to different technologies, based on all the collected data.

It is important to observe that the choice of the best technologies for treatment of hospital effluent should not necessarily lead to the complete removal of specific parent compounds, but to the removal of the estrogenic activity of the effluent itself, or more generally, a reduction in its ecotoxicological effects.

Bearing this concept in mind, processes including TiO_2 photocatalysis seemto be promising technologies as they are able to remove estrogenic activity of 17-β-estradiol (Byrne et al., 1998) and 17-α-ethinylestradiol (Coleman et al., 2000).

Table 6.7: Removal efficiencies expected for the different groups of compounds

Group	PAC	AOP	UV	Cl_2/ClO_2	Coag/Floc
Antibiotics	40-90	20-90	40-90	20-90	<20
Antidepressants	70-90	20-90	40-90	20-70	<20-40
Analgesics/Anti-inflammatories	>90	20-90	70-90	20-70	<20
Lipid regulators	>90		>90	20-70	<20
X-ray contrast media	70-90	70-90	20-90	20-70	<20-40
Disinfectants/detergents	>90	>90	40-90	>20	<20-40

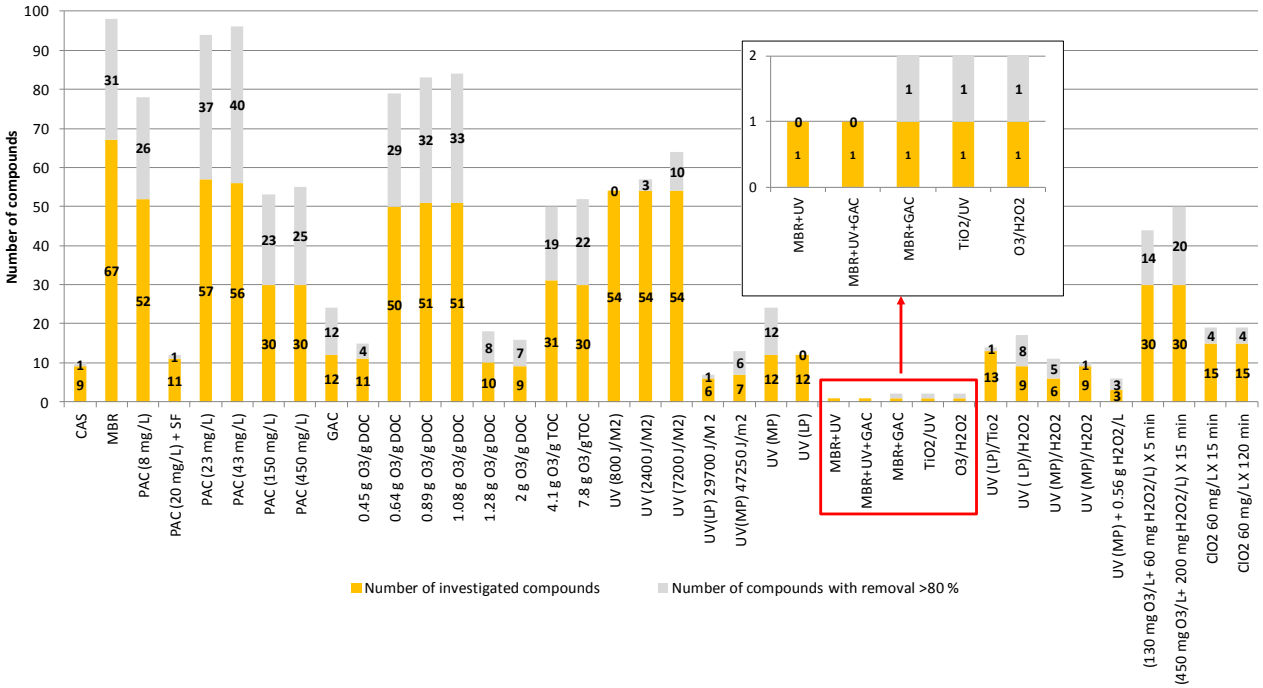


Fig. 6.13: Comparison among secondary and tertiary treatments of HWW with a view of the number of investigated compounds and of compounds exhibiting a removal efficiency greater than 80%.

AOPs seem to be the most promising technologies as they can be effective in removing compounds not affected by other technologies as discussed above, reactions are generally fast, resulting in more compact reactors, finally (no or) low chemical doses are required leading to (no or) lower residuals, but they may have undesirable drawbacks, namely:

unselective hydroxyl radicals, production of more hydrophiles and more difficult to treat byproducts than the original ones; as have been clearly listed by Suty et al. (2004).

The spread of disease due to pathogens and of specific strains of antibiotic resistant bacteria can be countered by a disinfection step (Korzeniewska et al., 2013). Some laws and regulations (including the Italian Deliberation by the Inter-ministerial Committee dated 4 February 1977) require treatment of the effluent from health care structures, blood analysis laboratories, and in particular, for the effluent from infectious disease wards. As an example, the effluent produced by the very large laboratory for blood analysis in Pievesestina (Cesena, North Italy, effluent flow-rate about 103 m^3/year) is subjected to ozonation and filtration through activated carbon prior to being immitted into the public sewage system and is then co-treated at the municipal WWTP. Alternatively, the addition of 10 mg/L of ClO_2 and a contact time of 30min guarantee an efficient removal of faecal and total coliforms, with a negligible increment of AOX (Nardi et al., 1995). This increment is consistent if the applied disinfectant is NaClO (Emmanuel et al., 2004). Due to the different nature of pollutants that may be present in hospital effluent (residues of PhCs, their metabolites, disinfectants and antiseptics, heavy metals, radio-elements, pathogens), the risk posed by this effluent may be toxic, radioactive and infectious.

Proper management of hospital effluent has to be considered and must include measures to mitigate the consequences at a WWTP level as well as towards the environment.

6.4.6 Removal efficiencies vs. physical–chemical properties of investigated compounds

Many studieswere developed in order to investigate potential correlations between observed pharmaceutical removal efficiencies achieved by the different wastewater treatments and pharmaceutical molecular properties (among them Cunningham, 2008; Joss et al., 2006; Rogers, 1996; Tadkaew et al., 2011). They underlined that it is always very difficult to find reliable correlations, because many factors (i.e., operational and environmental conditions) affect removal mechanisms of such complex molecules thus a wide range of variability is generally observed for the removal of a specific compound during a treatment. Studies referring to UWW led to rules of thumb that try to correlate the behaviour of a specific molecule on the basis of its properties: k_{biol}, K_d, K_{ow} and pK_a, as discussed and reported in Tadkaew et al. (2011) and Verlicchi et al. (2013). Lessons learned from UWW may be also useful in making a rough prediction of efficacy of specific treatments in HWW managing. Moreover attempts to correlate the behaviour of common parameters, such as COD or SS, and specific pharmaceuticals during hospital wastewater treatment were carried out, but unfortunately they did not suggest any reliable relationship (Emmanuel et al., 2004; Pauwels et al., 2006; Vasconcelos et al., 2009;Wilde et al., 2014).

6.5 Hospital effluent toxicity and environmental risk assessment

Interesting and useful research has been accomplished dealing with hospital effluent toxicity and assessment of the environmental risk posed by pharmaceutical residues in treated hospital effluent (Boillot et al., 2008; Perrodin et al., 2013; Emmanuel et al., 2004). This is quite a complex problem and is beyond the aim of this manuscript, but some lessons learned from published studies are discussed herein to point out concerns that merit further research. It is well known that hospital effluent is 5–15 more toxic than urban wastewater due to the high concentrations of detergent and disinfectants, often containing chlorine or aldehydes (such as sodium hypochlorite and glutaraldehyde), iodinated contrast media that lead to the generation of AOX in the drainage network, heavymetals (namely silver used in radiology departments), radio-elements injected or administered in nuclear medicine studies and completely excreted in urine and PhC residues. That being said, hospital effluent can inhibit the activity of the biomass in the aeration tank of a sewage facility by 7–8% as documented in Boillot et al. (2008) and Panouillères et al. (2007). Investigations

are often based on Microtox and acute D. magna tests (Emmanuel et al., 2004; Boillot et al., 2008), but also to batteries including different kinds of test (Perrodin et al., 2013).

Lessons learned from these studies suggest that different pollutants may induce or contribute to toxicity: namely free chlorine, AOX (Emmanuel et al., 2004), ethanol, propanol and metals including Zn, Cu, As and Pb (Boillot et al., 2008).

Environmental risk assessment of hospital wastewater is generally based on the risk quotient RQ, defined as the ratio between PhC concentration in the effluent and its predicted non-effect concentration (PNEC). According to the classification that was adopted in many studies (Straub, 2002; Verlicchi et al., 2012a; Santos et al., 2013) the risk is classified high if RQ ≥ 1, medium if 1 < RQ < 0.1 and low if RQ ≤ 0.1. Based on measured effluent concentrations Verlicchi et al. (2012a) and Santos et al. (2013) found that in raw hospital effluent a high risk is posed by azithromycin, clarithromycin, erythromycin, ofloxacin, sulfamethoxazole, metronidazole fluoxetine, ibuprofen, acetaminophen and iopromide. This fact pinpoints that adequate treatment is necessary for hospital wastewater to reduce its negative effect on the environment. Bearing this in mind, the frameworks provided by Al Aukidy et al. (2014), Emmanuel et al. (2005), Escher et al. (2011), Lienert et al. (2011) and Mullot et al. (2010)might help in evaluating and comparing the efficacy of different treatment trains.

6.5.1 Antibiotic resistance bacteria

Another source of risk in hospital effluent is correlated to the occurrence of antibiotics and consists in the potential development and release of antibiotic-resistant bacteria (ARB) and genes (ARG). The PILLS project pinpoints that the risk of the spread of resistance to specific antibiotic molecules is higher in hospital effluent than in urban WW. The efficiency of advanced biological and chemical processes varies in therange of 1–5 log units. Ultrafiltration MBRs guarantee a consistent reduction of this risk, whereas a following step including ozonation, sand or PAC filtration does not contribute to further reduction.

6.6 Costs

A summary of the investment and operational and maintenance (O&M) costs for the different scenarios is reported in Table 6.8 referring to economic evaluations carried out in the cited studies in a design step. Unfortunately they are not homogeneous and not always investment and operational and maintenance data are available. The investments are amortized over 10 or 15 years depending on the investigations. Table 6.8 just offers a rapid comparison of the different technologies and of the order of magnitude of the different treatment rains. Many considerations may arise from these reported values. For example, it emerged from previous discussion of collected removal data of PhCs that activated carbon seems a promising technology in reducing their occurrence in the final effluent. But activated carbon requires expensive maintenance operations in order to guarantee proper performance.

In this context, investment cost for an activated carbon filter is lower than that of another AOP treatment, but if DOC levels in the stream fed to the carbon filter are above 10 mg/L, carbon treatment could become uncompetitive against AOPs, due to frequent change out, regeneration and disposal of the exhausted carbon. Moreover, GAC and PAC do not destroy microcontaminants, but they allow their transfer from a liquid phase to a solid one. Operational costs should also include costs of final disposal of GAC and PAC. To have an idea of the potential cost of dedicated treatment of hospital effluent, total costs range between 4.1 €/m^3 and 5.5 €/m^3 in case of secondary treatment by means of an MBR and polishing AOPs with the exception of Kovalova et al. (2013) that reported lower total costs ranging around 2.4–2.7 €/m^3. These differences were not commented by the two research groups within the PILLS projects.

PART B

Table 6.8: Investment and O&M costs for hospital effluent treatment with different technologies

Author	Kajitvichyanukul and Suntronvipart 2006	Liu et al. 2010	Verlicchi et al. 2010a	Beier et al. 2012	Pills project 2012				Kovalova et al. 2012		Nielsen et al. 2013							
Place	Thailand	China	Italy	Germany	Netherlands				Switzerland		Denmark							
Type of treatment	Photo-Fenton	MBR	MBR+O_3+UV	MBR	MBR	MBR + GAC	MBR + O_3 + GAC	MBR +UV/H_2O_2 + GAC	MBR + PAC	MBR + O_3	O_3 82 mg/L x10 min	O_3 156 mg/L x 20 min	O_3+H_2O_2 (130+60) mg/L x5 min	O_3+H_2O_2 (450+200) mg/L x 15 min	PAC 150 mg/L	PAC 450 mg/L	ClO_2 60 mg/L x 120 min	MBR+O_3 156 mg/L
Investment cost (€/m^3)			3.6		3.25	3.35	3.5	3.65										
O&M cost (€/m^3)	0.38[1]	0.45-0.163[1]			1.45	1.65	1.75	1.85			0.22	0.4	0.34	1.08	0.31	1.06	0.3	1
Total cost €/m^3				4.1	4.7	5	5.3	5.5	2.7	2.4								

6.7 Current strategies and future perspectives in the treatment of hospital effluent — conclusions

Management and treatment of hospital effluent greatly vary in different countries. In developed ones they may be completely absent, meaning that HWW is directly discharged into a surface water body or they consist in simple chlorination, or primary clarification followed by a chlorination or primary and secondary treatments followed by chemical disinfection (Prayitno et al., 2014).

Various research projects have been carried out in these countries, aiming to evaluate the suitability of some (simple) treatment trains for hospital effluent. They generally refer to a discussion of the observed removal efficiencies of *conventional* contaminants and microorganisms, and the possibilities to directly re-use this reclaimed water for irrigation purposes as they have to face problems arising from water shortage (among them Chitnis et al., 2004; Shestha et al., 2001; Beyene and Redaie, 2011, Abd-El-Gawad and Aly, 2011). Suggestions to improve the adopted treatment are also provided with a view to their applicability in terms of land requirement, footprint, costs, installation, operation and maintenance. Some case studies have been reported herein. Direct reuse of reclaimed water should be evaluated, including the risk posed by persistent emerging contaminants and their (acute and chronic) effects on the environment and human health.

In European countries major concerns are devoted to improve removal of these persistent compounds by means of end-of pipe treatments and in this context, AOP technologies are the most researched ones. Studies generally refer to occurrence and removal of a consistent number of PhCs, as well as ecotoxicological evaluation by means of the risk quotient ratio, i.e. the ratio between maximum measured concentrations and predicted no-effect concentration (Verlicchi et al., 2012a; Escher et al., 2011). Different full scale WWTPs have already been constructed for the dedicated treatment of hospital effluent. Each one consists in preliminary treatment, MBR (Beier et al., 2011), MBR followed by ozonation and UV (Verlicchi et al., 2010a), ozonation and PAC (PILLS report, 2012), ozonation and GAC (Pharmafilter, 2013;Grundfos Biobooster, 2012).

An interesting approach has been adopted in France to manage and treat the effluent of the Centre Hospitalier Alpes Lemon in Annemasse. Thanks to dedicated piping, the HWW is conveyed to the near municipal WWTP where it is treated in a specific line and subjected to continuous monitoring to improve the removal of persistent compounds. This was a decision taken by the local authorities who have even drawn up a specific law for this site (Sibipel Report, 2014).
The best option in the management and treatment of hospital effluent is strictly correlated to hospital size and catchment area dimension and must be defined on the basis of a technical and economical feasibility study that would focus on the most appropriate measures able to reduce the (macro and micro) pollutant load discharged into the surface water environment.

Dedicated treatments for hospital effluent are recommended by many Authors worldwide, segregation and special treatment seems adequate for specific effluent including effluent generated in radiology wards, containing ICMs, the most recalcitrant compounds, at extremely high concentrations, but also for the effluent from laundries, oncological wards and clinical analysis laboratories, as in the case of the large and centralized Italian lab services discussed above. In any case, dilution with surface water should not represent the proper action to mitigate potential adverse negative effects of PhC residues in the environment.

A final remark is suggested by studies promoting the implementation of energy-intensive systems with indirect solar energy by aggregating photovoltaic cells for the generation of electrical energy. This may result in energy storage and in a balanced use of energy during periods in which light incidence is lower.

Reference

- Verlicchi P, Al Aukidy M, Zambello E. What have we learned from worldwide experiences on the management and treatment of hospital effluent? — An overview and a discussion on perspectives. Sci Total Environ. 2015;514:467-491.

Chapter 7:

7 Predicted and measured concentrations for selected PhCs in hospital effluent. Limits and advantages of the two approaches through the analysis of a case study.

7.1 Introduction

In the last decade some research groups have investigated hospital effluent in terms of micropollutants.

The review by Verlicchi et al., (2010a) focused on the comparison between the HWW (hospital wastewater) and UWW (urban wastewater) based on literature data referred to measured concentrations of the most common pharmaceutical compounds (PhCs) belonging to different therapeutic classes. They concluded that for PhCs the ratio between average PhC concentrations in HWW and in UWW ranges between 1-150, being the highest values found for contrast media. Nonetheless, these effluents usually do not undergo any specific treatment before being discharged into urban sewage network even if, recently, the problem of hospital effluent is becoming more important. Up to now studies dealing with the presence of PhCs in HWW are still fewer than those referring to UWW. This is due not only to high analysis costs of the investigations, but also by the difficulties in organizing water sampling campaigns inside health facilities.

Very few studies evaluated the content of these contaminants on the basis of predictive equations correlated to specific consumption inside the hospital (Kümmerer and Henninger 2003). Some Authors investigated the possibility to estimate PEC for selected compounds in hospital effluent was done, for instance, both in Swiss (Escher et al., 2011) and Australia (Le Corre et al., 2012, Ort et al., 2010a).

PhCs consumption varies from country to country, especially given the restrictions on the use of some pharmaceuticals (for instance, vancomycin is widely used as a first-line antibiotic in the United States, whereas its use in European countries is highly restricted), and from year to year, due to the progress in the development of new active ingredients. Moreover, analysis of the distribution of PhC consumption throughout the year evidences that there are *critical* months, (concentrations tended to be higher in winter than in summer) (Verlicchi et al., 2013).

Micropollutants compounds present in a hospital effluent are strictly correlated to the activities that take place inside the structure (research activities, diagnosis, surgeries, laboratories, wards as well as drugs that can be administered to patients) and are defined by protocols that are periodically revised. Moreover, characteristics of the hospital effluent seem to be influenced by the size of the structure (the smaller hospital discharged higher mean concentrations than the larger one).

The possibility to predict the concentration in hospital effluent in a reliable way could be useful to administrators and technicians who need to perform case-by-case analyses on a local scale, in particular during WWTP planning and design phases, in order to determine the best means of tackling the problem. Also risk assessment guidelines are based on equations that compared the predicted environmental concentration with toxicological data (EMEA, 2005).

As for urban WW, assessments may be conducted through either monitoring programs, providing measured environmental concentrations (MECs) or prediction models, based mainly on yearly consumption, which can be used to calculate local predicted environmental concentrations (PECs). A debate on which approach should be used or preferred is still open, as both approaches possess limitations (Johnson et al. 2008). On one hand, administered PhCs cover a wide spectrum of substances with different physico-chemical properties, while the analytical techniques available in laboratories are available only a small group, restricted also by the high cost associated to the analysis. On the other hand, prediction models, despite providing a cheaper way to evaluate concentrations of a larger number of compounds, might yield results that do not depict the real situation but that could be useful as the first step of a screening tool.

As for hospital effluent the comparison is still less investigated, the aim of this study is to compare measured and predicted concentrations of 39 PhCs in hospital effluent and to discuss the accuracy and usefulness of applied models in assessing their concentrations. The occurrence of the target compounds was monitored in the effluent of a large hospital structure and it has already been presented and discussed in a previous study (Verlicchi, Al Aukidy, Galletti, et al. 2012).

Information on the PhCs consumption during 2011 and data related to their excretion were used to predict the concentrations of the selected pharmaceuticals for the studied site. The measured and predicted concentrations were compared according to a criterion available in literature and already

adopted in similar studies (Coetsier et al. 2009). The results show that the comparison was acceptable for the majority of compounds while for others the difference between the measured and the predicted concentrations was very high. The main potential factors that influence both measured and predicted concentrations are identified by means of an assessment of the uncertainties in the measured values and sensitivity of all the parameters required for predicting concentration ones and the possible reasons for the discrepancies are widely discussed. The complications and the limitations of each technology are underlined. The efficacy of the predictions was hampered by the consumption report, that is only annually communicated, and by the big variability of available excretion rates.

7.1.1 The site under study

The hospital under study (Sant'Anna Hospital, Ferrara) is one of the largest health structures in the North Italy with 900 beds and 2000 members of staff. It is located in the center of a town of 135 000 inhabitants (bed density of 6.5 bed/1000inhab). It is a teaching hospital including a wide spectrum of healthy services and more than 50 wards. It offers a comprehensive set of medical services typical of a modern regional hospital such as general medicine, surgery, orthopedics, psychiatry, trauma, oncology, radiology, hemodialysis, obstetric, gynaecology and neonatality, intensive care units. Its effluent is directly discharged into the combined sewage network and it is conveyed to the local WWTP where it is treated together with the urban WW.

The hospital flow rate is regularly monitored by the internal Water and Wastewater Network Managing Body. The resulting average flow rate is equal to 603 m^3/d, corresponding to a specific water consumption of about 670 L bed^{-1} d^{-1}. (Verlicchi, Al Aukidy, Galletti, et al. 2012).

Fig. 7.1: Sant'Anna Hospital at Ferrara. Entrance

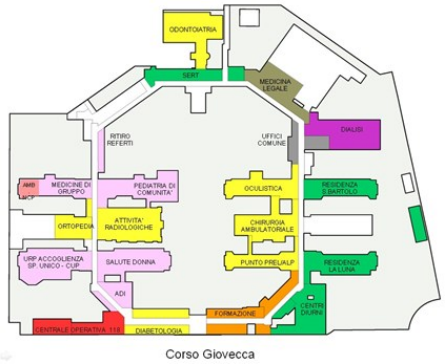


Fig. 7.2: Structure of the hospital after the demise. Ground floor.

Today, this hospital is not longer in operation because the wards were transferred to the newer hospital of Cona. In the centre of Ferrara a heath care structure is still remain. It is composed by a three floors building with the structure as follows.

Table 7.1: List of Sant'Anna Wards

Sant'Anna Wards
Anesthesia and intensive care, audiology, cardiology, center of eating disorders, Clinical Oncology, Clinical Pharmacology, Dentistry, dermatology, diabetology, Digestive Endoscopy, Ear nose and throat specialist (ENT), Endocrinology, First Aid and Emergency Medicine, Forensic Medicine and Social Insurance, Gastroenterology, Geriatrics, Haematology, Hospital Radiology, Immunohaematology Transfusion, Infectious Diseases and Tropical Diseases of Migrants, Infectious Diseases Hospital, Internal Medicine, Laboratory analyzes, Clinical chemistry and microbiology, medical clinic (medicine), Medical Genetics, Medical Physics, Neonatology and Neonatal Intensive Care, Nephrology, Neurology, Neurophysiology, Neuroradiology, Neurosurgery, Nuclear Medicine, Obstetrics-Gynecology, Occupational Medicine, Ophthalmology, Orthopaedics, Pathological anatomy, Pharmacy , Pneumology, Psychiatry, Radiation Oncology, Rehabilitation (Sector Rehabilitation Medicine - San Giorgio), Respiratory Pathophysiology, Rheumatology, surgery, Ultrasound Interventional, University Radiology, Urology, Vascular and Interventional Radiology, Vascular diagnostics.

7.1.2 Target compounds

The 11 therapeutic classes considered in this study are reported in Table 7.2 together with the corresponding compounds. The main physical-chemical properties of the PhCs are reported in Table A1 of Appendix A. They are drugs commonly prescribed in human medicine for prevention and treatment of several diseases and, consequently, they are expected to be found in hospital effluents as well as in urban sewage.

The 39 PhC compounds were selected on the basis of these criteria:

(1) available data of the consumption within the hospital (according to data provided by the Internal Pharmaceutical Office);
(2) available measured concentrations in the raw effluent of the same hospital
(3) potential adverse effects on water environment
(4) their occurrence and ubiquity in the aquatic environment

Table 7.2: Selected compounds listed according to the therapeutic class they belong to.

Therapeutic Class		Selected Compounds	Number of compounds
Analgesic/Anti-inflammatory	A	Acetaminophen, Codeine, Diclofenac, Ibuprofen, Indomethacin, Ketoprofen	6
Antibiotic	B	Azithromycin, Chloramphenicol, Chlortetracycline, Ciprofloxacin, Clarithromycin, Doxycycline, Erythromycin, Metronidazole, Norfloxacin, Ofloxacin, Sulfadiazine, Sulfamethoxazole, Tetracycline, Trimethoprim	14
Antidiabetics	C	Glibenclamide	1
Antihypertensive	D	Enalapril, Hydrochlorothiazide, Lisinopril	3
Antineoplastic	E	Tamoxifen	1
Beta-agonist	F	Salbutamol	1
Beta-blocker	G	Atenolol, Metoprolol, Propranolol, Sotalol, Timolol	5
Diuretics	H	Furosemide	1
Lipid regulator	I	Atorvastatin	1
Psychiatric drug	J	Carbamazepine, Diazepam, Fluoxetine, Lorazepam, Paroxetine	5
Receptor antagonist	K	Ranitidine	1

Table 7.3 reports the mean removal efficiencies for each selected compounds and underlines those that pose high environmental risk and that belong to different priority lists.

Chloramphenicol, glibenclamide, paroxetine, lorazepan and lisinopril (highlighted in grey in the table) do not belong to any of the reported priority list and seem not pose high environmental risk. While for the last two compounds no removal data are available, the other ones achieved elevated removal efficiencies in conventional secondary systems. There are compounds that are reported to be priority by many Authors even they are well removed in conventional WWTPs (*acetaminophen, ofloxacin and sulfamethoxazole*). This can be due to the high amount consumed or/and to high toxicity.

Clarithromycin and *erythromycin* instead are priority and exhibited a low removal efficiency.

Table 7.3 shows that conventional WWTP are not able to effectively remove *furosemide, carbamazepine* and *diazepam*. These compounds have to be considered with particular attention as generally presented in high concentration in hospital effluents.

The mentioned compounds are reported in bold in the table as have to be considered with particular attention for the reported reasons.

The priority classes of GWRC, (2008) are indicated with ordinal number. [7 priority criteria: Regulation; Consumption; physicochemical properties; Degradability/persistence; Resistance to Treatment; ecotoxicity; Occurrence in SW, groundwaters, drinking water and WW. I class satisfies more than 4 criteria; II class more than 2 criteria; III class 2 criteria].

7.2 Measured concentration in hospital effluent

The issue of PhC occurrence in hospital effluents has already been investigated by different Authors (Verlicchi et al., 2010a) finding concentrations generally from 1 to 150 times higher in HWW than UWW. Moreover, from the analysis of the percentage contribution of the hospital WW to the WWTP influent PhC load conducted by Verlicchi et al., (2012), it is evident that hospitals represent one of the main sources of pollutants (in particular antibiotics, receptor antagonists and lipid regulators).

In this investigation, the occurrence of 39 common PhCs from 11 different therapeutic classes in the effluent of Sant'Anna hospital in Ferrara in the Po valley are reported from the paper by Verlicchi et al., (2012) and analysed according to the aim of this work.

7.2.1 Sampling: mode, frequency and analysis

Four 24-h time-proportional composite samples were collected from the raw effluent of the hospital in dry days during August 2009 (summer) and March 2010 (winter). The HWW is not easily accessible. All the details of the sampling campaign and analytical methods were provided by Verlicchi et al., (2012a). Time proportional samples do not take samples weighted according to the flow in the sewer.

It is important to observe that the fraction of the selected pharmaceutical sorbed onto the suspended solids is removed during preparation phase and, as a consequence, the values of (measured) concentrations found correspond to the dissolved fraction of the investigated PhCs.

Ort et al., 2010 used a sophisticated continuous flow-proportional sampler to minimize the experimental uncertainties. Collecting representative samples requires a thorough knowledge of the sewer layout and awareness of potentially highly variable concentrations and loads in the course of a day. Ort et al., 2010 stated that continuously diverting a small flow-proportional side stream is conceptually the best solution to obtain representative samples for dissolved compounds. Moreover they prefer sampling over consecutive days to the alternative option of collecting samples on single days distributed over a longer period. Weissbrodt et al., (2009) suggested sampling periods over several weeks.

In the investigation on antibiotics by Diwan et al., (2013) it was found that continuous sampling showed lower concentrations in HWW than those obtained by grab sampling. This is due to dilution effect as the sample is collected and mixed with the total samples even when there is no PhCs in WW. Johnson et al., (2008) generally recommended integrated samples over time (e.g., a series of 24 h composite samples: time or flow proportional) for sampling rivers and streams. They added that installation of passive samplers can provide additional information. Flow weighted composite samples were collected also by Mullot et al., (2010) and Weissbrodt et al., (2009).

A method for estimating the required sampling frequency in order to not exceed a certain sampling error is described by Ort and Gujer, (2006).

The choice of the most adequate sampling mode depend on the question to be answered. Grab samples are suggested when the information is requested for a certain point in time, while continuous samples are suggested for dynamic systems in order to obtain a more realistic picture.

Table 7.3: Removal efficiencies achieved by CASs for each PhCs. The priority compounds and those posing RQ>1 are stressed. The ordinal numbers indicate the priority class the compounds belong to..

		Mean Removal in STP [%]	RQ>1					Priority lists					
		Verlicchi et al., 2012c	Verlicchi et al., 2012c	Verlicchi et al., 2012b	Mendoza et al., 2015	Kümmerer and Henninger, 2003	Santos et al., 2013	de Voogt et al., 2009	Roos et al., 2012	GWRC, 2008	European Directive 2015/495/EU	Daouk et al., 2015	RMMC, 2008
A	**Acetaminophen**	93		x	x		x	x	x	II			x
A	Codeine	68		x				x		II			x
A	Diclofenac	29			x			x	x	I	x	x	x
A	Ibuprofen	87	x	x	x			x		I		x	
A	Indomethacin	37		x									x
A	Ketoprofen	56											x
B	Azithromycin	44	x				x				x		x
B	Chloramphenicol	95											
B	Chlortetracycline	84											x
B	Ciprofloxacin	70				x	x	x		I		x	
B	**Clarithromycin**	40	x	x	x	x	x			II	x		
B	Doxycycline	71						x		III			
B	**Erythromycin**	26	x	x		x	x	x		I	x		x
B	Metronidazole	38					x					x	
B	Norfloxacin	68											x
B	**Ofloxacin**	60	x	x	x	x	x	x		II			
B	Sulfadiazine	93											
B	**Sulfamethoxazole**	52	x	x			x	x		I		x	x
B	Tetracycline	56	x										
B	Trimethoprim	40		x	x			x		II		x	x
G	Glibenclamide	45											
E	Enalapril	69								III			x
E	Hydrochlorotiazide	45								II			
E	Lisinopril	-											
N	Tamoxifen	-											
M	Salbutamol	61								III			x
G	Atenolol	38		x				x	x	I			
G	Metoprolol	24		x				x		II			x
G	Propranolol	39		x	x								x
G	Sotalol	29								II			
G	Timolol	-											x
H	**Furosemide**	5								II			
I	Atorvastatin	-											x
J	**Carbamazepine**	18						x	x	I		x	
J	**Diazepam**	14	x							II			x
J	Fluoxetine	56	x	x			x			III			x
J	Lorazepam	-											
J	Paroxetine	91											
K	Ranitidine	52						x		II			

7.2.2 Uncertainty on analysis

To evaluate the results it is important to communicate the expected uncertainty associated with the measurements or the estimations. Uncertainty associated with the measured concentrations of PhCs in hospital effluent consists of several components. Among them the uncertainties due to chemical analysis procedure, sampling mode and frequency contribute mostly to the total measurement uncertainty (Ort et al., 2010b; Kovalova et al., 2012). If the applied sampling protocol does not result in the collection of a representative sample, the large variation observed may not be ''true natural variation'' but ,instead, may simply be an artefact caused by inadequate sampling. The uncertainties are extremely hard to quantify if sampling is carried out with conventional (unsophisticated) devices, i.e. auto-samplers operated in a discrete sampling mode with (too) long time intervals, or grab samples (Ort, Michael G. Lawrence, Reungoat, et al. 2010). An accurate description of the optimal sampling modes is reported in Ort et al., (2010).

As the two error (on sampling and analysis) are independent, the total (overall) uncertainty was estimated by applying the Gaussian error propagation (for multiplication and divisions of independent uncertainties the squared relative standard deviations can be summed) from the individual uncertainties from sampling ($U_{sampling}$) and chemical analysis ($U_{analysis}$) as follows:

$$\sqrt{U_{sampling}^2 + U_{analysis}^2}$$

The uncertainty of chemical analysis was calculated from the relative recoveries (three spiked samples of each matrix), intra-day instrumental precision (six injections of standard at 50 ng/mL) and other uncertainty factors (i.e. 2%) as described in Kovalova et al., (2012) and in Verlicchi et al., 2014. The mixture of chemical standards was prepared just before the analysis, so the error associated with the stability of the solution may be considered negligible. The values obtained by different Authors are reported in Table 7.4. Ort et al., 2010a choose a random uncertainty (reproducibility) of 20% for all selected compounds.
For the applied sampling procedure, the associated uncertainty was estimated according to the studies of Ort et al. (2010b,c) and (Ort and Gujer 2006) considering the number of pulses containing the PhCs emitted by hospitalized patients (4.5 pulses) and out-patients who excrete also partly on the hospital site (1 pulse) (Weissbrodt et al., 2009) and the employed sampling mode and frequency (time-proportional sampling, Δt equal to 60 min). An estimation of this uncertainty is reported also in Verlicchi et al., 2014.
Table 7.4 summarizes also the total uncertainties associated with the PhC concentrations measured in hospital effluent according with Verlicchi et al., 2014 and Ort et al., 2010a.

Based on this table, it emerges that it is not possible to calculate total uncertainty for all the selected compounds. Observing data by Verlicchi et al., 2014, only 4 out of 12 PhCs (azithromycin, ciprofloxacin, clarithromycin and carbamazepine) could be quantified with an uncertainty lower than 32% (68% confidence interval). Extremely high uncertainties were estimated for erythromycin, metronidazole and ofloxacin. Ort et al., 2010a determine high uncertainty also for ibuprofen and trimethoprim.

Generally, the uncertainty due to the sampling procedure contributes the most to the total uncertainty: Uanalysis ranging from 2 to 11% while Usampling was higher than 30%, according to the vales observed in Verlicchi et al., 2014. Similar result was found by Lai et al., (2011).

Weissbrodt et al., (2009) reported a sampling uncertainty of 30-40% for contrast media and of 120-130% for antineoplastic drugs in hospital effluent. This great uncertainty is because only few patients are contributing to the daily load and the chance that toilet pulses are missed is high.

A complete uncertainty assessment was proposed by Lai et al., (2011). They remarked that any difference or change in measured concentrations smaller than total uncertainty cannot be considered to be significant.

For predictive model, it is a realistic assumption that uncertainties for both pharmaceutical (consumption, excretion)and hospital (water consumption, beds) data are mutually independent.

Le Corre et al., (2012) estimated a total uncertainty of predicted concentrations of 70%.

Table 7.4: Analytical, sampling and total uncertainties [%] together with the corresponding referencies.

	$U_{Analysis}$			$U_{Sampling}$	U_{Total}	U_{Total}
	Kovalova et al., 2012	Ort et al., 2010a	Verlicchi et al., 2014	Verlicchi et al., 2014	Verlicchi et al., 2014	Ort et al., 2010a
Acetaminophen	15-29	20				52
Codeine		20				45
Diclofenac	15-29	20				21
Ibuprofen		20				74
Indomethacin	<14	20				25
Ketoprofen						
Azithromycin	15-29		9	30	31	
Chloramphenicol		20				18
Chlortetracycline		20				11
Ciprofloxacin	30-100	20	8	30	31	43
Clarithromycin	<14		6	30	31	
Doxycycline			3	40	40	
Erythromycin		20	4	100	100	34
Metronidazole	30-100		5	100	100	
Norfloxacin	30-100	20	2	40	40	22
Ofloxacin			2	100	100	
Sulfadiazine	<14	20				33
Sulfamethoxazole +	<14	20				35
Tetracycline +		20	3	50	50	32
Trimethoprim +	30-100	20	11	40	41	60
Glibenclamide						
Enalapril						
Hydrochlorothiazide	<14	20				15
Lisinopril						
Tamoxifen						
Salbutamol						
Atenolol	<14	20				16
Metoprolol	<14	20				55
Propranolol	15-29					
Sotalol	<14					
Timolol						
Furosemide	15-29	20				9
Atorvastatin		20				5
Carbamazepine	15-29	20	5	30	30	24
Diazepam	<14	20				25
Fluoxetine	15-29	20				43
Lorazepam						
Paroxetine						
Ranitidine	15-29	20				38

7.2.3 Data - Measured concentration in the hospital effluent

Table 7.5 shows the range of PhC concentrations in hospital effluent and the corresponding mean in summer 2009 and winter 2010.

The highest average concentrations were found for acetaminophen (4.1 µg/L) and indomethacin (2.2 µg/L) in summer and ibuprofen (2.6 µg/L) in winter.

The concentrations of codeine are always greater than the maximum (378 ng/L) reported for 5 hospitals in Taiwan (max 2532 beds) by Lin et al., 2010. This may be due to the difference in the geographical position that may reflect differences in PhC consumption.

Among the antibiotics, the most prevalent compounds were: ciprofloxacin, ofloxacin and sulfamethoxazole respectively with concentration of 1.6, 3.7, 1.8 µg/L in summer and 21, 31 µg/L , 2.0 µg/L in winter. OFX and CIP are among the most widely used quinolones in hospitals and are found in wastewater in concentrations higher than 10000 ng/L by Brown (2006) and Thomas (2007). Results of Kümmerer et al., 2000 showed that it could be induced a 50% growth inhibition of gram negative bacteria Pseudomonas putida at OFX and CIP concentrations of 80000 ng/L and 10000 ng /L, respectively. A Previous study showed that fluoroquinolone antibiotics have been shown to exert genotoxic effects for the genetically modified bacterial strain, Salmonella typhimurium, at concentrations as low as 5000 ng/L for NOR and 25000 ng/L for CIP (Hartmann et al., 1998). Therefore, the concentration of CIP in this study showed negative effect on genotoxic of bacterial strain. The CIP hospital load contribution was 300% of the WWTP influent load in the investigation by Langford and Thomas, 2009.

Hydrochlorothiazide was the most present anti-hypertensive being detected at concentrations of 0.68 µg/L in summer and 2.2 µg/L winter.

The most represented beta blocker was atenolol (2.4 µg/L) in summer and in winter (5.1 µg/L). The psychiatric drug carbamazepine displayed the highest concentrations of its type.

Winter concentration were for the majority of compounds greater than those detected in summer. On the contrary: acetaminophen, indomethacin, chlortetracycline, doxycycline, tetracycline, trimethoprim, lisinopril, propranolol, furosemide and carbamazepine (see Table 7.5 and Fig. 7.3). These seasonal variations might be correlated to a variation of the PhCs or water consumptions.

Measured data refer to 24-h composite water samples, thus giving the corresponding average values of the micropollutant concentrations over the course of the day, ‘‘equalizing” the highest and lowest values of their instantaneous concentrations during the 24 h.

The reported value are in agreement with those reported in literature (Verlicchi et al., 2010a), except for erythromycin and propanolol (respectively two and one order of magnitude lower). As in other European studies (Lindberg et al., 2005) Trimethoprim and Ciprofloxacin have concentrations greater than those reported by Chang et al., 2010 that reported antibiotics concentrations in 4 hospital effluents in China.

The reported data show differences if compared with those reported by Lin et al., 2009 referring to Taiwan. In particular, Acetaminophene exhibited a mean concentration very higher if compared to that reported in this study. On the contrary, diclofenac, ibuprofen, ketoprofen. This fact could be due to the different size of the hospital and the different type and doses of PhCs consumption. Similar concentrations were found for sulfamethoxazole and propranolol. Sulfamethoxazole, ciprofloxacin and ketoprofen exhibited slightly higher concentration in the investigation by Mullot et al., (2010) that referred to three small-medium hospitals in Paris. Mendoza et al., (2015) confirmed the highest concentrations for acetaminophen, ofloxacin and trimethoprim.

Other concentrations are reported by Saussereau et al., (2013) referring to a French HWW. The PhC concentration found in hospital WW was listed by Orias and Perrodin, (2013) together with the corresponding PNEC.

Table 7.5: Ranges of concentrations [ng/L] of pharmaceuticals in the hospital effluent (adapted from Verlicchi et al., (2012a))

		Summer	Summer	Summer	Winter	Winter	Winter	Year	Year	Year
		Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
A	Acetaminophen	3450	4658	4054	1426	3390	2536	1426	4658	**3143**
	Codeine	422	636	529	410	3167	1886	410	3167	**1343**
	Diclofenac	176	271	223	476	527	510	176	527	**395**
	Ibuprofen	380	813	597	2230	3220	2623	380	3220	**1813**
	Indomethacin	895	3409	2152	403	607	533	403	3409	**1181**
	Ketoprofen	829	1417	1123	1066	1765	1400	829	1765	**1289**
B	Azithromycin	46	50	47	577	1044	797	46	1044	**497**
	Chloramphenicol	<lod	<lod	<lod	<lod	10	8	4	6	**5**
	Chlortetracycline	62	93	77	<lod	<lod	<lod	62	93	**77**
	Ciprofloxacin	1379	1889	1634	14944	26167	21389	1379	26167	**13487**
	Clarithromycin	50	64	57	9330	13500	10943	50	13500	**6589**
	Doxycycline	56	97	76	<lod	<lod	<lod	56	97	**76**
	Erythromycin	79	86	82	91	227	157	79	227	**127**
	Metronidazole	261	392	326	853	1057	956	261	1057	**704**
	Norfloxacin	23	44	34	224	513	347	23	513	**222**
	Ofloxacin	3262	4049	3656	24538	36538	30949	3262	36538	**20032**
	Sulfadiazine	77	119	98	271	383	328	77	383	**236**
	Sulfamethoxazole	900	2670	1785	936	3364	2011	900	3364	**1921**
	Tetracycline	<lod	33	17	<lod	<lod	<lod	3	3	**3**
	Trimethoprim	449	860	654	68	359	182	68	860	**371**
C	Glibenclamide	66	71	68	72	113	96	66	113	**85**
D	Enalapril	85	176	131	244	404	311	85	404	**239**
	Hydrochlorothiazide	536	816	676	1838	2388	2185	536	2388	**1582**
	Lisinopril	89	337	213	<lod	<lod	<lod	89	337	**213**
E	Tamoxifen	<lod	<lod	<lod	<lod	<lod	<lod	<lod	<lod	**<lod**
F	Salbutamol	26	30	28	99	140	121	27	140	**83**
G	Atenolol	2208	2586	2397	5050	6550	5750	2208	6550	**4409**
	Metoprolol	507	970	739	862	1193	1054	507	1193	**928**
	Propranolol	76	94	85	30	61	43	30	94	**60**
	Sotalol	352	613	483	3306	6723	5074	352	6723	**3238**
	Timolol	<lod	<lod	<lod	22	39	33	22	39	**33**
H	Furosemide	6389	7717	7053	5297	6281	5766	5297	7717	**6280**
I	Atorvastatin	80	173	127	244	308	268	80	308	**212**
J	Carbamazepine	758	1183	971	748	1083	947	748	1183	**956**
	Diazepam	<lod	<lod	<lod	21	38	31	21	38	**31**
	Fluoxetine	24	33	29	35	69	56	24	69	**45**
	Lorazepam	167	198	183	464	698	601	167	698	**433**
	Paroxetine	<lod	<lod	<lod	56	76	67	56	76	**67**
K	Ranitidine	1077	1511	1294	1407	4107	3033	1077	4107	**2338**

Table 7.3 shows that conventional WWTP are not able to effectively remove furosemide, carbamazepine and diazepam. These compounds are to be considered with particular attention as generally present in high concentration in hospital effluents. Fig. 7.3 reports a comparison between the mean concentration in summer and winter.

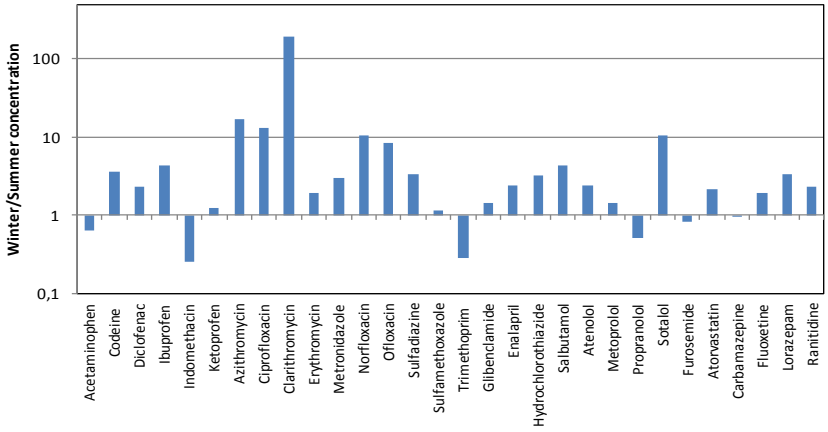


Fig. 7.3: Ratio Winter/Summer for the PhC concentration at Ferrara's Hospital

It is clear that in winter season the concentration of most compounds are much higher than in summer. In this hospital it is possible to examine a high variability between summer and winter.

This is an expected result (although not expected in this size) because in winter the use of antibiotics (mainly ciprofloxacin, clarithromycin, ofloxacin for example) or analgesics/anti-inflammatories (like Codeine and Diclofenac) is really relevant, comparing with the summer use. It is assumed that this fact reflects a variation in the pharmaceutical consumption other than a variation of the flow rate. For other compounds the ratio is nearer to 1 as the consumption does not follow a seasonal pattern but it is more related to the needs of the patients.

7.2.4 Comparison between urban and hospital wastewater

In order to compare MECs in hospital effluent and the values obtained by other Authors, literature data were collected and show in Fig. 7.4. In the same figure also PhC occurrence in urban WW was reported. As it is vital that the quality of literature data is assured, the references selected for this comparison provide a description of the analytical methodology and the quality assurance programme adopted for sampling, analysis and elaboration.

It is evident that the mean values considered in this work are inside the ranking of concentrations derived considering data published in other works.

Fig. 7.4: Occurrence of selected PhCs in urban and hospital wastewater together with the mean MEC values.

[Data from: - UWW Verlicchi et al., 2012c; Li et al., 2013a;- Hospital WW: Verlicchi et al., 2010a; Santos et al., 2013; Fatta-Kassinos et al., 2011; Verlicchi et al., 2012b; Al Aukidy et al., 2014]

The most investigated compounds in both raw UWW and HWW are analgesics and antinflammatoris, antibiotics and the psychiatric drug carbamazepine, even if these effluents may be an important point source of some other classes. For other compounds only few data are available (as glibenclamide, paroxetine). The range of concentration varies among compounds from several ng/L to several Mg/L. Maximum measured concentrations were found for acetaminophen and ibuprofen in urban WW (higher than 100 μg/L) and erythromycin and sulfamethoxazole in HWW; concentrations between 10 and 100 μg/L were found for 12 compounds in urban WW and for 11in hospital WW. The maximum average concentrations were found for acetaminophen (38 μg/L), ibuprofen (37 μg/L) for UWW and for ciprofloxain (38 μg/L) and acetaminophen (32 μg/L) for HWW.

Based on this spectrum of compounds, antibiotics seems to be most present in hospital effluent rather than in urban raw wastewater, whereas for the other classes the two wastewaters seem to have similar characteristics. Studies based on a more populated selection of pharmaceuticals (Verlicchi et al., 2010a, Santos et al., 2013, Al Aukidy et al., 2014) found that concentrations in HWW are quite often higher than in urban WW. For this reason, at a European level a wide debate is ongoing on the question if hospital effluent has to be considered of the same pollutant load of urban one and on its best management and treatment.

An in-depth analysis by Verlicchi et al., (2010a) report a ratio for comparison of the PhC therapeutic classes amount administered in hospitals and households. It is noticeable that there is a great variation if considering different therapeutic classes. Obviously, the greatest difference is on ICM and Platinum compounds that are administered only in hospital: only a fraction of patients excreted them at home. In general, higher concentrations are observed in HWW than in UWW (see Table 7.6).

Table 7.6: Ranges and average concentrations for the main classes of micropollutants in HWWs and UWWs (μg/L). (Galletti 2010)

Therapeutic class	HWWs	UWWs	$\frac{HWWs}{UWWs}$
Analgesics	100	11.9	8-15
Antibiotics	11	1.17	5-10
Cytostatics	24	2.97	4-10
β-blockers	5.9	3.21	1-4
Hormones	0.16	0.1	1-3
ICM	1008	6.99	70-150
AOX	1371	150	7-15
Gadolinium	32	0.7	35-55
Platinum	13	0.155	60-90
Mercury	1.65	0.54	3-5

7.3 Predicted concentration

With widespread awareness of the issue of pharmaceuticals in aquatic environments, public agencies like EMEA (European Medicine Agency) and FDA (Food and Drug Administration) have supported predictive models of drug concentrations in receiving waters. They require pharmaceutical consumption as an input, with the output being a concentration, either in wastewater or in natural water.

This approach is a valid method for screening purposes that identify the potential PhCs (Le Corre et al. 2012)of concern and could be an additional step for the prioritization but it should be validated by an experimental campaign(Johnson et al. 2008).

In this case, the predicted concentrations for each compound i in the raw hospital effluent were calculated according to eq. 7.1, used previously ,for instance, by Escher et al., 2011 and Mullot et al., 2010.

$$PEC_{HWW,i} = \frac{M_i * E_i}{Q} \qquad \text{(eq. 7.1)}$$

where M_i is the annual amount of each compound i consumed in the hospital [kg/year], Ei is the fraction excreted of the unchanged compound i, and Q is the volume of water consumed in this hospital in a year.

The PEC model used in this study is built upon the strong assumption of conservative mass transfer of PhCs from excretion to the hospital effluent. It was assumed that the consumption occurs on site and that no wastes are produced. Q amounted to 198 500 m^3/year, that means on average 544 m^3/d. It was assumed that mean water consumption was not change among the two considered years and that correspond to the water exiting the hospital structure. Consumption, excretion rate and flow rate were considered to vary as described in the following sections. Mi was summed up from all amounts mi (g) of PhCs consumed in the different drug preparations. mi was derived from the units consumed for each drug preparation Ui and the amount of active ingredient contained in each unit, mUi (g).

$$M_i = \sum_{i=1}^{n} m_i = \sum_{i=1}^{n} U_i m_{U_i} \quad \text{(eq. 7.2)}$$

The following approach was proposed by Heberer and Feldmann (2005) where they considered also the absorption rate R_p, the release rate of the compounds from the individual formulation si, and both the fractions excreted as parent compound (E_p) and as conjugate (E_c).

$$M_{week} = M_i \times s_i \times ((1 - R_p) + R_p(E_p + E_c)) \quad \text{(eq. 7.3)}$$

This equation provides the maximum values the minimum value of the absorption rate and the maximum values of excretion are considered for the PhC compounds under consideration. As the absorption rate is strongly influenced by the mode of application, this should be included in the analysis of PEC.

Other equation have been proposed referring to PEC in sewage (Carballa, Omil, and Lema 2008), in urban WWTP influent (Kümmerer and Henninger 2003) and effluent (Coetsier et al., 2009; Johnson et al., 2007) or in surface water in a urban catchment area (Bound and Voulvoulis, 2006; Castiglioni et al., 2004; Liebig et al., 2006; Oosterhuis et al., 2013).

In the first case they are mainly based on the consumption (calculated multiplying the number of daily defined doses by the defined daily dose (DDD), (Verlicchi et al. 2012a), considering the proportion of the population being treated daily with a specific drug substance. In these approaches the drugs purchased without prescription or those used for veterinary purposes are usually not considered. DDD refers to the maximum dose per day but the adherence to therapy of patients can be reduced. For serious illnesses this may be more elevated.

The second case the removal rate within the WWTP is also considers or mass balances are implied.

In the latter case the dilution factor (DF) within the receiving water body is also taken into account (Daouk et al. 2015). Liebig et al., 2006 compared three different models for predicting environmental concentrations (EMEA, 2001; EMEA, 2003; EC, 2004) finding that PEC underestimated MEC for highly lipophilic compounds as models do not come up the complexity of the real environment.

Other approaches were be proposed by Heberer and Feldmann, 2005 and Feldmann et al., 2008 who have taken into account also metabolites. Le Corre et al., (2012) proposed a method for evaluating the hospital contribution to the PhC residue load in municipal wastewater, considering also the population and the PhC consumption of the catchment area.

Other authors (Lin et al., 2010, Daughton and Jones-Lepp, 2001) proposed with success procedures to back-calculate the consumption of drugs based on levels in sewage.

Many reports give PhCs consumption data in terms of costs. This data cannot be used to predict PhCs concentration as are not representative of the real amount of PhC consumed.

7.3.1 Hospital PhC consumption data

The consumption of PhCs is increasing in hospitals (Deo and Halden 2013) but varies from country to country, especially for variation in restriction of use of some compounds, and from year to year. Annually, changes in quantity and quality of medicaments may result due to new legislation, the introduction of new active pharmaceutical ingredients or the disappearance of others following medical progress (Verlicchi et al., 2010a).

Pharmaceutical consumption data from the investigated hospital were made available by the Hospital Pharmacy Service for the year 2011. This has an internal office that manages the hospital drugs warehouse and provides PhC types and amounts necessary to the different medical units, laboratories, clinics and wards on the basis of their request along the year. It excludes the direct distribution that is the dispensing of medicines to be administered at home and over-the-counter products.

All these requests are recorded in a database specifying specific code, generic name, brand name, description (tablets, ointments, syrup, suspension), form (tablets, suppositories, tubes, vials, bottles, sachets) and quantity dispensed (expressed as number of tablets, vials, tubes, bottles, sachets).

It is assumed that all the PhCs dispended by the pharmaceutical hospital services are assumed and excreted in the same structure. Ort et al., (2010), on the contrary, derive the amounts exclusively used for hospitalized in-patients and not those given to out-patients.

Globally data of consumption are reported in Table 7.7.

Table 7.7: Report of the PhC numerousness in 2008 and 2011.

	2011
Number of products	1129
Number of active ingredients	689
Major consumed compound	Pantoprazole (161169 units)

For comparison, in the study of Jean et al., (2012) said that 960 substances were consumed in 2007 in the second largest hospital structure in France (5200 beds). In the hospital investigated by Le Corre et al., (2012) 548 compounds are used during 2008 (882 beds).

In the following tables pooled data are considered for 15 therapeutic classes among those most investigated in literature, in order to identify among them the most administered ones.

The more administered therapeutic classes were identify considering only the administered units and not the total mg.

Table 7.8: Ranking of the most administered therapeutic classes based on the percentage of units consumed in hospital during 2011

Therapeutic class	**%**
Analgesic/Anti-inflammatory	26,03
Antiviral	20,00
Antibiotic	11,76
Antihypertensive	11,06
Psychiatric drug	10,12
Beta-blocker	5,87
Antiseptics	3,86
Antineoplastic	3,61
Receptor antagonists	2,92*
Lipid regulator	1,94
Antihistamines	1,05
Antifungals	0,76
Hormones	0,51
Immunosuppressants	0,26
B-agonists	0,24

Analgesic/Anti-inflammatories, Antibiotics, Psychiatric drugs, Antihypertensives and Beta-blockers are the most administered therapeutic classes.

Taking into consideration the total mg consumed, disinfectants are the most consumed as they are used in large quantities for the disinfection of surfaces, instruments and skin, in glue and size production and use, and in food processing. In particular, 893403 g of alcohol were used within the hospital in 2011. As reported in Verlicchi et al., 2012d among disinfectants, alcohol is one of the most common. When measured detergents, they are usually subjected to global measurement (anionic, cationic and non-ionic).

Moreover it was possible underline from the analysis of the whole lists of compounds that also other therapeutic classes are administered in hospitals but for them literature data are still scarce or inexistent.

Other classes administered in considerable amount are cortisones, antineoplastics, cardiovascolar and drugs, neuromuscolar blocking agents and general anesthetics as well as but in minor quantities antihistamines and contrast media (Verlicchi, Galletti, et al. 2012).

An in depth analysis of the therapeutic classes consumption was done by Oliveira et al., (2015). In that study different percentages were found, but they also stated that PPCP concentrations are very dependent on the hospital, prescription habits and country. All in all analgesic are also in that study among the most consumed classes .

Also in Verlicchi et al., (2010a), Le Corre et al., (2012) and Verlicchi et al., (2012d) antibiotics and analgesics/anti-inflammatories are among the main therapeutic classes administered in hospital. At these ones Daouk et al., (2015) added laxatives and antivirals. The use of the latter is steadily increasing.

Boillot et al., (2008) confirmed that analgesic, antibiotic, psychiatric drugs, beta-blockers, lipid regulators and antineoplastics are frequently found in hospital effluents. The same Authors stated that detergent and disinfectants take up the majority on the quantitative level. They observed that the few kg of medicaments appear negligible in term of quantity in comparison to the consumption of detergents and disinfectants. These quantities should not be neglected as high levels may have the potential to inhibit biological treatment.

Hormones, lipid regulator and beta-blockers could be found in high concentrations in hospital WW also because some in-patient bring their own medications for being consumed inside the hospital.

Herrmann et al., (2015) analyzed in phychiatric hospitals and nursing home the consumption of group of PhC according to anatomical therapeutic chemical (ATC) code finding that antiepiletics are the most significant in the group of nervous system.

Jean et al., (2012) observed that hormones, antineoplastic agents, antidepressants and antihistmines are the therapeutic classes with the highest bioconcentration factor. Moreover, hormones and antineoplastic agents are considered responsible to cause endocrine disruption type effects.

Some PhCs are especially used in hospitals (contrast media, antineoplastics), while others are used also in the community (Beta-blockers, antibiotics, anti-inflammatories).

There are some hospital specific molecules (propofol) that have no been monitored even if are of special interest for hospital water quality assessment (Mullot et al. 2010).

For the selected compounds Table 7.9 and Fig. 7.5 show the consumption for the active ingredients consumed more than 350 units during 2011. A “plus” after the name of compound indicate that the respective brand product contain more active ingredients.

An analysis of the total kg consumed in the 2 different years was not possible. Daouk et al., (2015) calculated the amount of the administered drug under the hypothesis that 100% of the administered drugs are consumed inside the hospital equal to 90 g/patient. Calculating the total amount considering the outpatients this ratio became 4.8 g/patient.

Table 7.9: Hospital consumption of the selected pharmaceuticals. Year 2011

		pharmaceutical product	UM	Quantity	mg/UM	mg	kg/year
A	**Acetaminophen**						**101,31**
A	Acetaminophen	ACETAMOL 125 MG/TACHIPIRINA!	SUP	780	125	97500	
A	Acetaminophen	PERFALGAN 12FL 100ML 10MG/	FL	38.820	1.000,00	38820000	
A	Acetaminophen	TACHIPIRINA 250MG SUPP.+	SUP	1.550	250,00	387500	
A	Acetaminophen	TACHIPIRINA 500MG SUPP. +	SUP	1.400	500,00	700000	
A	Acetaminophen	TACHIPIRINA CP 500 MG	CP	45.310	500,00	22655000	
A	Acetaminophen	TACHIPIRINA FL 120ML +	FL	392	120	47040	
A	Acetaminophen	TACHIPIRINA*16CPR DIV 1000MG	CP	17.760	1.000,00	17760000	
A	Acetaminophen+	CO-EFFERALGAN CP/TACHIDOL BUSTE	CP	41.695	500,00	20847500	
A	**Codeine**						**1,251**
A	Codeine +	CO-EFFERALGAN CP/TACHIDOL BUSTE	CP	41.695	30,00	1250850	
A	**Diclofenac**						**1,071**
A	Diclofenac	DICLOREUM 50 MG/DICLOFENAC CP	CP	3.150	50,00	157500	
A	Diclofenac	DICLOREUM/VOLTAREN R.P. 100 M	CP	2.280	100,00	228000	
A	Diclofenac	VOLTAREN 75MG/DICLOREUM+	FF	9.138	75,00	685350	
A	**Ibuprofen**						**6,912**
A	Ibuprofen	BRUFEN 600MG BUSTE	BUS	11.520	600,00	6912000	
A	**Indomethacin**						**0,0573**
A	Indomethacin	INDOXEN 50MG/METACEN CPR +	CP	1.125	50,00	56250	
A	Indomethacin	LIOMETACEN 50MG @	FF	12	50	600	
A	Indomethacin	INDOCOLLIRIO 0,1% 5 ML COLL!	FL	95	5	475	
A	**Ketoprofen**						**3,751**
A	Ketoprofen	IBIFEN/FLEXEN 100MG FF EV	FF	28.956	100,00	2895600	
A	Ketoprofen	KETOSELECT/ IBIFEN 50 MG CP	CP	10.890	50,00	544500	
A	Ketoprofen	ORUDIS/ KETOPROFENE 100 MG IM	FF	3.108	100,00	310800	
B	**Azithromycin**						**1,943**
B	Azithromycin	ZITROMAX 500MG EV @	FL	1.492	500,00	746000	
B	Azithromycin	ZITROMAX SOSP.*BB OS FL 1500MG	FL	3	1.500,00	4500	
B	Azithromycin	ZITROMAX/TROZOCINA 500MG CPR	CP	2.385	500,00	1192500	
B	**Chloramphenicol**						**0,866**
B	Chloramphenicol	IRUXOL	TUB	866	1000	866000	
B	**Chlortetracycline**						**0,1158**
B	Chlortetracycline	AUREOMICINA 1% POM.OFT.@	TUB	1.288	35,00	45080	
B	Chlortetracycline	AUREOMICINA 3% DERMICA@	TUB	166	426	70716	
B	**Ciprofloxacin**						**20,7**
B	Ciprofloxacin	CIPROFLOXACINA/CIPROXIN 200 MG	FL	24.326	200,00	4865200	
B	Ciprofloxacin	CIPROFLOXACINA/IBIXACIN 500MG	CP	31.738	500,00	15869000	
B	**Clarithromycin**						**2,13**
B	Clarithromycin	KLACID 250 MG/MACLADIN+(ESAU)	CP	1.128	250,00	282000	
B	Clarithromycin	KLACID 500MG EV/MACLADIN+	FF	318	500	159000	
B	Clarithromycin	KLACID 500MG/MACLADIN+(ESAU)	CP	3.052	500,00	1526000	
B	Clarithromycin	KLACID SCI/MACLADIN 125/5ML 2,	FL	65	2.500,00	162500	
B	**Doxycycline**						**0,062**
B	Doxycycline	BASSADO 100 MG+	CP	620	100	62000	
B	**Erythromycin**						**0,534**
B	Erythromycin	ERITROCINA 10% SOSP.@	FL	44	10000	440000	
B	Erythromycin	ERITROCINA 600MG @	CP	156	600	93600	
B	**Metronidazole**						**6,037**
B	Metronidazole	FLAGYL 250MG/VAGILEN+	CP	7.020	250,00	1755000	
B	Metronidazole	METRONIDAZOLO 500MG/DEFLAMON F	FL	8.564	500,00	4282000	
B	**Norfloxacin**						**0,040**
B	Norfloxacin	NORFLOXACINA/UTICINA 400 MG CP	CP	84	400	33600	
B	Norfloxacin	THEANORF 0,3% COLL.COLL	FL	412	15	6180	
B	**Ofloxacin**						**0,0178**
B	Ofloxacin	EXOCIN 0,3 % 10 ML.COLL @ (esa	FL	481	30	14430	
B	Ofloxacin	EXOCIN 0,3 % 3,5G.POM.OFT.@	TUB	320	10,5	3360	
B	**Sulfadiazine**						**0,233**
B	Sulfadiazine	SULFADIAZINA 500MG(NO SCORTA)	CP	100	500	50000	

B	Sulfadiazine	CONNETTIVINA PLUS*CREMA 25G	TUB	624	250	156000	
B	Sulfadiazine	SOFARGEN 1% 50GR + ESAU	TUB	54	500	27000	
B	**Sulfamethoxazole**						**2,7772**
B	Sulfamethoxazole	BACTRIM @	FF	659	400	263600	
B	Sulfamethoxazole	BACTRIM 80/400 MG SCI+	FL	23	8000	184000	
B	Sulfamethoxazole	BACTRIM 800 MG + 160 MG	CP	2.912	800,00	2329600	
B	**Tetracycline +**						**0,0001**
B	Tetracycline +	PENSULVIT POMATA OFT.@ ESAU	TUB	1	65	65	
B	**Trimethoprim +**						**0,555**
B	Trimethoprim +	BACTRIM 80/400 MG SCI+	FL	23	1600	36800	
B	Trimethoprim +	BACTRIM @	FF	659	80	52720	
B	Trimethoprim +	BACTRIM 800 MG + 160 MG	CP	2.912	160,00	465920	
C	**Glibenclamide**						**0,0221**
C	Glibenclamide	DAONIL 5MG/GLIBORAL/GLIBEN	CP	300	5	1500	
C	Glibenclamide	GLIBOMET+/SUGUAN M+	CP	4.120	5,00	20600	
D	**Enalapril**						**0,1316**
D	Enalapril	ENAPREN20MG/CONVERTEN/NAPRIL EN	CP	6.580	20,00	131600	
D	**Hydrochlorothiazide**						**0,407**
D	Hydrochlorothiazide	MODURETIC @ *	CP	5.840	50,00	292000	
D	Hydrochlorothiazide	ESIDREX 25 MG@	CP	4.600	25,00	115000	
D	**Lisinopril**						**0,0146**
D	Lisinopril	ZESTRIL/PRINIVIL 20MG+	CP	728	20	14560	
E	**Tamoxifen**						**0,003**
E	Tamoxifen	NOMAFEN/NOLVADEX 10 MG CP	CP	330	10	3300	
F	**Salbutamol**						**0,176**
F	Salbutamol	BRONCOVALEAS 0,5%15ML SOL @	FL	1.180	75,00	88500	
F	Salbutamol	VENTOLIN AER/BRONCOVALEAS+	FL	632	20	12640	
F	Salbutamol	VENTOLIN FIALE 100@	FF	270	0,1	27	
F	Salbutamol +	CLENIL A MONODOSE ~	FL	46.900	1,6	75040	
F	Salbutamol	VENTOLIN FIALE 500@	FF	650	0,5	325	
G	**Atenolol**						**0,785**
G	Atenolol	ATENOL 100MG/ATENOLOLO ESAU	CP	6.850	100,00	685000	
G	Atenolol	ATENOLOLO 50 MG!	CP	2.000	50,00	100000	
G	ATENOLOL	TENORMIN 5MG/10ML @	FF	80	5	400	
G	**Metoprolol**						**3,293**
G	Metoprolol	LOPRESOR/SELOKEN 100MG/METOPRO	CP	32.790	100,00	3279000	
G	Metoprolol	SELOKEN 5MG 5ML EV@	FF	2.815	5,00	14075	
G	**Propranolol**						**0,27**
G	Propranolol	INDERAL 40MG @	CP	6.750	40,00	270000	
G	**Sotalol**						**0,2**
G	Sotalol	RYTMOBETA/SOTALOLO 80 MG	CP	2.500	80,00	200000	
G	**Timolol**						**0,0046**
G	Timolol	XALACOM 2,5 ML COLL.(DIST.DIR.	FL	20	12,5	250	
G	Timolol	GANFORT*COLL 3ML 300MCG+5MG/ML	FL	5	15	75	
G	Timolol	COMBIGAN*COLL FL 5ML 0,2%+0,5%	FL	4	25	100	
G	Timolol	DUOTRAV*COLL FL 40MCG/ML+5MG/M	FL	2	12,5	25	
G	Timolol	CUSIMOL 0,50% COLL./TIMOLOLO	FL	166	25	4150	
H	**Furosemide**						**6,78**
H	Furosemide	FUROSEMIDE/LASIX 250MG/25 ML A	FF	3.690	250,00	922500	
H	Furosemide	FUROSEMIDE20MG/2ML/LASIX	FF	57.555	20,00	1151100	
H	Furosemide	LASIX 25MG/FUROSEMIDE+	CP	84.870	25,00	2121750	
H	Furosemide	LASIX/FUROSEMIDE 500MG!	CP	5.180	500,00	2590000	
I	**Atorvastatin**						**0,343**
I	Atorvastatin	TORVAST 10 MG CP	CP	1.170	10,00	11700	
I	Atorvastatin	TORVAST 20 MG CP	CP	3.240	20,00	64800	
I	Atorvastatin	TORVAST 40 MG CP(CONF 30 CP)	CP	5.760	40,00	230400	
I	Atorvastatin	TORVAST*30CPR RIV 80MG	CP	450	80,00	36000	
J	**Carbamazepine**						**2,66**
J	Carbamazepine	TEGRETOL /CARBAMAZEPINA 200 MG	CP	8.250	200,00	1650000	
J	Carbamazepine	TEGRETOL 400 MG R.C.	CP	1.830	400,00	732000	
J	Carbamazepine	TEGRETOL/CARBAZEPINA 400 MG	CP	690	400	276000	

J	**Diazepam**						**0,121**
J	Diazepam	DIAZEPAM 0,5% GTT/TRANQUIRIT	FL	885	100	88500	
J	DIAZEPAM	MICROPAM /MICRONOAN 10MG MICRO	PZ	96	10	960	
J	DIAZEPAM	MICROPAM/ MICRONOAN 5MG MICRO	PZ	60	5	300	
J	Diazepam	VALIUM 10MG/DIAZEPAM	FF	3.144	10,00	31440	
J	**Fluoxetine**						**0,0073**
J	Fluoxetine	FLUOXETINA/XEREDIEN CPR SOL. 2	CP	364	20	7280	
J	**Lorazepam**						**0,0633**
J	Lorazepam	TAVOR 1MG/LORANS/CONTROL+	CP	24.240	1,00	24240	
J	Lorazepam	TAVOR 2,5MG/LORANS/CONTROL +	CP	14.020	2,50	35050	
J	Lorazepam	TAVOR 4 MG IM IV@	FF	1.015	4,00	4060	
J	**Paroxetine**						**0,0437**
J	Paroxetine	SEREUPIN/DAPAROX/PAROXETINA 20	CP	2.184	20,00	43680	
K	**Ranitidine**						**5,287**
K	Ranitidine	RANIDIL 50MG/RANITIDINA FIALE	FF	17.670	50,00	883500	
K	Ranitidine	RANITIDINA 150 MG CPR	CP	29.360	150,00	4404000	

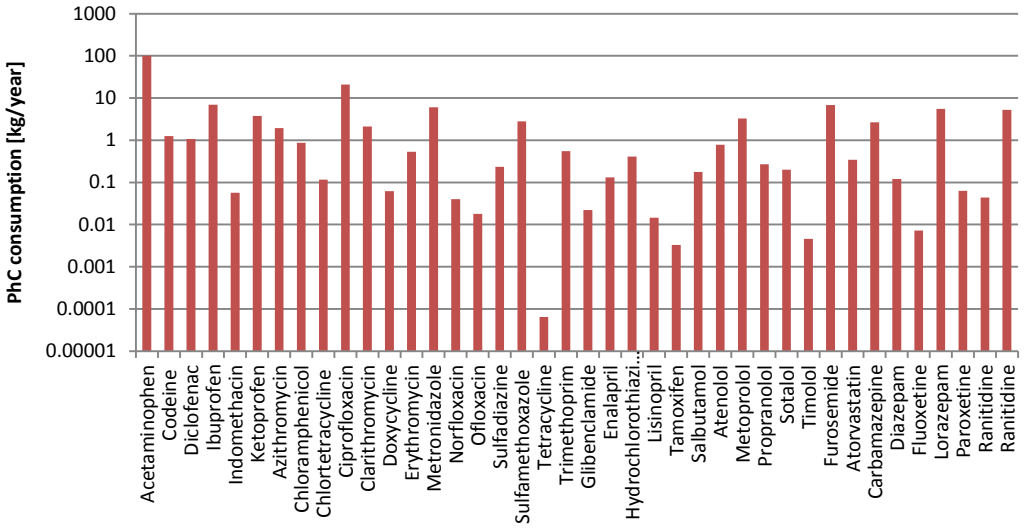


Fig. 7.5: PhC consumption during 2008 and 2011.

The table and Fig. 7.5 show that there is a great variability among the different compounds.

Among the selected compounds and basing on consumption data Acetaminophen and Ciprofloxacin are the compounds dispensed in the greatest quantities. In is reasonable to think that these compounds are also consumed inside the hospital, as they are sold also by pharmacies in the territory. Tetracycline is the lowest consumed in 2011.

The consumption is also calculated referring to the number of beds of the Sant'Anna hospital and compared with those reported by Ort et al., 2010. Data are shown in Table 7.12.

The consumption data are affect by several biases (consumption of drugs outside the hospital for leaving patients, taking into account the drugs only purchased as a total package, lack of patient compliance, etc.) but they provide a first assessment of the consumption of pharmaceuticals by in-patients (Jean et al. 2012). Weissbrodt et al., 2009 (in their supporting data) reported the consumption variation of some contrast media between 2005 and 2006. Some new compounds are consumed only in 2006 and generally the concentration increased chronologically.

A mean of the consumption of the two year has been calculated and reported below together with the percentage range variation.

Table 7.10: Consumption data (g/year^{-1}bed^{-1}) and comparison with literature data by Ort et al., (2010a) (190 beds).

	2011	Ort et al., 2010
	[g/year bed]	[g/year bed]
Acetaminophen	112,57	681,53
Codeine	1,39	4,73
Diclofenac	1,19	0,65
Ibuprofen	7,68	123,10
Indomethacin	0,06	1,25
Ketoprofen	4,17	-
Azithromycin	2,16	-
Chloramphenicol	0,96	0,00
Chlortetracycline	0,13	-
Ciprofloxacin	23,04	2,85
Clarithromycin	2,37	-
Doxycycline	0,07	-
Erythromycin	0,59	4,09
Metronidazole	6,71	-
Ofloxacin	0,02	-
Sulfadiazine	0,26	-
Sulfamethoxazole	3,09	-
Trimethoprim	0,62	4,80
Glibenclamide	0,02	-
Enalapril	0,15	-
Hydrochlorothiazide	0,45	0,19
Lisinopril	0,02	-
Tamoxifen	0,00	-
Salbutamol	0,20	-
Atenolol	0,87	0,48
Propranolol	0,30	0,15
Timolol	0,01	-
Furosemide	7,54	4,18
Atorvastatin	0,38	1,20
Carbamazepine	2,95	3,07
Diazepam	0,13	0,70
Fluoxetine	0,01	0,09
Lorazepam	0,07	-
Paroxetine	0,05	-

7.3.2 Excretion rate

In evaluating PEC, the influence of excretion rate is quite important. If we consider the human body as a black box, once a pharmaceutical is administered, it is partially absorbed and partially excreted as unchanged compound (parent), or as metabolites, depending on the reactions involved in the metabolism process, as reported in the review by Monteiro (2010).

The excretion factor corresponds to the fraction of the compound excreted in urine and faeces in unchanged form (Jean et al., 2012). The metabolites are not considered, even if many of them still remain active.

Eq. 7.4 estimates excretion rate E as the sum of the fraction of non-absorbed compound (1- Rp, being Rp the absorbed fraction) and the fraction of the parent compound that was absorbed but not metabolized in the human body (R_p x_p, being x_p the portion of the active ingredient that is excreted unchanged after its absorption).

$$E = (1 - R_p) + R_p x_p \quad \text{(eq. 7.4)}$$

The table in Appendix D reports excretion rate literature data for the selected compounds together with the corresponding references.

Table 14 reports the excretion rate together with the corresponding range of variability for each selected compound.

It is worth noticing that E refers to the excretion (in urine and faecis) of the unchanged compound and not to its metabolites (when present).

Excretion rates of active ingredients greatly vary (from 0 to 99.9 %) from one compound to another mainly depending on their chemical and physical characteristics (Jjemba et al., 2006). Moreover, it is strictly correlated with individual human characteristics who assumed them (Dautghton and Ruhoy, 2009): human health condition, age, gender, diet, body weight, ethnicity) and to the mode of use (pharmaceutical administered doses, mode of applications (oral, rectal, dermal, parental), time of assumption, interference with other administered drugs). Johnson and Williams (2004) reported the excretion rates of hormones by five groups of population (men, menstrual women, pregnant women, menopausal women and postmenopausal women using hormone replacement therapies) finding great variations.

Lienert et al., (2007), in evaluating the convenience of urine separation systems, evaluated the excretion pathway underlining that some compounds are mainly excreted via feces, like cytostatic. Generally, they found that 22 % and 19 % of the parent compounds are excreted in urine and feces respectively. As meabolites, 49 % was excreted via urine and only 3 % via feces. They confirmed that half of the PhCs are metabolized before excretion.

The analysis has been carried out considering the mean value and the corresponding range of variability for the parent compounds. Active metabolites are not considered.

A conservative approach (E=1) is also used in this study for assess a risk analysis with the aim of prioritizing the choose the compounds for which doing further investigations.

Table 7.11: Excretion rate [%] and corresponding range of variability [%]

PhC compound	min	max	mean	Delta -	Delta +
Acetaminophen	2	80	29,0	27,0	51,0
Codeine	3	40	19,7	16,7	20,3
Diclofenac	5	39	15,0	10,0	24,0
Ibuprofen	1	25	9,8	8,8	15,2
Indomethacin	10	20	15,0	5,0	5,0
Ketoprofen	10	90	58,3	48,3	31,7
Azithromycin	6	8	7,0	1,0	1,0
Chloramphenicol	5	10	7,5	2,5	2,5
Chlortetracycline	20	70	45,0	25,0	25,0
Ciprofloxacin	20	83,7	47,9	27,9	35,8
Clarithromycin	20	25	22,5	2,5	2,5
Doxycycline	41	70	55,5	14,5	14,5
Erythromycin	5	15	10,0	5,0	5,0
Metronidazole	40	40	40,0	-	-
Norfloxacin	30	69	46,3	16,3	22,7
Ofloxacin	46,5	95,2	70,6	24,1	24,6
Sulfadiazine	44	57	50,5	6,5	6,5
Sulfamethoxazole	6	90	30,2	24,2	59,8
Tetracycline	70	90	80,0	10,0	10,0
Trimethoprim	30	80	55,2	25,2	24,8
Glibenclamide	5	5	5,0	-	-
Enalapril	36	36	36,0	-	-
Hydrochlorothiazide	24	24	24,0	-	-
Lisinopril	100	100	100,0	-	-
Tamoxifen	30	30	30,0	-	-
Salbutamol	28	28	28,0	-	-
Atenolol	50	90	70,0	20,0	20,0
Metoprolol	10	39	23,5	13,5	15,5
Propranolol	0,5	24	12,3	11,8	11,8
Sotalol	80	80	80,0	-	-
Timolol	20	20	20,0	-	-
Furosemide	40	40	40,0	-	-
Atorvastatin	5	5	5,0	-	-
Carbamazepine	1	15	5,3	4,3	9,8
Diazepam	1	1	1,0	-	-
Fluoxetine	2,5	60	24,5	22,0	35,5
Lorazepam	0,3	85	42,7	42,4	42,4
Paroxetine	3	3	3,0	-	-
Ranitidine	6	79	45,0	39,0	34,0

7.3.3 Water consumption in the hospital

Hospitals require a significant quantity of water per day for the different purposes and services depending on the activities which take place within the structure. The quantity of WWs produced in a hospital depends on different factors: bed numbers, hospital age, accessibility to water, general services present inside the structure (laundry, kitchen and air conditioning), type and number of wards and units, institution management policies and awareness in managing the structure and in safeguarding the environment, climate and cultural and geographical factors.

There is not a clear correlation between specific hospital consumptions (expressed as L bed-1 d-1) and hospital size (that is bed numbers), as shown by the data reported in Fig. 7.6 , which refers to hospitals in different countries around the world. In Fig. 7.6 data are spread between 200 and 1200 L bed^{-1} d^{-1} with the highs values coming from industrialized countries.

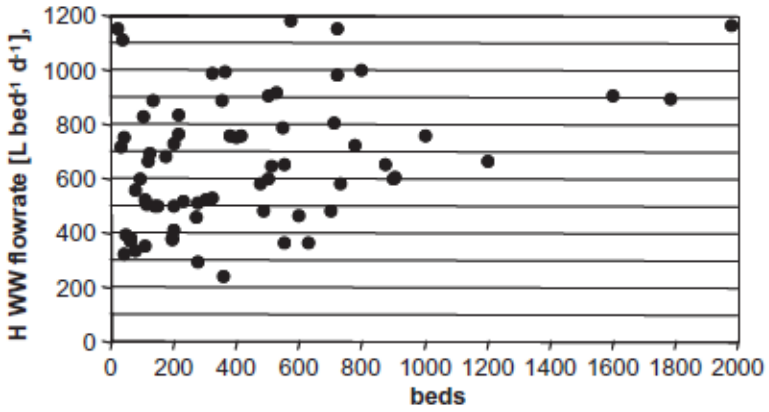


Fig. 7.6: Water consumption per day and per bed with respect to hospital size (P. Verlicchi et al. 2010)

Generally, from a quantitative point of view, hospitals consume 400 to 1200 L of water per day per bed (Boillot et al., 2008).

Peaking coefficients for hospital flow rates are reported in Table 7.12 (P. Verlicchi et al. 2010).

Table 7.12: Peaking coefficient for HWW (P. Verlicchi et al. 2010)

Peaking coefficient	HWWs	UWWs (small community)
Monthly	1,5-1,8	1,2-2
Daily	2-2,8	2-5
Hourly	3,5-4	3-4

Verlicchi et al., 2010a stated that for an hospital of 900 beds the water consumption varies from 600 to 900 L/ bed day. Le Corre et al., (2012) confirmed a consumption of 627 m^3/d for an hospital of 882 beds while the hospital of 1000 beds analyzed by Mendoza et al., (2015) consumed 500 L/bed day. In future the consumed water volume will drop because of the water saving measures.

In this study, a first analysis is completed by using the water consumption (198 500 m^3/year) drawn from the municipal supply pipe and assuming an equal flow of wastewater for the full year. Considering a bed density of 6.5 beds/1000 inhab, the specific hospital consumption is of about 600 L $beds^{-1}$ d^{-1}.

The water consumption is in good agreement with data reported by Metcalfe and Eddy (2004). Wangsaatmaja reported in her thesis a consumption of 297323 m^3/year for an hospital of 538 beds in 1996 but it is estimated to be 2-3 times higher than those reported in other studies. The main activities that produce wastewater are: Laundry, Kitchen, Laboratory Chemical, Boiler, Shower/Toilet, Cooling Tower.

Water consumption is the highest in wards (30 % of the total consumption) and that of outpatients is very low (1 %) (Wangsaatmaja, 1997).

From data provided by the “Assessorato alla sanità” of the “Emilia Romagna region”, other water contribution must be considered to the tap water consumption. This amount derived from purified water bags (5-10 bags, 5 litres each one) used during surgical operations. Estimating 20 operations/day per five days per week, 260 m^3/year are to be added to consumption of tap water.

Moreover, also the liquid generated from urine reach the sewage network. For this case study, this amount could be calculated as follow:

- Patients: 900 x 1.2 (percentage of patient and relatives using the toilet inside the hospital) x 2 L/day x 365 days= 788 m^3/ year

- Staff member: 2300/3 (percentage of staff inside the hospital) x 2 L x 365 days = 560 m^3/year

These amounts reach the sewage network but they not derived from tap water supply. If compared with the tap water consumption, these amount can be neglected (they are equal to about 0,7%).

Daily flow fluctuations

Boillot et al., (2008) analysed daily flow fluctuation curves and they correlated them to the hospital activities over one day:

- 6.30 -7 a.m. to 1 p.m disinfectants cleaning of the units, care activities and laboratory operations
- -1 to 5 p.m. cleaning of the operating theatres
- 5 to 7 p.m progressive termination of all the activities
- -7 p.m. to 6.30 a.m. night.

Maternity and emergency function 24-h and these activities are reflected in the night period.

They took 5 samples from HWW in a day taking into account this pattern of activities. The obtained flow rate pattern is shown in Fig. 7.7.

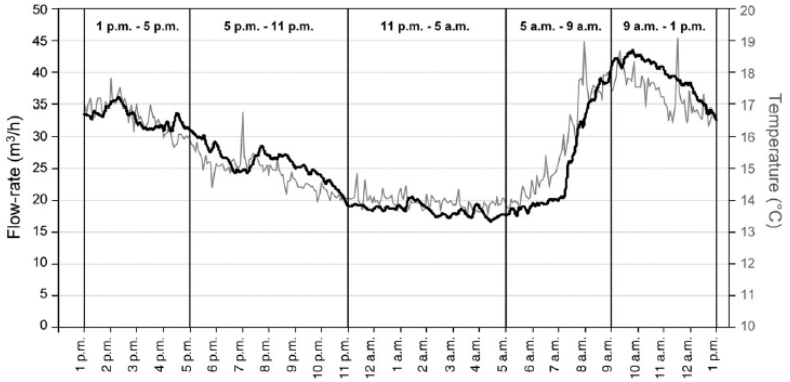


Fig. 7.7: Flow rate and temperature of the hospital effluent investigated by Boillot et al., 2008. A total volume of 652 m^3 was recorded over 24 h.

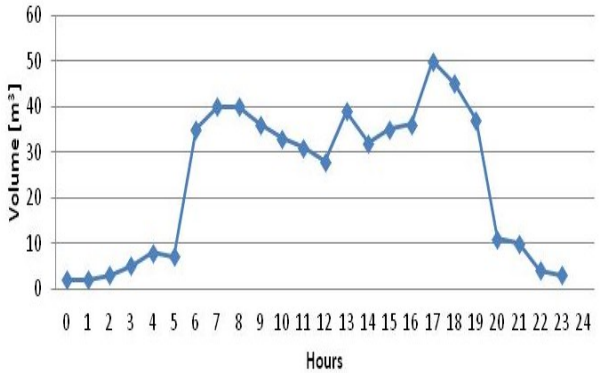


Fig. 7.8: Water consumption at SSRN Hospital, 566 beds in Rep. of Mauritius (Mohee, 2005)

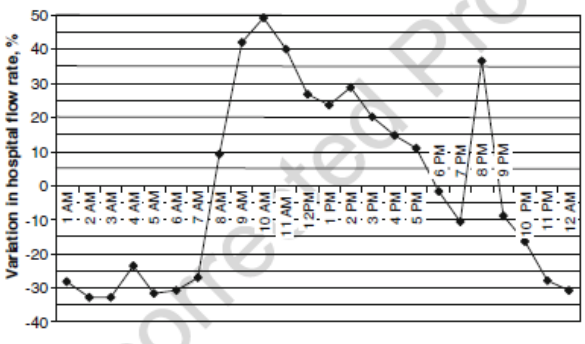


Fig. 7.9: Percentage variation in hourly hospital consumption (flow rate) with respect to its corresponding average value (27.3 m3/h, 700 beds) from (Boillot et al. 2008)

Generally, water consumption varies during the day: with respect to the daily average flow rate, increasing by up to +20% between 8 a.m. and 4 p.m., reaching a maximum about at 10.00 a.m., and decreasing to -30% between 1 a.m. and 8 a.m., as it is shown in Fig. 7.8, Fig. 7.9 and Fig. 7.10.

Effluent flow rate trend of an hospital of medium size can be considered similar to that of urban WW of small urban center that could varies according to the specific activities of the urban settlement and fluctuation in the density of the resident population.

Weekly variation

Weissbrodt et al., (2009) analysed flow variation during a week of the wastewater that reflected the water consumption. They found that higher values occur on Wednesday as shown in Fig. 7.10.

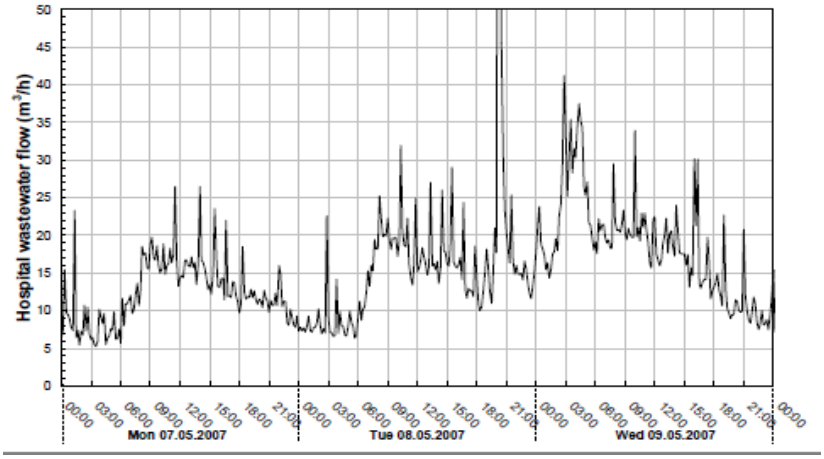


Fig. 7.10: Hospital wastewater flow rate at the sampling point by Weissbrodt et al., (2009)

Monthly variation

Further differences occur during the year, with higher average values during hotter months (Joss et al., 2005; Mohee, 2005; Boillot et al., 2008; Verlicchi et al., 2008) in part due to irrigation.

Fig. 7.12 shows the changes in the water consumption on March, April and May. It is clear that the water consumption has a cyclic pattern and that the higher vales occurred in the hotter months. This trend is still more clear by observing Fig. 7.13.

The variation in water consumption with respect to average monthly value is included between -41 % in March and 70% in July.

Table 7.13: Monthly variation of water consumption

	Summer	Winter
Monthly variation	70%	-41%

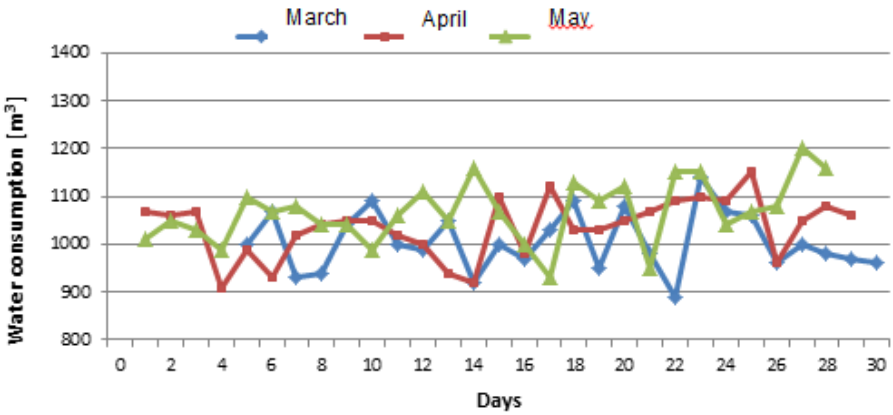


Fig. 7.11: Water consumption during three months at Bangkok Children Hospital, 538 beds. (Wangsaatmaja 1997)

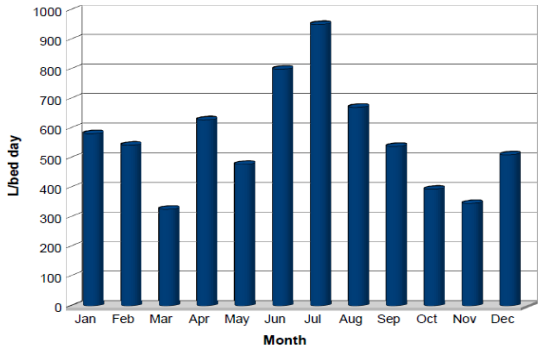


Fig. 7.12: Monthly Rimini Hospital water consumption (450 beds) (Galletti 2010)

As shown in Table 7.14, the most water demanding services are the some wards, the laboratories and the blood centres (30-40 %) (Wangsaatmaja 1997).

By considerations of technical staff, it is know that in urology is common the use of sterilized water pockets (also 5-10 bags of 5 liters each) that after their use are channeled into the sewage system (these volumes of water are not derived from the aqueduct, but end in the sewer).

Table 7.14: Hospital services that require water

	General services	Diagnostic services	Water demanding Wards
Uses	kitchens laundry Air conditioning fume purification Greenery Toilet Technical services	laboratories Sanitary Ambulatory radiological Ambulatory blood centers	general medicine surgery hemodialysis First aid orthopedics urology

In this study the variation of the water consumption along the year is not considered as the PhC consumption is given on annual basis.

In general, health facilities can conserve water resources by closely metering water use, installing water-efficient fixtures and technologies and making sure that leaks are quickly repaired. For even greater impact on overall usage, hospitals in a number of countries are harvesting rainwater. Others treated recycle water for process purposes.

The report by Health Care Without Harm suggests the following actions:

- Establish a framework that aspires to “net zero water use” within the hospital
- Implement water conservation strategies: install efficient faucets and toilets, routinely check plumping and pipes to prevent leaks, eliminate seal and cooling water on medical air compression and vacuum pumps, and retrofit refrigeration systems.
- Switch from film-based radiological imaging equipment, which uses large quantities of water, to digital imaging, which uses no water and no polluting radiological chemicals.
- Landscape grounds using drought resistant plants to minimize water use.
- Consider harvesting rainwater and/or recycling water for process water uses.
- Eliminated bottled water facility-wide if high quality potable water is available
- Regularly analyze water quality
- Implement on-site wastewater treatment technologies when no municipal service is available.
- Develop joint project with the community to improve and protect water supplies; support initiatives for public systems to improve water quality, water delivery and wastewater systems for the entire population.

Wastewater derived from consumed water

-As it was estimated (Metcalfe and Eddy, 1991) wastewater flow rate represents the 85% of the water consumption, the analysis was performed calculating the effluent wastewater flow rate by reducing the water consumption **(-25%)**. A water loss could be due mainly to leakages in some water supply installations (taps, reservoir etc.), water was also used for buildings construction for instance. This is in perfect agreement with the hospital WW volume indicated by (Lin and Tsai 2009). They indicated a daily WW volume of 585 m^3/d for an hospital of 1174 beds, slightly higher than the one of this study.

7.3.4 Variation of PhCs concentration in HWW with time

The variation of PhC concentration reflects both the variation of water and PhC consumption.

Analysing the concentration variability without considering to what this is due permit to report the following considerations.

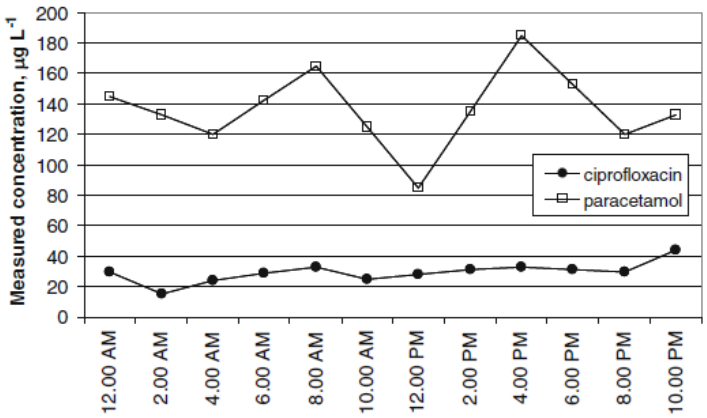


Fig. 7.13: Variation of hospital effluent concentrations of ciprofloxacin and acetaminophen over 24-h period (Verlicchi, Galletti, et al. 2012)

Fig. 7.14 shows that the patter of excretion of each PhCs could be very different. For this reason a complete analysis of these aspects is necessary to define the most adequate sampling campaign.

Nelson et al., 2011 observing the effluent of a WWTP receiving also HWW found different pattern concentration along the day. Referring to selected compounds sulfamethoxazole has a morning peak of excretion, azithromicin has concentrations elevated most of the day. Carbamazepine concentration is constant throughout the day. This investigation underline the importance of choosing the best way of sampling: a grab sample could under or overestimate the daily concentration.

Mendoza et al., (2015) analysed the total PhC concentrations along the week founding the highest concentration on Thursday and the lowest on Wednesday . Weissbrodt et al., (2009) found that the total emission of contrast media and antineoplastic drugs varies substantially from day to day, reaching maximal level on Friday and Wednesday, respectively when the wards operated at their highest capacity. There was a good correlation between the consumption and the measured data.

The studies of Coutu et al., (2013) and Verlicchi et al., 2012a confirm the high spatial and temporal heterogeneity of the hospital contribution of to the total load in WTP influent. However, little variability was observed from one year to the next in the contribution of hospitals to the total antibiotic load consumed over the WTP basin, whereas these fluctuations can be marked when observed at a monthly time scale. As a consequence, field campaigns that aim to estimate hospital pharmaceutical contributions to WTPs from comparison of measurements in hospital effluent and WTP influent need to account for this variability in hospital releases.

Diwan et al., (2013) analysed the seasonal variation of PhC concentration in HWW observing that in summer the concentrations of antibiotics are lower than in winter. This may be due to higher activity of microorganisms, intense sunlight and high temperatures during summer, which could

cause more biodegradation and photodegradation. On the contrary, they did not find specific patter in detection of antibiotic residues over 24-h with continuous sampling.

Daouk et al., (2015) stated that largely consumed API such as anti-inflammatory (ibuprofen) or analgesic drugs (acetaminophen) exhibited much lower variations than the lowest consumed drugs like some cytostatics.

An interesting work has been published by Lindberg et al, 2004 reporting the variation of the concentration in 13 hours for 6 antibiotics. They observed large variations of the concentrations between the different sampling times, caused by variation in the flow rate and in consumption.

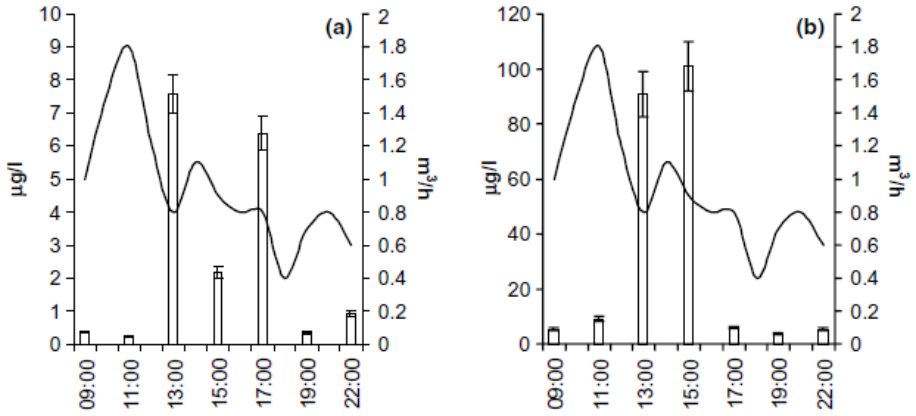


Fig. 7.14: Concentrations of ofloxacin (a) and ciprofloxacin (b) in hospital WW by Lindberg et al., (2004)

This rapid variations have to be taken into consideration when evaluating a sampling campaign. The same could be important in the development of bacterial resistance (Lindberg et al. 2004).

7.3.5 Data- Predicted concentrations in hospital effluent

A first evaluation was made considering:

1. uniform water consumption throughout the year and equal to 198500 m^3/year
2. mean excretion rate among those reported from literature
4. uniform PhC consumption throughout each year

The obtained results for PEC are listed in Table 7.18 together the corresponding MEC. In the last three columns is specify which between PEC or MEC have the greater values.

Observing Table 7.18, the highest PEC value is referred to Acetaminophen. This compound has been indicated also by Daouk et al., 2015 for having high value of PEC in comparison with the other PhCs. For same compounds the values are very similar (Codeine, Clarithromycin, Sulfadiazine), while in other cases the values are some orders of magnitude major for PEC (Acetaminophen, Ketoprofen, Chloramphenicol, Metronidazole, Furosemide) or for MEC (Indomethacin, Ofloxacin, Hydrochlorothiazide, Sotalol).

A more detailed analysis are carried out on the base of the ratio PEC/MEC and discussed in the dedicated paragraph together with the reasons for the discrepancies.

Kümmerer and Henninger, (2003) provided a list of PEC for various type of antibiotics but data are relative to 1996-98. As it has been over a decade, they are not considered for a comparison.

Kümmerer and Henninger, (2003) underlined the development and the spread of resistant bacteria due to an high use of antibiotics. This has to be prevented avoiding inappropriate use of these substances.

Table 7.15: PEC [ng/L] value for the selected compounds and comparison with MEC [ng/L]

		PEC	MEC	
		Mean	Mean	Major
A	Acetaminophen	148016	3143	PEC
A	Codeine	1239	1343	MEC
A	Diclofenac	809	395	PEC
A	Ibuprofen	3412	1813	PEC
A	Indomethacin	43	1181	MEC
A	Ketoprofen	11023	1289	PEC
B	Azithromycin	685	497	PEC
B	Chloramphenicol	327	5	PEC
B	Chlortetracycline	263	77	PEC
B	Ciprofloxacin	50034	13487	PEC
B	Clarithromycin	2414	6589	MEC
B	Doxycycline	173	76	PEC
B	Erythromycin	269	127	PEC
B	Metronidazole	12165	704	PEC
B	Norfloxacin	93	222	MEC
B	Ofloxacin	63	20032	MEC
B	Sulfadiazine	593	236	PEC
B	Sulfamethoxazole	4225	1921	PEC
B	Tetracycline	0,262	3	MEC
B	Trimethoprim	1545	371	PEC
C	Glibenclamide	6	85	MEC
D	Enalapril	239	239	MEC
D	Hydrochlorothiazide	492	1582	MEC
D	Lisinopril	73	213	MEC
E	Tamoxifen	5	<loq	PEC
F	Salbutamol	249	83	PEC
G	Atenolol	2770	4409	MEC
G	Metoprolol	3899	928	PEC
G	Propranolol	167	60	PEC
G	Sotalol	806	3238	MEC
G	Timolol	5	33	MEC
H	Furosemide	13673	6280	PEC
I	Atorvastatin	86	212	MEC
J	Carbamazepine	703	956	MEC
J	Diazepam	6	31	MEC
J	Fluoxetine	9	45	MEC
J	Lorazepam	136	433	MEC
J	Paroxetine	7	67	MEC
K	Ranitidine	11987	2338	PEC

7.4 Sensitivity analysis

A sensitivity analysis was carried out in order to quantify the influence on the PEC values of a variation of each of the factors included in the adopted model (see eq. 7.1).

For each parameter, a specific variability range was set according to collected data (Mi) or according to data available in literature (Ei, Q).

In this analysis, only one parameter could change its value at a time within its defined range, while the others assume constantly the corresponding mean value equal to those reported in Table 7.9 for PhC consumption, Table 7.11 for excretion rate and equal to 198500 m^3/year for water consumption.

Generally, this analysis allow to determine:

-the parameters that must be most investigated for better evaluate the output;

-the parameters that do not influence the output (and that could be eliminated by the model)

-the parameters that must be considered in a subsequent analysis of the uncertainty of output values

By means of eq. 7.1 a "new" values of PEC are evaluated while the percentage variation with respect to the corresponding values are calculated by mean of eq. 5 and reported in Table 18.

$$\frac{PEC_{new} - PEC_{average}}{PEC_{average}} \times 100 \qquad \text{(eq. 7.5)}$$

The correlations between percentage variation in PEC vs variability range of each parameter are discussed underling the most influencing parameters. It is possible to pinpoint the parameters whose expected variability can mostly influence the predictive value of PECs.

- Variation of the PhC consumption

It is difficult to ascertain the yearly distribution patterns of PhC consumption inside the hospital as data are not readily available.

Pill project monitored the variation of consumptions between two years for some compounds. Table 7.16 reports the percentage of variation. There are slight differences between the two years.

Table 7.16: PhC consumption [mg] in the CHEM Hospital in Luxemburg during 2012 and 2013.
IV = Intravenous; OA = oral administration

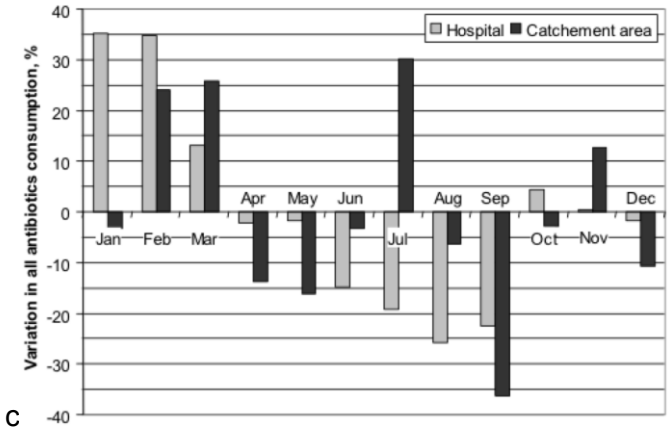
		2012	2013	Percentage of variation from 2012 to 2013
A	Diclofenac OA	2.418.875	2.116.825	-12
	Diclofenac IV	139.125	132.450	-5
	Naproxen OA	3.546.000	4.187.000	18
B	Erythromycin OA	-	-	
	Erythromycin IV	204.000	116.000	-43
	Ciprofloxacin OA	14.220.000	13.350.000	-6
	Ciprofloxacin IV	918.400	765.200	-17
	Sulfamethoxazole OA	2.972	3.366	13
	Sulfamethoxazole IV	-	-	
J	Carbamazepine OA	1.015.200	745.600	-27

Le Corre et al., (2012) compared two years the hospital consumption and found differences between 22 and 44 %. For considering the temporal variability they assumed an uncertainty of 50%. For these reasons cautiously a yearly consumption variability equal to **50%** was considered.

Monthly basis - Antibiotics

It was not possible to state accurately the PhCs variation along the year but, generally, it is possible to consider that for antibiotics the annual variation of consumption is more marked than for PhCs more regularly consumed throughout the year (i.e. diuretics and beta-blockers).

The greater antibiotic hospital consumption in winter is confirmed by the following figure.



C

Fig. 7.15: Monthly variation on the antibiotics consumption in Urbans and Hospitals users in Lagosanto. (Galletti 2010)

On the contrary, seasonality was not evident in hospital consumption in other studies (i.e. Coutu et al., 2013). They observed no seasonal pattern for antibiotics consumption in hospital. This is explained by the seemingly random, behaviour of hospital antibiotic consumption, which dominates over any periodic seasonal consumption. Ciprofloxacin is annually high consumed but reported low monthly fluctuations. In general, these results suggest that hospital antibiotic use is largely disconnected from non-hospital use, perhaps due to different protocols used for hospital and non-hospital patients. Another possibility is that drugs are used to treat different diseases in hospitals than in the community.

Considering only antibiotics, it was possible to complete the sensitivity analysis considering a monthly consumption by dividing the annual consumption by 12 months. This monthly consumption was varied between **+35 %** (from October to March) for winter and **-25%** (from April to September) for summer months.

Monthly basis – Carbamazepine

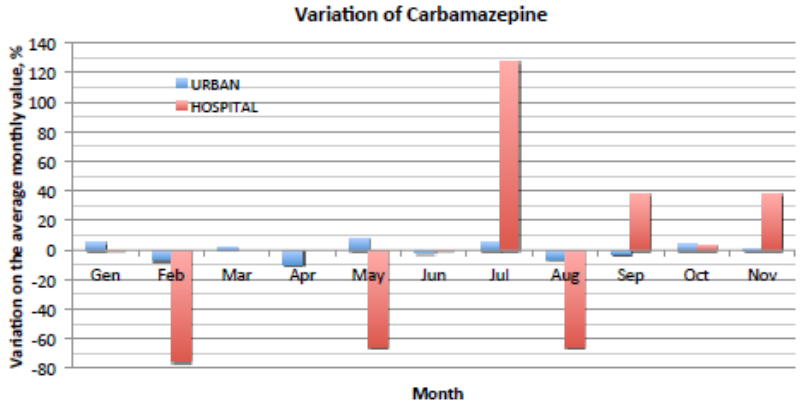


Fig. 7.16: Monthly variation on Carbamazepine consumption in Urbans and Hospitals users in Lagosanto. (Galletti 2010)

Considering only carbamazepine, it was possible to complete the sensitivity analysis considering a monthly consumption by dividing the annual consumption by 12 months. This monthly consumption was varied between **+130 %** and **-80%.**

- Variation of the excretion rate

The uncertainty due to a wide range of reported excretion rates may suggest that the predicted concentration is subject to even higher uncertainty (Lai et al. 2011). On the other hand, the limitation of the excretion data available in the literature have been highlighted. For example, most of the published clinical data originate from urinary analysis of a very limited number of young or healthy men and adults. Furthermore, poly-consumption of drugs (i.e. drug-drug interactions) and different administration routes such as intravenous, intranasal, smoked and/or the combination with alcohol can result in a higher or lower excretion rate which is also not taken into account. The range of variation considered for each selected compound is reported in Table 7.14.

- Variation of water consumption

The water consumption has been decreased of 25 % for considering the water loss due mainly to leakages in some water supply installations (taps, reservoir etc.) (Metcalfe and Eddy, 1991).

Only for antibiotic, the analysis was repeated also increasing the average monthly water flow (16 540 m^3/month) by multiplying it for the peak 1.8 considering the summer months from April to September (see Table 7.12. Observing Fig. 7.13 the minimum flow rate, typical of winter from October to March, was estimated assuming a coefficient of 0.5.
Johnson et al., (2008) said that hydrology (dilution) is often seen to be the major determining factor in sensitivity analysis for assessing the removal rate in the environment. To account for seasonal or day-to-day variability of dry weather wastewater volumes and flow measurement errors a total uncertainty of 50% was assumed by Le Corre et al., (2012).
The compounds are excreted every day, also during the weekend and at night, when the water consumption in hospitals is lower and therefore the concentrations in those moments are higher.

This uncertainty can only be reduced with a conscientious calibration of flow meters.

7.4.1 Results on a multi years basis

Table 7.16 summaries the criteria for varying each parameter considered in the sensitivity analysis that consider the variation of consumption from one year to another.

Table 7.17: Criteria for varying each parameter in the sensitivity analysis

Parameter	Notes
Q	Equal for all compounds: -25 % of water consumption
E	Variable for each compounds: see Table 7.11.
PhC consumption	±50 %

Table 7.18 report the percentage variation of PEC_{new} with respect to PEC mean. Obviously the variation there is not variation in PEC new if compared with PEC mean.

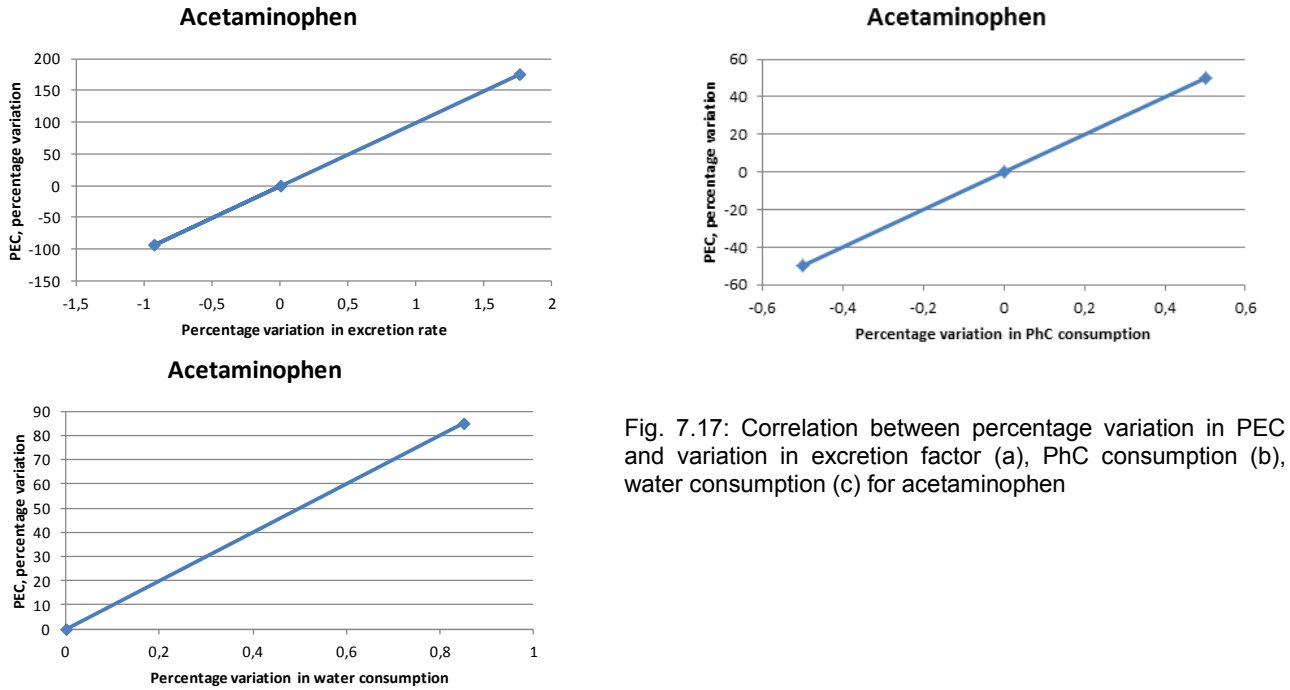


Fig. 7.17: Correlation between percentage variation in PEC and variation in excretion factor (a), PhC consumption (b), water consumption (c) for acetaminophen

Table 7.18: Percentage variation of PEC by varying each parameter of the quantities considered in previous paragraphs.

	-	Excretion	Excretion	PhC consumption	PhC consumption	Water consumption
	Mean	Min	Max	Min	Max	Min
	PEC %	PEC %	PEC %	PEC %	PEC %	PEC %
Acetaminophen	0,00	-93,10	175,86	-50,00	50,00	85,00
Codeine	0,00	-84,75	103,39	-50,00	50,00	85,00
Diclofenac	0,00	-66,67	160,00	-50,00	50,00	85,00
Ibuprofen	0,00	-89,80	155,10	-50,00	50,00	85,00
Indomethacin	0,00	-33,33	33,33	-50,00	50,00	85,00
Ketoprofen	0,00	-82,86	54,29	-50,00	50,00	85,00
Azithromycin	0,00	-14,29	14,29	-50,00	50,00	85,00
Chloramphenicol	0,00	-33,33	33,33	-50,00	50,00	85,00
Chlortetracycline	0,00	-55,56	55,56	-50,00	50,00	85,00
Ciprofloxacin	0,00	-58,25	74,74	-50,00	50,00	85,00
Clarithromycin	0,00	-11,11	11,11	-50,00	50,00	85,00
Doxycycline	0,00	-26,13	26,13	-50,00	50,00	85,00
Erythromycin	0,00	-50,00	50,00	-50,00	50,00	85,00
Metronidazole	0,00	0,00	0,00	-50,00	50,00	85,00
Norfloxacin	0,00	-35,25	48,92	-50,00	50,00	85,00
Ofloxacin	0,00	-34,10	34,91	-50,00	50,00	85,00
Sulfadiazine	0,00	-12,87	12,87	-50,00	50,00	85,00
Sulfamethoxazole	0,00	-80,13	198,01	-50,00	50,00	85,00
Tetracycline	0,00	-12,50	12,50	-50,00	50,00	85,00
Trimethoprim	0,00	-45,65	44,93	-50,00	50,00	85,00
Glibenclamide	0,00	0,00	0,00	-50,00	50,00	85,00
Enalapril	0,00	0,00	0,00	-50,00	50,00	85,00
Hydrochlorothiazide	0,00	0,00	0,00	-50,00	50,00	85,00
Lisinopril	0,00	0,00	0,00	-50,00	50,00	85,00
Tamoxifen	0,00	0,00	0,00	-50,00	50,00	85,00
Salbutamol	0,00	0,00	0,00	-50,00	50,00	85,00
Atenolol	0,00	-28,57	28,57	-50,00	50,00	85,00
Metoprolol	0,00	-57,45	65,96	-50,00	50,00	85,00
Propranolol	0,00	-95,92	95,92	-50,00	50,00	85,00
Sotalol	0,00	0,00	0,00	-50,00	50,00	85,00
Timolol	0,00	0,00	0,00	-50,00	50,00	85,00
Furosemide	0,00	0,00	0,00	-50,00	50,00	85,00
Atorvastatin	0,00	0,00	0,00	-50,00	50,00	85,00
Carbamazepine	0,00	-80,95	185,71	-50,00	50,00	85,00
Diazepam	0,00	0,00	0,00	-50,00	50,00	85,00
Fluoxetine	0,00	-89,80	144,90	-50,00	50,00	85,00
Gabapentin	0,00	0,00	0,00	-50,00	50,00	85,00
Lorazepam	0,00	-99,30	99,30	-50,00	50,00	85,00
Paroxetine	0,00	0,00	0,00	-50,00	50,00	85,00
Ranitidine	0,00	-86,67	75,56	-50,00	50,00	85,00

The color help to indicate the parameters that major influence PEC. Dark green indicate the parameter with the greatest negative variation and on the contrary dark red indicate the parameter with the greatest positive variation.
It is evident that excretion rate influence PEC more than the other parameters for the majority of compounds. For a lot of compounds this parameter has no influence in the variation because only one value was found in literature. For this reason for those compounds there is the need to have more data. The variation in this parameter imply that variation of PEC vary from -99 % (lorazepam) to +198% (Sulfamethoxazole). For these compounds a better estimation of E is needed for reducing the variability in PEC. Considering a decrease in the flow imply that PEC increase of 85 % for all the selected compounds.

7.4.2 Results on yearly basis

A sensitivity analysis that consider the monthly variability was carried out only for antibiotics and carbamazepine because data of hospital consumption variation along the year was available only for these compounds. The class of antibiotics drugs represent the most problematic class of compounds for the aquatic system and carbamazepine is an anthropogenic marker (Daouk et al. 2015).

Table 7.19: Criteria for varying each parameter in the sensitivity analysis

Parameter		Min	Max	Notes
Q		-41% Winter	+70% Summer	See Fig. 7.12, See TAble 7.13
E		Variable for each compounds		see Table 7.11
PhC consumption	Antibiotics	-25 % Summer	+ 35 % Winter	See Fig. 7.15
	Carbamazepine	-80% Summer	+130% Winter	See Fig. 7.16

Also considering seasonal variations, excretion rates greatly influence PEC for the majority of compounds. The relative variations vary from -81% (carbamazepine) and +198 % (sulfamethoxazole). Also in the work by Daouk et al., 2015 The excretion rate is the most influencing factor.

The variation of water consumption along the year influence PEC more than the variation of PhC consumption. The color in Table 7.20 show the more influencing parameters for each compound with the same color scale of Table 7.18.
Comparing the results of the sensitivities analysis of PEC with the uncertainties of MEC, it can be observed that generally the variability associated to PEC is greater than the uncertainties of MEC. Only for erythromycin, metronidazole and ofloxacin the uncertainty on sampling and on analysis is greater than the variability of the parameters involved in calculating PEC.

Table 7.20: Percentage variation of PEC by varying of the quantities considered in Table 7.19 each parameter.

PEC percentage variation						
	Excretion	Excretion	phC consumption	phC consumption	Water	Water
	Min	Max	Min	Max	Min	Max
Azithromycin	-14,29	14,29	-25	35	-41	70
Chloramphenicol	-33,33	33,33	-25	35	-41	70
Chlortetracycline	-55,56	55,56	-25	35	-41	70
Ciprofloxacin	-58,25	74,74	-25	35	-41	70
Clarithromycin	-11,11	11,11	-25	35	-41	70
Doxycycline	-26,13	26,13	-25	35	-41	70
Erythromycin	-50,00	50,00	-25	35	-41	70
Metronidazole	0,00	0,00	-25	35	-41	70
Norfloxacin	-35,25	48,92	-25	35	-41	70
Ofloxacin	-34,10	34,91	-25	35	-41	70
Sulfadiazine	-12,87	12,87	-25	35	-41	70
Sulfamethoxazole	-80,13	198,01	-25	35	-41	70
Tetracycline	-12,50	12,50	-25	35	-41	70
Trimethoprim	-45,65	44,93	-25	35	-41	70
Carbamazepine	-80,95	185,71	-80	130	-41	70

7.5 Comparison between predicted and measured concentration

It is important to examine just how close the two approaches come to agreement when tested in the field than established what of the two is the true one.

In this study consumption data of 2008 and 2011 were compared with a sampling campaign carried out during 2010. Consumption pattern might have shifted to some extent however these differences are expected to be marginal for the selected compounds. The same consideration was done also in the monitoring of regional sales data by Oosterhuis et al., (2013) for the prediction of the concentrations in surface water.

A comparison of the predicted and measured concentrations of the investigated compounds is first performed by means of the ratio PEC/MEC, to establish whether the predicted equations used tend to underestimate or overestimate measured values.

This approach has already been followed in other investigations concerning urban WW (among them Coetsier et al., 2009; Morasch et al., 2010, Verlicchi et al., 2014) and hospital effluent (Heberer and Feldmann 2005). Obviously, few works are published referring to PhC PEC in HWW.

Varying the available excretion rate data, the min, max and mean values of PEC were compared with those measured in the hospital effluent.

Coetsier et al., (2009) proposed an accuracy evaluation criteria based on limits between witch the ratio PEC/MEC should be included. This is presented in Table 7.21.

Table 7.21: PEC/MEC evaluation criteria

Coetsier et al., (2009)	PEC/MEC < 0.2	not acceptable
	0.2< PEC/MEC <1	slightly underestimated
	1< PEC/MEC <4	slightly overestimated
	4<PEC/MEC <8	significantly overestimated
	PEC/MEC>8	Strongly overstimated

The obtained values were ranked in accordance with the criteria proposed by Coetsier et al., (2009). Jean et al., (2012) used estimations as a comparative criterion for assess the necessity of further studies on the estimated priority compounds.

7.5.1 Results

The following figures show the comparison between the PEC obtained for the two considered year and the mean values of MEC.

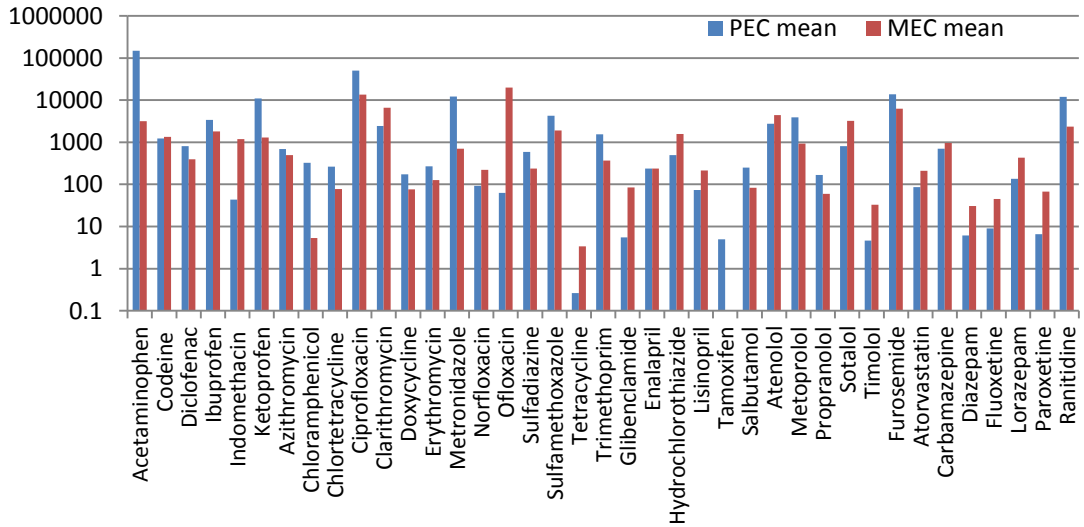


Fig. 7.18: PEC and MEC

Even though the reports on the consumption Tamoxifen show that it is used within the hospital, it was not detected in the effluent. It could be explained as when the PEC are lower than their corresponding MDLs it could be expect that these PhCs are undetected on the field campaign. An example is Tamoxifen whose is predicted to be about 4 ng/L and it is not detected in HWW as its lod is 14 ng/L.

A ratio between PEC mean and MEC mean was calculated for comparing the two values. PEC mean was calculated by using the mean value of all the parameter used in eq. 7.1. MEC mean has been reported in Table 7.5.

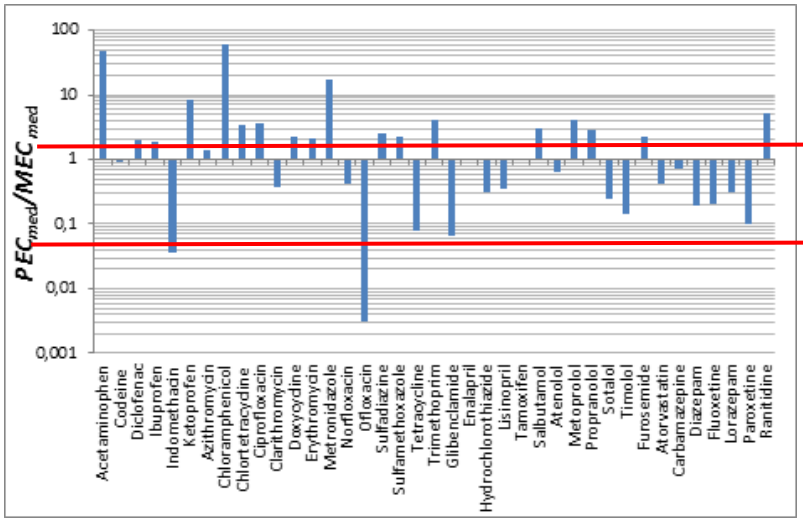


Fig. 7.19: Comparison of PECs and MECs of the selected compound calculated by means of their ratio. Red lines represent the levels of prediction accuracy.

As it is clear from Fig. 7.19 the majority of the compounds are slightly over or under estimated. For Acetaminophen, Ketoprofen, Chlorotetracycline, Clarithromycin, Metronidazole and Trimethoprim PEC values overestimated the measure. Ofloxacin has measure valued greater than the predicted ones, as well as for Indomethacin, Tetracycline and Glibenclamide (but for these last compounds it is less evident). When there is an under estimation a potentially dangerous drug may escape further assessment. On the contrary false positive could lead to not necessary further investigations.

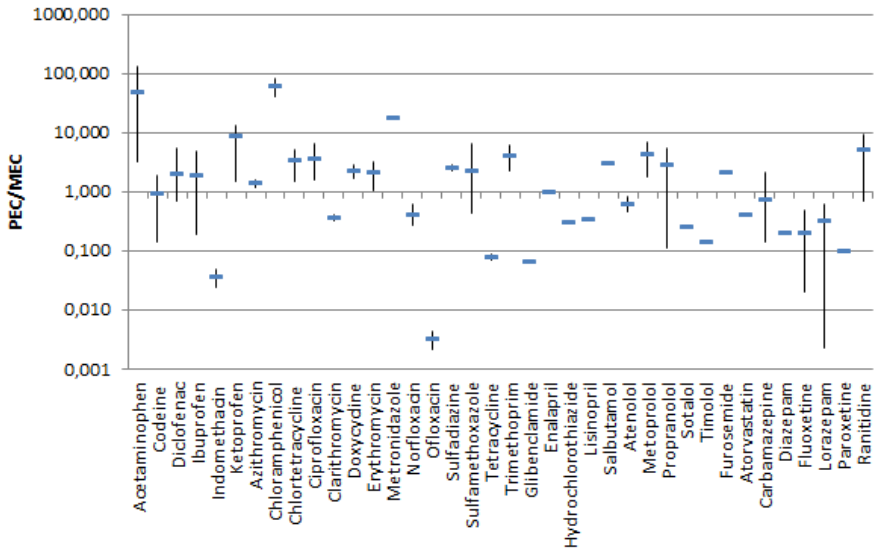


Fig. 7.20: PEC_{mean}/MEC_{mean} ratio together with their range of variability as defined in sensitivity analysis

Fig. 7.20 reports the mean value of the ratio reported in Fig. 7.19.
A vertical bar represents the range of variability of the ratio obtained considering, for each compound, the maximum and the minimum value of each parameter considered for evaluating PEC in the sensitivity analysis.

As PEC differ from MEC for most of the compounds, this may not be considered sufficient as a complete investigation but the results of PEC calculations and subsequent risk assessments would provide valuable insight into the prioritisation of drugs of concern in hospital effluents. The comparison between PEC and MEC carried out by Mullot et al., (2010) showed a satisfactory correlation for those compounds with short elimination half-lives and weak human metabolism.

Table 7.22 Half-lives of some compounds proposed by Castiglioni et al., (2004)

Compound	Half-lives
Ibuprofen	t50 < 1 d
Ciprofloxacin	Stable for > 40 d in close bottle test
Erythromycin	t50> 1 y, 11,5 d (20°C)
Atenolol	Stable for 40 d (5-25°C)
Furosemide	Stable for 90 d (pH 5,2); Stable 96 % 240 d pH 5,2
Ranitidine	Stable 160 h, pH 6,18 65°C

Observing Table 7.22 that reports some values of half-lives for some compounds, it is not possible to confirm the statement of Mullot et al., (2010) because for those compounds the ratio is quite close to one even for compounds with long half-lives.

7.5.2 Scenarios of seasonal variation

Considering the same seasonal variation listed in Table 7.19 and a mean value for excretion rate, it was possible to define a seasonal scenario calculating PEC for the worst condition in in summer and winter. It is evident that for all selected PhCs the worst scenario occur in a month in Winter as in that season there is an increase of the PhC consumption and a decrease of the water flow rate.

Table 7.23: PEC estimated for winter and summer

	[g/month]	[g/month]
	PEC	PEC
	Winter	Summer
Azithromycin	308,3	47,6
Chloramphenicol	147,2	22,7
Chlortetracycline	118,1	18,2
Ciprofloxacin	22515,1	3474,6
Clarithromycin	1086,2	167,6
Doxycycline	78,0	12,0
Erythromycin	121,0	18,7
Metronidazole	5474,4	844,8
Norfloxacin	41,8	6,4
Ofloxacin	28,5	4,4
Sulfadiazine	266,7	41,2
Sulfamethoxazole	1901,4	293,4
Tetracycline	0,1	0,0
Trimethoprim	695,1	107,3
Carbamazepine	550,7	13,0

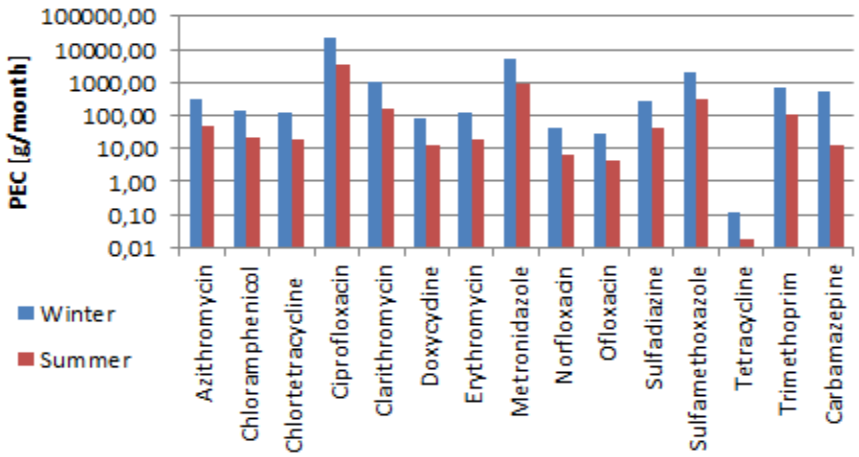


Fig. 7.21: PEC calculated for winter and summer scenario

Calculating PEC by using seasonal data the PEC are higher in winter than in summer due to higher consumption of the selected compounds and lower flow rate.

These data were compared with the corresponding MEC for winter and summer, obtaining the results shown in Fig. 7.25. The comparison with MEC has not been improved by considering the different scenario.

Chloramphenicol, metronidazole and trimethoprim are still out of the range of acceptability.

Ciprofloxacin, erythromycin , sulfadiazine and sulfamethoxazole have a ratio close to 1 in winter while in summer the differences are more evident.

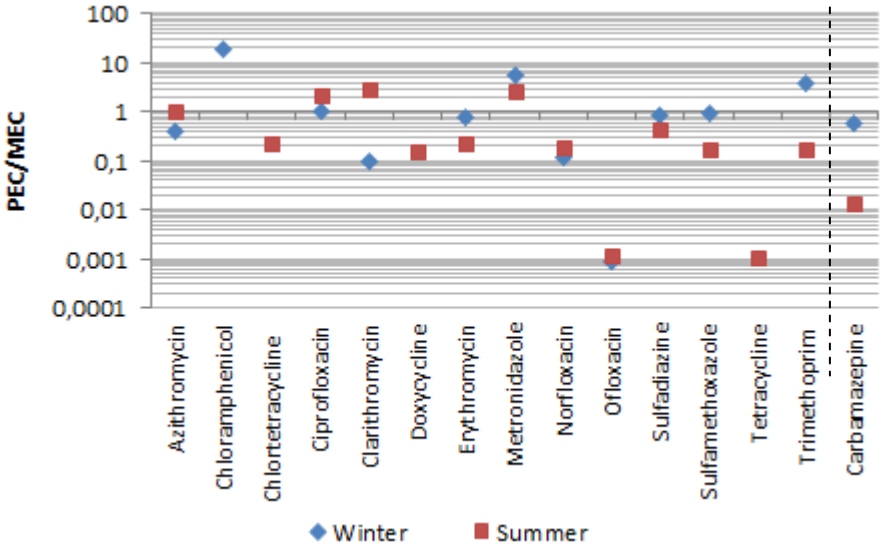


Fig. 7.22: Comparison between PEC and MEC for winter and summer

7.5.3 Reasons of discrepancies between MECs and PECs

Both predicted and measured concentrations can be affected by a variety of factors, depending on both the compound itself and the investigated point. For organic micropollutants, the PEC and MEC have mostly been investigated in surface water (Bound and Voulvoulis, 2006)

The prevailing opinion is that predictive models could be very useful tools, but intrinsic uncertainties are unavoidable, due to the necessary adoption of default or literature values, which should be carefully evaluated case by case in order to reduce the inaccuracy of the estimation. A further limitation of prediction models is that results are average values in both time, as compared to measured concentrations that are typically associated with a certain point in time (Verlicchi et al. 2014).

Potential factors influencing PEC

1. Wrong estimation of PhC consumption

PEC values are estimated basing on the PhC consumption. This datum generally contains all the PhCs dispensed by the hospital structure to in patients and out patients.

In predicting the PhC concentrations the following factors should be keep in mind.

- Many in-patient have to bring with them their usual medicaments from home to the hospital when they are hospitalised. Thus these compounds are not considered among the hospital consumption data. "A patient who regularly takes histamine blockers at home is likely to take them with him if he is being hospitalized for any treatment not related or interfering with histamine blocker; this is also to assumed to be valid for beta-blockers and diuretics. National consumption data to calculate this contribution appears to be good predictors (Ort, Michael G. Lawrence, Reungoat, et al. 2010).

- Some in-patients could also excrete in hospital residues of PhCs assumed in another site. On the contrary, a lot of day-hospital people present only for few hours in a day in the hospital for analyses or therapy requiring specific agents, such as antineoplastics, or diagnosis agents…) or out-patients do not excrete totally in the structure the administered compounds (Weissbrodt et al., 2009, Mullot et al., 2010). Escher et al., 2011, confirmed a large ammount of pharmaceuticals consumed in the hospital but excreted at home by out-patients (50% out-patients for X-ray contrast media and 70%). In the Swiss study (Weissbrodt et al. 2009) a maximum of 7.5% of the cytostatics were quantified in the hospital's effluent, implying that the remaining part is most likely ''carried home'' by patients and excreted in household toilets. In this study the predicted concentration of Tamoxifen was always greater than the measured but considering the outpatients it could be reduced. Lenz et al. (2007) report that for some PhCs merely a small fraction of the amounts administered in the hospital were actually found in its effluent (i.e. 0.1–0.2% for doxorubicin, 0.5–4.5% for 5-fluorouracil and 27–34% for total platinum).
- Moreover, the hospital pharmacy provide the PhCs to discharged patients or outpatients for starting or continuing at home the cures. It is the case, for instance, of antineoplastics and psychiatric drugs (Verlicchi et al., 2010a, Bianchi et al., 2011). All this amount is neither administered nor excreted in hospital. Antivirals may be prescribed and delivered in the hospital but are likely to be excreted at home by outpatients (Daouk et al., 2015). Moreover, the pharmacy data can differ from real consumption in the services due to lack of patient compliance, outside consumption for leaving patients, etc. (Jean et al., 2012).

For these reasons, it should be very important to assess the amount of PhCs dispensed for internal use or provided to outpatients or discharged patient.

Feldmann et al., 2008 stated that thanks to very precise data of administration, a small difference between PEC and MEC was observed.

2. Variation of consumption over the year

A description of the variation of PhC consumption over the year has been discussed in paragraph 4. There are class of compounds that show a variation along the year (antibiotics) while other are more constant. Kümmerer and Henninger, (2003) confirmed an higher antibiotic consumption in winter than in summer.

For the compounds that have a great variation along the year it is very important to decide the most adequate sampling campaign. Measuring only in a season may imply an over or under estimation of the yearly load. In calculating PEC , the consumption should be considered on monthly base for the compounds that have a strong seasonal variation.

3. Differences between the pharmacy consumption data and the effective administration

It has to be noted that the hospital consumption data in the database correspond to the amounts supplied by the pharmacy to the individual wards and not to the amounts effectively administered. Some unused drugs for in-patients may be collected on the wards and returned to the pharmacy for reuse or proper disposal. It is generally not the hospital's policy to discard drugs to the (solid or liquid) waste system, both from a financial and environmental point of view. Hence, these drugs do not contribute to the load in the HWW. However, in discussion with relevant hospital staff these

amounts are considered to be very limited (Ort, Michael G. Lawrence, Reungoat, et al. 2010) and so the discrepancies due to this cause. Morover, there could be a lag time between the delivery to the ward and the actual consumption.

4. Inaccuracy in the excretion rate assumed for the evaluation of PEC

As previously said, the excretion rate varies with individual human characteristics who assumed them. The estimated value should considered excretion data of a large set of individuals as the variations of little number of patients are not significant.

Moreover, quite often for a given active ingredient, literature provides ranges of excretion rate resulting by different studies, showing minimum-maximum values observed for it. In many cases excretion rates refer to investigations of some decades ago (Jjemba et al., 2006; Kümmerer and Henninger, 2003). The pharmaceuticals of new generation (i.e. gatifloxacin and moxifloxacin, (Jia et al. 2012)) are designed to provide better therapeutic effect improving human absorption rate and at the same time reducing the excretion rate.

It is questionable if it is still correct, from a scientific view point, to assume existing (and old) literature data for the these compounds. This could lead to a overestimation of the predicted concentrations.

When adopting the excretion rate for a given compound, particular attention must be paid to the correct values as they may refer to the unchanged compound or to the corresponding metabolites. If both are considered for the evaluation of the predicted concentrations, an overestimation will occur. Moreover attention is required for the application mode of the active ingredient resulting in different excretion rate (Heberer and Feldmann 2005).

Moreover, another difficulty is to accurately evaluate the fraction of the sorbed drug eliminated unchanged during each of the following days (Mullot et al., 2010). However, the selected PhCS are mainly polar and not subject to a significant absorption on suspended matter.

Le Corre et al., (2012) suggested of considering a total excretion of each PhC for counterbalancing other uncontrolled parameters (i.e. improper disposal or unused PhCs). By this way there could be only an overestimation and false negative results are prevented.

When estimating PEC, the variation in excretion rate may be one of the major cause of variation (Verlicchi et al., 2014).

5. Different mode of application

Excretion rate for the same compound is strongly influenced by the mode of application. Heberer and Feldmann, (2005) identified dermal application as the main source for the occurrence of diclofenac residues in the hospital effluent, as a low absorption rate is reported for this type of application. This could lead paradossally to a low recovery of the compounds as they may be absorbed by clothes or bandages. If a laundry is present at the hospital, part of these compounds might be found in its effluent. Therefore, they suggested that it is not sufficient to acquire only the total amounts of the administered pharmaceuticals but the individual amounts for each formulation.

Liquid are more frequently wasted than pills or tablets.

6. Lag time for excretion

In considering consumption data for calculating PEC, lag times for excretion should be considered (Heberer and Feldmann, 2005).

These times are influenced by pharmacokinetic data. Pharmacokinetics describes how the body affects a specific drug after administration through the mechanisms of absorption and distribution, as well as the chemical changes of the substance in the body. Pharmacokinetic properties of drugs may be affected by elements such as the site of administration and the dose of administered drug: these may affect the absorption rate.

Different lag time influenced the moment in which the administered compounds reach the HWW.

7. Wastewater flow variations

Wastewater flow is usually estimated from the water consumption that have daily variations as reported in paragraph 2.4.3. In the paragraph of sensitivity analysis is explain the variability of PEC associated to the variation of this parameter. The variation in the flow rate should be combined with the variation of consumption during a day but obtaining this data are very difficult.

For this reason, it should be important at least consider the variation of water consumption along the year to be combined with the variation of PhC in the same period.

8. Lack of patient compliance

The lack of patient compliance is to be considered in particular when PEC is calculated considering the prescriptions. Bianchi et al., (2011) found that for antipsychotic the mean adherence to therapy was equal to 64%. In this study, it was assumed that data of consumption were referred to PhC effectively administered to patient by specific personal. Nevertheless Jean et al., (2012) considered this aspect as a potential bias also for estimating the PhC consumption by in-patients.

9. Improper disposal of unused medicines (in household waste or via the toilet)

Improper disposal of unused medicines, i.e., by flushing them down the toilet or throwing them out with the household waste rather than returning them to a pharmacist, will also affect the prediction accuracy (Verlicchi et al. 2014). In the case of hospital, this factor could be of minor importance respect to investigations carried out for urban WW as the disposal of medicines is managed by the personal of the structure that should return the waste PhCs to an authorized supplier or reverse distributor. Nowadays, the traditional method of disposal for "left over" substances is to squirt any liquid into toilet and to crushed and mix with water pills as the recommendations for disposal are confusing and conflicting. In the study by Mankes and Silver, (2013) it was found that from 1/2 to 1/3 of codeine was wasted so it was necessary to find disposal alternatives.

For registered entities such as hospitals there are no clear guidelines for the disposal of PhCs in U.S (Mankes and Silver 2013) but any such disposal must be done in accordance with local environmental regulations. Usually U.S. Drug Enforcement Administration (DEA) may dispose of controlled substances by returning them to the manufacturer, by transferring them to a reverse distributor, or by destroying them by a procedure specified by federal regulation (as of this date no such procedures exist). They remarked that liquids are more frequently discharged than those dispensed in tablet form. They found in particular that 50 % of dispensed acetaminophen and codeine were wasted in the analysed academic centre hospital.

Mankes and Silver, (2013) reported that for most of the analysed compounds incineration was the method of disposal recommended even if there is a great opportunity for waste reduction changing, for instance, dispensing, sizes and stokes.

10. Metabolites hydrolyzed back to the patent compound

In the investigation by Kovalova et al., (2012) the hospital effluent was found to be a dynamic system in which conjugates of PhCs deconjugate and biological transformation products are formed (in some cases are PhCs themselves). Mankes and Silver, (2013) remarked that 13 of the 15 analyzed compounds formed potentially reversible conjugates (predominantly glucuronides) that were excreted but that can hydrolyzed back to the patent compound once into the environment. The same was observed also by Bound and Voulvoulis (2006).This may lead to greater than expected concentrations. In the investigation by Mullot et al., (2010), it was assumed that the conjugated metabolites were completely hydrolyzed in wastewater. This resulted in a over estimation of the annual load of ketoprofen and of the anesthetic propofol. The relatively modest concentrations in the hospital effluent of tamoxifen, ketoprofen and carbamazepine could be explained as they are excreted as conjugates with higher concentrations of the parent compounds (Langford et al., 2009).

11. Experimental half-lives

Mullot et al., (2010) found a satisfactory correlation between measured and calculated concentrations for the compounds with short elimination half-lives. For the others compounds, pharmacokinetic data and molecule stability in the effluent should be considered in the model.

Considering, when available, half-lives in the evaluation of PEC generally produced reductions in the PEC values (Castiglioni et al., 2004). Often the reductions are not significant as the molecules considered are not easily degraded in the environment, at least under the experimental conditions considered. See Table 7.25 for some half-lives values.

Since reliable ‘‘environmental half-lives’’ were not available, the values considered for calculation were the experimental half-lives. It must be taken into account that experimental conditions can produce outcomes that do not reflect real behavior in the environment or in the sewage network. (Castiglioni et al., 2004)

For compounds with short half-lives that are not stable with slightly alkaline or neutral pH the MEC could be lower than PEC.

12. Neglected biodegradation/biotransformation or adsorption processes occurring in the sewage system before entering in the WWTP

Lai et al., (2011) assumed that the effect of biodegradation was more or less constant within a given sewer system and over a short sampling period (i.e. days) and that inter-day variability is negligible, as in this case. This may not hold true when data among different locations or within a location over a longer time span (i.e. year, seasonal effects) are compared.

Compounds with high sorption potential, like a azithromycin, may be affected by desorption processes as they may sorb onto sludge and particles present in the sewer and can also be released at a later time depending on environmental conditions (Verlicchi et al. 2014). The following table provide a rule for assessing which compounds be strongly sorb onto particles reducing or retarding their presence in the sewerage. See properties of PhCs in Appendix A.

Table 7.24: Sorption potential – Rule of thumb (Verlicchi, Zambello, and Al Aukidy 2013)

Parameter	Conditions	Rule of thumb
K_d Log K_d	> 500 L/kg > 2.67	High sorption
K_d Log K_d	< 500 L/kg < 2.67	Low sorption

13. Sewage system

The rain water could dilute the concentration if the sewage system is combined (Kümmerer and Henninger 2003)

14. Number of patients treated during the sampling periods

Assessing PEC with data of consumption, the variation between the average number of the patients treated in hospital should not be considered. In other cases the explanation may be sought in a higher or lower than average number of patients being treated during the sampling period in the hospital [Ort et al. 2010a].

Potential factors affecting MEC

a. Sampling protocols

Sampling protocols, namely sampling frequency and sampling mode, as clearly remarked by Ort et al., (2010b) and Johnson et al., (2008) as well as instrumental and human errors, may cause a high uncertainty in MEC (and also discrepancies between MEC and PEC), especially for those compounds detected at very low concentrations (several ng/L). Depending on the compounds and the characteristics of the hospital structure, it is therefore crucial to define sampling frequency and sampling mode (grab or composite samples) in order to be sure that the water samples are representative of a certain period of time (Ort, Michael G. Lawrence, Reungoat, et al. 2010).

Additionally, matrix effect, as clearly discussed in Ternes and Joss (2006) and in Gros et al. (2006, 2009) could be carefully evaluated (Verlicchi et al. 2014).

According to (ISO 1980), “the times and frequencies of sampling in any program can be properly decided only after detailed preliminary work, in which a sampling frequency is necessary.” Perhaps preliminary investigations are simply not reported or not recognized to be sufficiently important. The high analytical costs per sample appear to be another reason: preliminary work at one location and one point in time may not be transferable and would have to be repeated, adding substantial costs to monitoring campaigns without answering the final research questions.

Relevant sampling guidelines have existed for decades yet a review of 87 papers comprising 267 different sewer sites reveals that these published procedures and methods were not cited nor heeded (Ort, Michael G Lawrence, et al. 2010). As a result it is not possible accurately understand how the concentration of a chemical is represented by the sample that often have small volume.

The Authors evaluate uncertainty related to two possible approaches for measuring concentrations in the sewerage: "high frequency grab sampling" and "different composite sampling modes".

This uncertainty may become a dominant source of error if not managed. For reducing the uncertainties, the Authors suggest a precautionary high sampling frequency (<5 min) if the dynamics for the substances of interest or to considered different composite sampling modes considering that the choice highly depend on the site-specific boundary conditions. Different, commonly applied composite sampling modes which were presented as appropriate in previous literature to obtain a representative average sample from wastewater in a sewer are likely to lead to unreal variations (sampling artifacts), in another situation.

When sampling we can find: real variation (due to pattern consumption of PPCPs) and additional variation due to analytical error (including transport preservation, storage, preparation and instrumental error). A continuous flow proportional sampling mode is conceptually the most accurate (true and precise) sampling mode when sampling for loads of dissolved compounds (Ort, Michael G Lawrence, et al. 2010).

For estimating risk a grab sample in the hour of maximum discharge may be a better choice as acute toxicological aspects are not only related to the load and even the maximum concentration must be considered. Ort et al., (2010b, 2010c) have discussed the main aspects to be considered to ensure the reliability of the measured data and reduce the relative uncertainty.

a. instrumental (and human) errors

This aspect is considered when calculating the uncertainties related to chemical analysis.

These kind of error may cause high uncertainties especially for those compounds detected at very low concentrations (some ng/L) (Verlicchi et al. 2014).

Johnson et al., (2008) measured different subsamples of the same sample in different laboratories reporting that the Phc concentrations did not guarantee accurate results with these compounds as the standard deviation ranging up to 60%.

b. Analytical methods limitations

The analytical methods analyse only the compound dissolved in the water phase. For the compounds having high sorption potential, a fraction might sorbed to suspended solids phase and consequently is not analysed in the water samples.

b. Fluctation of the concentration during a day

For most compounds, measured concentrations keep quite low during the night and exhibit several peaks in the morning as well as in the afternoon, following different consumption and excretion patterns (Kummerer et al., 1998, 1999; Kummerer and Helmers, 2000; Joss et al., 2005; Duong et al., 2008). These discrepancies with respect to the corresponding daily average value confirm that analytical investigations on pharmaceutical compounds must be performed on 24 h composite water samples in order to measure average concentrations for the different compounds which would better represent the potential impact of the hospital wastewater (Verlicchi et al., 2010a).

Boillot et al., 2008 found that for of physicochemical, ecotoxicological and microbiological variations were correlated with hospital activities.

c. Sewage system

For assessing the sampling campaign the sewage system kind must be considered. If the sewerage is combined, the weather condition should be taken into account as rain weather may dilute the samples. With the newest sewage networks this problem is less marked.

d. Photodegradation processes during sampling and trasportation

In the investigation by Diwan et al., (2013) a possible reasons for fewer antibiotics detected in wastewater in summer could be intense sunlight and high temperatures, which could have caused more biodegradation and photodegradation.

e. Deconiugation of metabolites

There is some uncertainty when looking at the hospital contributions of pharmaceutical compounds for which deconjugation seems to occur; for these drugs effluent concentrations were higher than in the influent. When measuring only the parent compound it is assumed that no deconjugation occurs in the sewage system before reaching the treatment plant. In reality it is possible that deconjugation occurs throughout the wastewater system so measuring the compounds in their conjugated form would be necessary in order to confirm the load coming from hospitals compared with that from the public (Fatta-Kassinos et al. 2011).

It emerges that the most influencing factors already discussed according to the sensitivity analysis carried out cannot always completely explain the discrepancies which occurred in the case study.

Due to all these factor it is not surprising that a over or under estimation was observed. These variations may be compound specific

7.5.4 Advantagies of PEC and MEC

Often the advantage of one approach is the disadvantage of the other one, so it is suggested to use them both in a complementary manner. The use of PECs is recommended to reduce the cost of the sampling campaigns which are however necessary when greater precision is required. Predicted approach can be used with some confidence for substances where no analytical method exists to experimentally determine concentrations and loads or where the LOQ is not low enough (Ort, Michael G. Lawrence, Reungoat, et al. 2010).

Table 7.25: Comparison of strengths and weakness of measured and predicted approaches (adaped from Johnson et al., 2008)

	Strengths	Weakness
MEC	- measurement indifferent to source information issues (availability and quality) - may pick up unanticipated contaminants	- problem when concentrations are close to LOD -necessity of preliminary works -high analytical costs per sample - different practitioners using same method could get the different results - applicable only to small scale monitoring campaign - different analytical method required for each different chemical - fraction sorbed onto sample is not analyzed in water phase. -only give a here and now snapshot of the situation
PEC	- applicable when chemical is below LOD; - different practitioners using same model and same data get the same results -applicable to different catchments at local, regional or national scale - capable of simulating all possible flow scenario - capable of predicting concentrations for several different chemicals	- possibility to use different model - initial model setup require a lot of data -highly dependent to the quality information on source and excretion - difficult to be apply to diffuse source - do not anticipate other source - do not allow for serendipitous discoveries of other emerging contaminants

7.6 Load from hospital effluent

The average annual mass load in hospital effluent has been evaluate for both PEC and MEC.

For the first ones, the load of each PhC was estimated as follow:

$$L_i = \overline{c_i} * \overline{E_i} * Q$$

where $\overline{c_i}$ and $\overline{E_i}$ are respectively the mean consumption and excretion for the compound i and Q is the annual flow rate.

The load are derived from MEC multiplying them by Q.

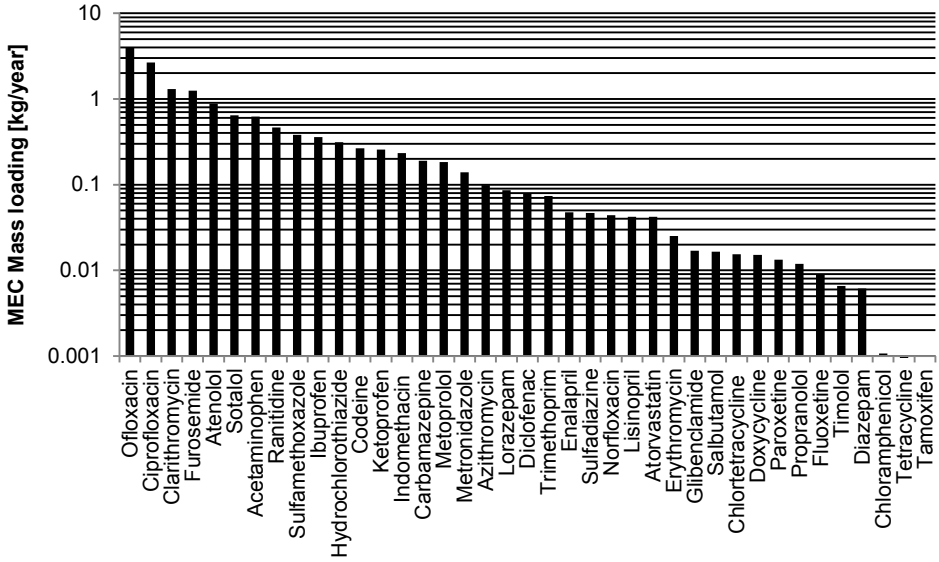


Fig. 7.23: Average annual mass loads evaluated from MEC values

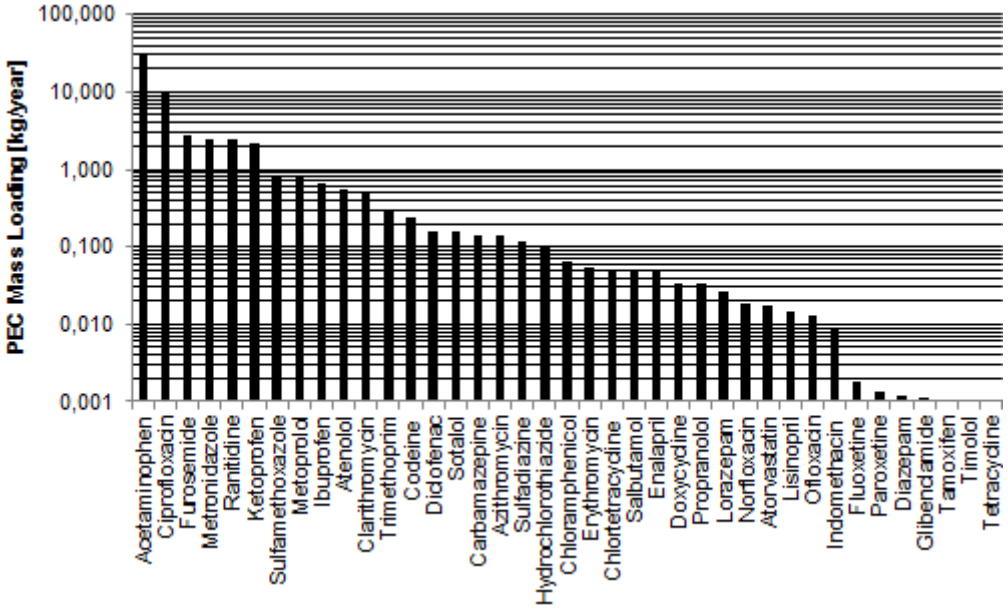


Fig. 7.24: Average annual mass loads evaluated from PEC values

Using PEC the load is higher than that obtained by using MEC. Also the ranking of the compounds change. Ofloxacin, for instance, is the first considering MEC but in in the lower position considering PEC.

Ciprofloxacin, furosemide and ranitidine are in the first position in both the figure. Tamoxifen, diazepam, tetracycline and timolol have a low mass low in hospital effluent in both the figures.

A rough comparison with the load observed in UWW reported referring to mg/(d*1000 inhabitants) by Verlicchi et al., (2012b), show that the compounds with the highest load are different. Only atenolol show high load in both the effluents.

Considering the load calculated by means of MEC and data of the relative catchment area reported in (Verlicchi, Al Aukidy, Galletti, et al. 2012) the highest contributions oh HWW to the WWTP influent were found for ofloxacin (67%), azithromycin (67%), clarithromycin (53%), ranitidine (52%) and metronidazole (45%). This confirms that antibiotics represent a critical class of compounds due to their high consumptions inside the hospital and their stability once excreted.

Le Corre et al., (2012) observed that for trimethoprim and roxithromycin the hospital contributions to the influent STP total load are 13 and 19 % respectively, while for all the other investigated compounds it is lower. (Ort, Michael G. Lawrence, Reungoat, et al. 2010) for the same compounds found a contribution of 18 and 56 % respectively.

Considering data about the WWTP receiving the HWW of the hospital here analysed reported in Verlicchi et al., (2012a) the contribution of trimethoprim in HWW with respect to total WWTP influent load is equal to 3,2% considering MEC.

Obviously for comparing the load derived from hospital with that derived from urban area, the bed density is very important. In In fact, Beier et al., 2011 found the highest hospital contribution of almost all compounds administered in a hospital that had an high value of bed density (33.5) indicating the importance of this parameter.

The study performed by Langford and Thomas (2009) shows that point source discharges from hospitals typically make a small contribution to the overall pharmaceutical load when compared with municipal areas. However, this varies from substance to substance and is not true when a drug's use is primarily hospital-based.

An in depth analysis was carried out by Santos et al., (2013) considering the most representative therapeutic classes. They observed that the daily mass loads of pharmaceuticals from urban wastewater would be greater than those from hospital effluents even that its concentrations were, in general, lower as WWTP influent as an higher flow rate. They considered four hospital connected to the same WWTP and found high contribution to the total load for analgesics (51 %) and antibiotics (41%). Escher et al., (2011) found that the amount of pharmaceuticals discharged into the WWTP from households totals to 62% of the total pharmaceutical load in the WWTP and the remaining 38% stems from the hospital.

NSAIDs, analgesics and antibiotics are amongst the groups with highest loads coming from hospitals, whereas antihypertensives, psychiatric drugs or lipid regulators do not have a very significant contribution (<10%), being most of the input of these kind of pharmaceuticals attributed to public wastewaters (Santos et al. 2013).

7.7 Risk assessment

Close attention must be paid to hospital wastewater as remarked by World Health Organization (WHO, 1999). Wastewater from hospital contains various potentially hazardous substances and precisely: antibiotics, genotoxic (citotoxic and antineoplastics agents) drugs and radioactive isotopes (Bottoni, Caroli, and Caracciolo 2010).

The article of Jean et al., (2012) present a method for selecting the PhCs discharged in hospital effluents that have the worst impact on the aquatic ecosystem, in particular due to their bioaccumulation potential.

RQ for HWW can thus help hospital manager to focus on priority compounds and to elaborate strategies to reduce their input into the urban network.

Although it is not realistic to calculate a risk for hospital effluents, because the exposure of living organisms is null in hospital sewers, risk quotients are useful tools to evaluate the potential risk of hospital effluents once they reach the aquatic environment Daouk et al., (2015).

European Medicines Agency (EMEA) proposes an environmental risk assessment procedure based on two phases: the estimation of exposure (phase I) and the environmental fate and effects analysis (phase II) (EMEA, 2006).

The European Medicines Agency's guideline also advises to include persistence, bioaccumulation, and toxicity (PBT) assessment in the phase I of risk assessment of pharmaceuticals. PBT properties that are not available for many compounds lead researchers to use model to predict them. For pharmaceuticals exceeding a log K_{ow} of 4.5 phase II is needed (EMEA, 2006). Phase II deals with the calculation of environmental risk as the ratio between exposure (PEC) and effects (PNEC).

EMEA (2006) recommended the use of chronic toxicity data for the calculation of PNEC, or, if no chronic data are available, acute toxicity data. PNEC value are still scarce and all the risk analysis depends on the exhaustiveness and the quality of the available data.

Mendoza et al., (2015) underlined the necessity to assess for PhCs both the environmental hazard by the PBT index and the environmental risk by the risk quotient according with the EU guidelines (EU, 2003). They found that some compounds (i.e paroxetine, indomethacin) have a low risk even if the hazard index is medium or high. Daouk et al., (2015) compared the two approaches (RQ and OPBT occurrence, persistence, bioaccumulation, and toxicity) finding that 45% of the compounds were among the 20 top priority of both methods. Some compounds have an high hazard risk not for high occurrence but for their high toxicity and bioaccumulation potential, as for sertraline.

When PNEC values are high, both the approaches still be accurate enough to provide valuable information (Johnson et al. 2008).

An alternative approach was proposed by Le Corre et al., (2012). They assessed the risk through a comparison of PEC with an effect threshold (ET) derived from guidelines for water recycling or estimated on the base of the acceptable daily intakes. The ratio between ET and PEC represents the margin of exposure (MOE). If MOE is > 100 the PhC is unlikely to present a risk to reach water supply and to affect human health. In this assessment they also considered the influence of the uncertainties but the comparison with this approach is not possible as the set of analysed compounds is different. Kümmerer and Henninger, (2003) conducted an approximate risk assessment comparing the PEC with the minimal inhibitory concentration ($MICs_{50}$)

Generally, those pharmaceuticals with a high consumption are selected for further investigation and risk assessment, which is reflected by many studies on these compounds. However, those pharmaceuticals are not necessarily the most relevant ones with respect to their environmental risk as present the analysis indicated by Escher et al., (2011) and by Orias and Perrodin, (2013) that proposed also a matrix approach for evaluating the impact of the whole HWW on the tested organisms. They stated that for risk assessment also excretion rate, biodegradability, bioaccumulation, ecotoxicity should be taken into account for choosing the compounds to be monitored. PEC combine consumption and excretion rate.

When the objective of the work is to assess the environmental risk a particular attention also to the sampling mode has to be paid because the maximum concentration should be measured and considered. In composite samples flow proportional this value might be underestimated if related to a low flow rate. For this aim a time proportional sample might be a better option.

In this evaluation only an evaluation of the RQ was carried out. Both MECs (maximum values) and PECs are considered and compared with PNEC.

Since it is impossible to make an exact mass balance of which pharmaceuticals are excreted where, the maximun excretion rate (E=100%) was assumed. It was assumed that all pharmaceuticals administered in the hospital would also be excreted there. Likewise, the pharmaceuticals brought in by patients was not considered.

As chronic data are less readily available PNEC values are referred to acute toxicity data and are reported from Verlicchi et al., 2012c. The variability of PNEC among all PhCs investigated is more than seven orders of magnitude (Escher et al. 2011).

Generally PNEC is derived from toxicological data by applying an extrapolation factor (EF) that decrease when more data are available (see Table 7.26).

Table 7.26: Extrapolation factor (EF) to derive PNEC from toxicological data by Orias and Perrodin (2013)

Available data	EF
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, daphnia and algae)	1000
One long term chronic data (either fish or invertebrate)	100
Two long term from species representing two trophic levels (fish and/or daphnia and/or algae)	50
Long-term NOECs from at least three species (fish and/or daphnia and/or algae and/or decomposers) representing three trophic levels	10
Statistics method (e.g. species sensitivity distribution)	5–1 (justify case by case)
Field data or model ecosystem	Reviewed case by case

Table 7.27: RQ level

RQ value	RQ level
<0.1	Low
0.1<RQ<1	Medium
RQ>1	High

7.7.1 Results of Risk assessment

The following figures report RQ calculated by using MEC and PEC.

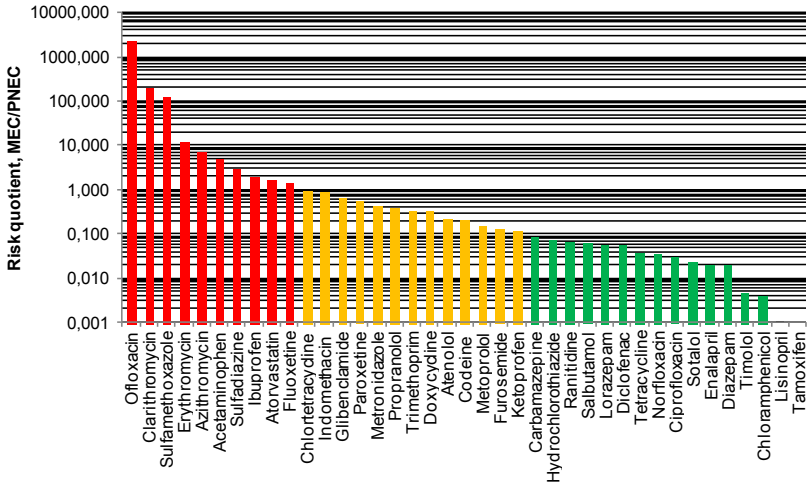


Fig. 7.25: Risk quotient obtained by MEC/PNEC

The result shown in Fig. 7.25 are in agreement with those reported in (Verlicchi, Al Aukidy, Galletti, et al. 2012). These analyses reveal that 9 substances (the two analgesics/anti-inflammatories acetaminophen and ibuprofen, the six antibiotics ofloxacin, clarithromycin, sulfamethoxazole, erythromycin, azithromycin and sulfadiazine, the lipid regulator atorvastatin and the psychiatric drug fluoxetine) pose a potential high ecotoxicological risk calculated by using MEC.

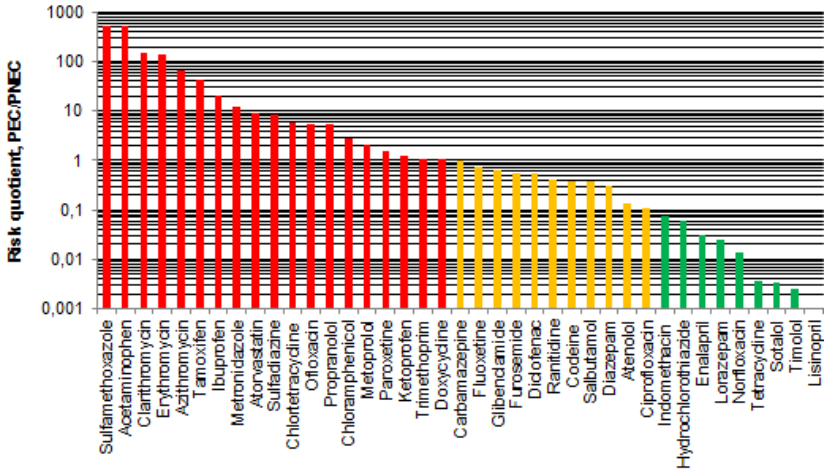


Fig. 7.26: Risk quotient obtained by PEC/PNEC

In Fig. 7.26 more compounds have an high risk as it was assumed that they are totally excreted and the maximum consumption along the two years was considered.

The case of tamoxifen should be considered with particular attention as it is usually excreted at home. This may explain why it shows a low risk if measured and high risk if predicted. Model of prevision do not consider this aspect in the model.

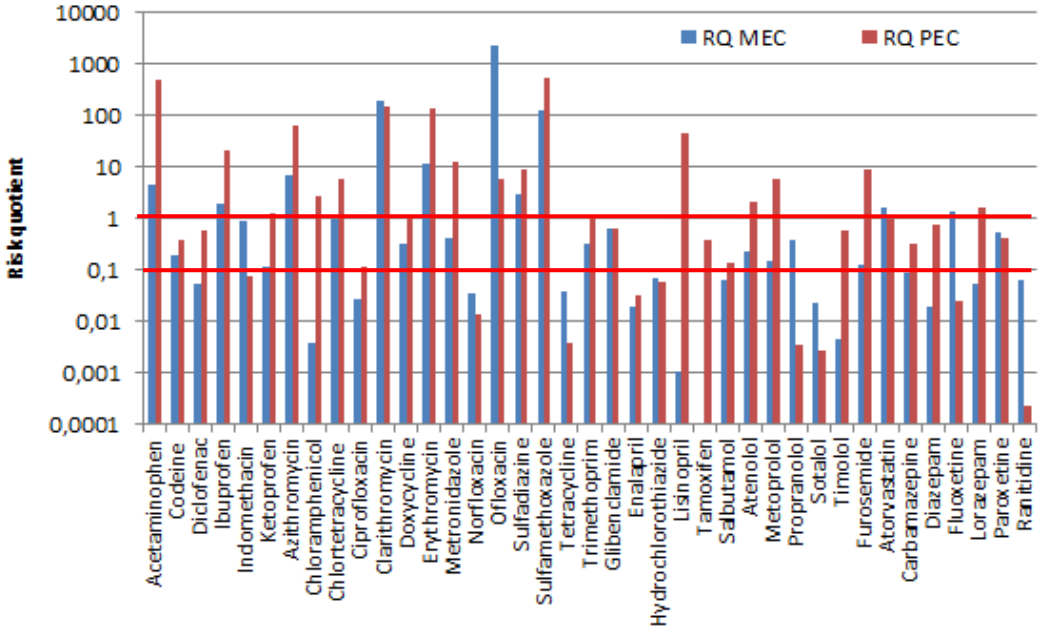


Fig. 7.27: Comparison between RQ obtained by MEC and PEC

As it is evident observing Fig. 7.27 PEC is usually greater than MEC with the exceptions of indomethacin, norfloxacin, ofloxacin, tetracycline, hydrochlorothiazide, lisinopril, atenolol, sotalol and timolol, fluoxetine and lorazepam. The causes might be searched between those listed in paragraph 7.5.3.

For comparison with literature data referred to HWW, the compounds showing high risk quotient are listed in Table 7.28 together with the corresponding references.

Analgesic/Anti-inflammatory and Psychiatric drugs

Codeine and diazepam were evaluated to be hazardous to aquatic life in the study by Mankes and Silver, (2013) as they have a high PBT index and toxicity (only for diazepam). Despite this they seem pose an "insignificant" risk based on the PEC/PNEC ratio. This is confirmed by this results. For this reason a PBT approach should be suggested for these compounds.

Diclofenac shows an high risk equally driven by exposure and effect (Escher et al. 2011). In that investigation diclofenac, ibuprofen , acetaminophen and carbamazepine showed the highest concentrations and the highest risk in both the hospitals investigated (general and psychiatric).

High risk was also found by Orias and Perrodin, (2013) for diclofenac and ibuprofen even if their relatively low EF. In this investigation only ibuprofen , acetaminophen and paroxetine have risk greater than one.

Antibiotics

Antibiotics belonging to several important groups were not readily biodegradable. Due to their antimicrobial properties and their role in the propagation of resistance, antibiotics remain one of the most hazardous pharmaceutical classes for the aquatic environment (Daouk et al., 2015). For this reason they have to be considered with particular attention.

Following the OPBT approach, Daouk et al., (2015) found that the hospital fraction of ciprofloxacin represent a risk to aquatic organisms.

The study by Kümmerer et al., (2000) found that ciprofloxacin, ofloxacin, metronidazole were not biodegraded and their genotoxicity was not eliminated.

Sorption for these groups could be more important than biodegradation in removing these compounds. Enrichment of sewage sludge may lead to the easily liberation into the environment of the absorbed compounds if the sludge is used in agriculture (Kümmerer and Henninger, 2003; Verlicchi et al., 2015).

In this study only some of these compounds seem to pose high risk but considering these other works also the others compounds should be taken in mind.

Others

Orias and Perrodin, (2013) found concentrations of ranitidine 100-fold higher than the corresponding PNEC, while in this work it does not seem pose high risk.

Even if this class is not considered in this work, among hormones, considering the maximum concentration they found RQ equal to 28750 for estradiol.

In particular, furosemide, carbamazepine as to be considered with particular attention as they have low removal efficiencies in WWTP. Even if they are present in HWW (furosemide in particular) they do not seem have high risk thanks for their eco-toxicological properties.

Table 7.28: compounds showing high risk quotient in different works

Compound	**Escher et al., (2011),**	**Daouk et al., (2015)**	**Kümmerer and Henninger, (2003)**	**Orias and Perrodin, (2013)**	**Santos et al., (2013)**	**(Verlicchi, Al Aukidy, Galletti, et al. 2012)**	**(Mendoza et al. 2015)**
Acetaminophen	x	x			x		x
Diclofenac	x			x			x
Ibuprofen	x	x		x			X
Azithromycin					x		
Ciprofloxacin			x			x	
Clarithromycin			x				x
Erythromycin			x			x	x
Metronidazole					x		
Ofloxacin			x			x	x
Sulfamethoxazole					x	x	
Carbamazepine	x						
Fluoxetine					x		

7.8 Conclusions and Final remarks

Observed differences between PEC and MEC varied among the selected compounds, confirming that both predicted and measured concentrations are plagued by different kind of uncertainty. To obtain annual measured data, monitoring campaigns would become even complex and expensive. With this view predicted value could be useful to estimate the PhC concentration in hospital effluent. However, as the consumption has been extrapolated from annual data, PEC values can only be considered theoretical values. Predictive models might include terms accounting "generation" mechanisms (mainly due to desorption of the PhC or reactions among its metabolites leading to the parent compound itself).

The question of whether predicted concentrations should be used at all the subject of a debate which has interested different researchers, as noted by Bound and Voulvoulis (2006), Castiglioni et al. (2004), Coetsier et al. (2009), Liebig et al. (2006) and Ort et al. (2009).

The huge discrepancies between MECs and PECs documented by our findings, as well as in other studies, are discouraging, indicating that calculation models still need considerable refinement to increase model reliability and discriminative power. A first analysis aiming to identify the main factors influencing PECs and MECs was carried out by means of an estimation of uncertainty for MECs and an assessment of sensitivity for PEC models. This showed that uncertainty in sampling is the greatest contributory factor in the first case, while in the second excretion factor is the most critical parameter followed by water consumption.

An accurate estimation of PEC could enable the authorities to evaluate the risk posed by hospital effluent in a catchment area as proposed in the framework described by Al Aukidy et al., (2014), but more experimental data are necessary to increase their reliability with particular attention to seasonal variations. As exposure assessment is the first step in environmental risk assessment, it is vital that PECs should not underestimate actual environmental concentrations, otherwise the environment will be put under considerable strain considering also that Diwan et al., (2013) remarked the presence of antibiotics in HWW may lead to a selection of resistant bacteria.

PEC is a useful tool to define classes of compounds to be monitored or to point out which compound form should be measured (Carballa et al., 2008), but it has strong limitations. It is indeed built upon several assumptions: the total consumption of the delivery data, the conservative mass transfer of substances during their transport in the urban wastewater network and surface waters (Daouk et al., 2015).

The approach adopted in this study can be easily transposed to any other hospitals, which have the will to look at the contamination of their effluents by API expecially when measured data are not available or are difficult to be obtained.

PEC is a suitable tool to determine discharges of PhCs over long period of time. In this fact, it is the approximate concentration range that is of interest and not the exact concentration at a certain time point or during a very short time period.

The choice of the approach should be done taking into account the aim.

Proposal

A proposal of analysis could be to put certain hospitals of different sizes and in different locations to a careful experimental investigation on the occurrence of the target compounds. These situations account for the situations to be referenced.

For other hospitals PECs of the target compounds could be estimated and compared with those of the hospital sample monitored for location and size closed to that of interest.

Evaluate discrepancies. To make environmental risk analysis or to take the management of hospital wastewater the maximum concentration between PEC and MEC for each compound can be taken into consideration.

For a further investigation in order to better assess PECs, it could be useful to create a questionnaire to be distributed to all wards for the cumulative and anonymous recording of the number and type of medication administered to the patients as done in the investigation of the No-Pills project.

Part C

Combined sewage overflows

Occurrence and Management

Chapter 8:

8 *Contribution of combined sewage overflow to the load of E.Coli and PhCs. The case study of Comacchio.*

Abstract

Many urban areas are drained by an unique sewage network that combines, during rain events, the urban WW with the runoff water. In case of intense or prolonged rain events, the sewer system can be unable to collect all the water flows to the WWTP or this one could be unable to treat all the flow. In these cases, part of the water flow is discharged in the receiving water body prior to reaching sewage treatment facilities by combining sewer overflow (CSO). CSO events impact the aquatic environment because the urban pollution is discharged without any treatment. The high amount of organic matter associated to the raw WW could cause oxygen depletion in the surface body. Moreover it is observed an increase of the concentrations of suspended solid, metal, micro-pollutants, pathogen and fecal micro-organisms (Passerat et al., 2011). Up to now, there are no study that quantify the contribution of CSOs in term of faecal indicator bacteria (FIB) and micropollutants in the studied area.

The aim of this study is studying the impact of CSO system in the Comacchio's area.

8.1 Introduction

In this chapter the quantification of the monthly summer load of some pharmaceuticals (PhCs) and bacteria indicators of fecal pollution (FIB), relative to both combined sewage overflows (CSOs) of an urban drainage and wastewater treatment plant (WWTPs) effluents, is presented. By this way, it is possible to assess the contribution of both types of discharge onto the quantity of the targeted substances in receiving surface water bodies.

The considered urban drainage system is about of the City of Comacchio. It is a very complex network, which uses numerous pump stations to remove the waste and rainwater from population centers, conveying it to the WWTP and/or, in the case of particularly intense rainfall events by means of CSOs, directly to the drainage channels which, finally, discharge into the Adriatic sea. This system will be described in detail in the next paragraphs.

It should also be noted that the estimated load of microorganisms released through CSOs in surface waters of the basin, is currently mainly used to compare the contribution of these discharges with the contribution of bacteria from the runoff of large farming areas, which are also drained by the channels that carry the water to the sea. The managing body of the sewerage system (CADF SpA) is above all interested in this assessment: coastline subject to these discharges is really interesting for tourism, due to the presence of bathing areas where it is very important to guarantee an adequate water quality or prevent bathing when some conditions are not respected.

A previous monitoring program underline that the presence of E.Coli in channel and sea water is evident. The same investigation underline that it is not only due to the input of the CSO and WWTP effluent in channel but it is also due to the input of water coming from the agricultural land.

The presence of PhCs in surface water is becoming an issue of growing international attention because of the possible effects on human health and the environment. Therefore, the quantification of these substances, with reference to all possible sources of emissions, is essential to understand what are the best strategies to adopt in order to manage pollution related to them.

In particular, the presence of drugs in surface waters around urban areas was highlighted by several researchers during the last decade (Bendz et al, 2005; Zuccato et al, 2006; Al Aukidy et al,

2012) since the usual wastewater treatments, typically activated sludge, are not able to completely eliminate at all these substances. Recently, numerous researches have studied different technical solutions to solve this problem by proposing different technologies to be considered during the design of new urban treatment plants but also to improve existing ones (Miege et al., 2009).

Until now, the current legislation does not impose mandatory limits under which it must maintain concentrations of these compounds but some recent assessments of environmental risk showed that the concentrations of some PhCs in aquatic environment may exceed the threshold concentration below which there are no negative effects (predicted no-effect concentrations, PNEC) (Kim et al. 2007).

In this context, scientists and engineers concluded that reducing the load of drugs deriving from urban areas is a matter of high priority (Ternes and Joss, 2006).

However, the effluent of the purifier is not the only way in which drugs reach the surface water. As already said, during rainfall events that cause exceeding the capacity of the treatment plant, mixtures of urban wastewater and stormwater are discharged into surface water bodies, by means CSOs inserted upstream of the WWTP, along the entire sewerage. This leads to an increase in receiving waters of the concentration of micro-pollutants, as well as other organic and inorganic substances and unwanted microorganisms (Phillips et al., 2012). These discharges are rarely taken into account, despite the fact that they can greatly contribute to aquatic contamination, which can pose a risk to living organisms (Phillips et al., 2012) and compromise, even only temporarily, the usability of water resources due to a deterioration of its quality.

As remarked in the work of Phillips et al. (2012), the dilution is the main factor that controls the concentrations of micro-pollutants in CSOs but a great dilution is also associated to an increasing of hydraulic load, resulting in reduced efficiency of the biological WWTP. In addition, it is noted that, while referring to the effluent of the WWTP the legal limits for microbiological pollution control are normally met, in all the operating conditions (dry weather and rain), for CSOs discharges limits are not imposed to the protection of human health and environment. Therefore, for these discharges none specific techniques to contain the spilled load of bacteria and other pollutants are applied, and the reduction of concentrations is achieved only thanks to the dilution capacity of rainwater and water courses receptors. In fact, concentrations of organic pollutants, inorganic and biological agents are the result of several phenomena that occur simultaneously: dilution of waste water by rainwater, the contribution of the internal drainage system by resuspension of sedimented material and external contribution of water runoff (Madoux-Humery et al., 2013).

For these reasons it would certainly be desirable to reduce the overflow events, to whom are not applied suitable treatments. However, it must be assessed case by case, the number of situations where such discharges occur, establishing their real impact on the contribution of macro and micro pollutants, even compared to all the other sources from which the undesirable compounds originate.

With this in mind then, we can state that in the literature there are studies that characterize the CSOs in terms of common physical and chemical parameters, organic matter, nutrients and total suspended solids (Chambers et al., 1997); while on the other hand no so much data available concerning the concentrations of microbiological contaminants (Madoux-Humery et al., 2013) and, as already said, of micro-pollutants to whom belong also pharmaceutical substances (Eriksson et

al., 2008, Chèvre et al., 2013; Rossi et al., 2009, Del Rio et al., 2013; Ryu et al. 2014). From these researches, however, it was shown that concentrations of FIB and PhCs measured in CSOs vary by several orders of magnitude, reflecting differences in duration and intensity of rain, the local characteristics of the drainage basin, sampling procedures (number and frequency of samples collected) and of the type of samples (grab against composite) (Ort et al., 2010 b).

It is also interesting to note that some studies have proposed PhCs (CAF, CBZ, ACE) as tracers of contamination in surface water due to the discharges of WW not or not sufficiently treated by WWTP and as indicators of untreated WW released through the CSOs. These studies have therefore shown a link also between presence of these PhCs and FIB (Madoux-Humery et al., 2013). However, further investigations are necessary to evaluate these relationships and to better characterize the fluctuations in the concentration of FIB and PhCs in CSOs.

All considered, this chapter wants to characterize the CSOs in terms of PhCs and microbiological pollutants, through an estimation of their actual loads spilled during overflow events that occurred in summer 2014 for the drainage system under consideration. It has been made a comparison with the loads spilled during the same events by the WWTP effluent. For this period, in fact, the managing body CADF Spa has made available rainfall data, cycles of operation of the pumps responsible for the overflow of water in excess, treated daily flows and concentrations of bacteria (E. coli) collected during a sampling campaign. With this data it was possible also to evaluate the variability of concentrations of FIB during overflow events.

The estimation of summer monthly loads of the selected PhCs was done creating a simplified model that is essentially based on the mass balance for the urban drainage system considered (based on the Substance Flow Analysis carried out by Chèvre et al., 2013 and 2011, Guignard, 2008). Moreover the estimation of loads of PhCs is also performed starting from the concentrations found in the literature, not having measured concentrations.

The estimated load of FIB is instead made from concentrations measured by CADF SpA. The model can then be refined by reducing or eliminating the uncertainties related to the various components that constitute it. In a second step, it will be possible also using a free software for modelling sewerage systems (SWMM agency US EPA), able to represent the hydrologic-hydraulic operation of drainage system even in unsteady conditions, and able to consider the trend of concentrations or loads of pollutants in the same sewer system.

Moreover, the same management authority has already conducted models of the hydraulic behavior of the network in question with the simulation software InfoWorks CS (IW), a commercial product of the company Wallingford Software. This can then be used for later comparison.

To validate the results for the selected PhCs it will be necessary a sampling campaign aimed to analyze the actual concentrations of the substances in CSO discharges.

8.2 The study area

The basin under consideration is that of the Municipality of Comacchio. It located in the north-east part of Italy in the province of Ferrara and has an area of 28478 ha. The coastal strip is a few meters above the sea level while most of the part of the inland area is under the sea level. For this reason, several pumping stations are necessary to allow the drainage of agricultural land and urban sewerage permitting water of reaching the sea. Many areas are occupied by stretch of fresh water and brackish marsh: the valleys of Comacchio belonging to the Park of River Po Delta. The portion of the coastal strip included within the City of Comacchio is swimming for a length of 21.17 km, from near the Lido di Volano, to about 300 meters north of the mouth of the channel Bellocchio, which is situated just south of Lido di Spina.

The human activities are mainly dominated by agriculture and tourism in the summer.

Maintaining the current hydraulic balance is hampered by the worsening of weather conditions, abasement of land (subsidence), from rising sea level, and the reduction of the absorption capacity soil due to expansion of impervious areas, due to the general and progressive urban development. Moreover, it is relevant the human impact due to agricultural intensification, causing eutrophication and spreading of synthetic chemicals compounds into the environment.

Fig. 8.1: Picture of Comacchio's Valleys

Fig. 8.2: Porto Garibaldi separated from the Lido of Estensi through the Canale Navigabile and in distance Comacchio.

The geographic area is described by means of regional mapping of Emilia-Romagna, with the topographic map in scale 1:250000 and numerous sheets, in raster format, at 1:5000 (Fig. **8.3**).

Fig. 8.3: The framework of union of the technical regional maps of the study area (1:5 000 each map)

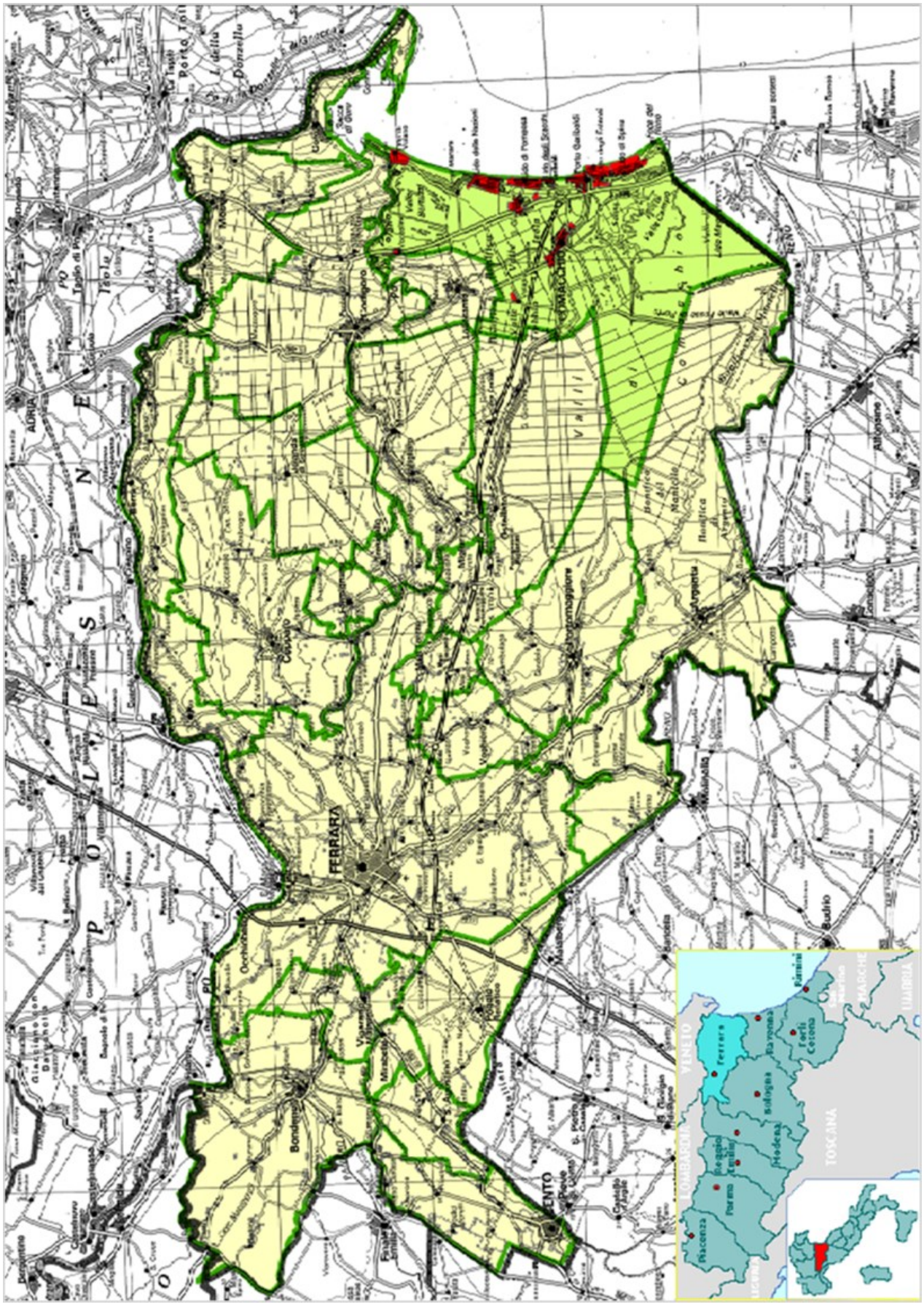


Fig. 8.4: Framing of the study area on the topographic map in scale 1: 250,000 of the region Emilia Romagna (display in scale 1: 310000 from ArcMap-ArcGis)

8.3 The sewage network

The area is drained principally by a unique sewer network. All the sewage network is shown in Fig. 8.5.

The main sewage, mainly combined, is long about 120 km and consists of two main parts that collects WW to the municipal WWTP:

- the first collect the urban (mixed) wastewater of Comacchio

- the second collects wastewater from the fractions located on the coast; this is in turn divided into three sublines connected by a the pump station “Torrino” from which a pipeline DN 1200 starts towards the treatment plant:

  - The first serving northen area of the beaches of Volano (only black water), Nazioni, Scacchi, Pomposa and the industrial zone of San Giuseppe; a series of lifting cascade revived water until Torrino station without any CSO. The network of northern part is 49 km long and collect the water of area of 600 ha. The main collector has a diameter of 1000-1600 mm ;
  - The second serves the town of Porto Garibaldi; this part is equipped with CSO into Canale Navigabile;
  - The third line serves the southern area of Spina and Estensi; a series of plants in cascade relaunch the water up to the Torrino station.

Fig. 8.5: The sewer network of the area of Comacchio visualized by Google Earth.

All pipes, including those of the main collector, compose a gravity sewer system, with the exceptions of those who send WW by pumping systems from S5, S6 and S7 to Torrino pumping station, of the conduct that connects Torrino to WWTP and of the conduct that from S14 leads waters (derived from Comacchio) to WWTP.

As already noted, the sewerage system in question is **almost unitary** for all its extension. Some areas, however, are drained by **separate** networks, which convey, in a distinct way, the rain water (white) to those discharged from urban utilities (black). Among that kind of area it is recognizable:

- The "Lido di Volano" area, from which only black flow rate is sent by means of pump S0 towards Comacchio's WWTP.
- Some areas of the "Lido delle Nazioni", in which the separation of the network is only partial and, in any case, both the kind of wastewater (black and white) merge in the main collector that is unique. By this way, the separation is made not effective in draining rainwater to an another address, different from that of the mixed network.
- The industrial zone of San Giuseppe, for which the white water are effectively removed directly to the drainage canal, and are therefore not conveyed to the WWTP.
- A portion of the urban area of civil San Giuseppe (about 25%), from which the rain water are separately conveyed to the drainage canals.
- The locations "La Fattoria" or "Parco del Sole", in which there is a separation of networks for rainwater and the water discharged from the houses, which, however, are attached to the same pump, called S, and therefore also in this area can be considered mixed.
- The "Lido di Spina", whose rainwater are drained separately from the black, although it appears to have been detected illegal connections of the latter in white net. Both network types come in a distinct manner to the pump station S8.

To overcome the exceeding capacity of the WWTP and of the drainage system in case of rain events, the excess flows are discharged, before reaching the treatment plant, by means of **combined sewage overflows** (S6, S8, S13, S14 and MD).

The location of all the overflows of the drainage system considered is shown in Fig. 8.6 and in greater detail in Figures from 8.7 to 8.14 in which it is possible to note the point of exit and the route accomplished by the discharged water. These images document also points where water samples for analysis of concentration of pollutants (FIB, conductivity, N-NH_3) have been collected.

Fig. 8.6: Satellite image of the study area, circled in red is the purifier.

One of these overflows (MD) is placed immediately upstream of the WWTP and is constituted by a special valve whose degree of opening is adjustable according to step 24, to each of which corresponds a different value of flow rate discharged. This is discharged into the near Canale Adige just upstream of the WWTP effluent. Together, they are conveyed to the scooping plant of Guagnino. This sent the water, through the downstream channel, into "Canale Navigabile" (see Fig. 8.7 and Fig. 8.8). The 24 steps and the corresponding flow rate values are indicated in the table below.

Fig. 8.7: Satellite image indicating the exit of the overflow upstream of the purifier MD

Fig. 8.8: MD (S dep) and WWTP effluent (Eff Dep) collected to the Guagnino plant through Canale Adige.

Table 8.1: flow discharged from overflow upstream of the WWTP as a function of the degree of valve opening

step	Q [m3/sec]		step	Q [m3/sec]		step	Q [m3/sec]
1	0,004		9	0,449		17	1,580
2	0,016		10	0,536		18	1,716
3	0,037		11	0,651		19	1,852
4	0,065		12	0,775		20	2,023
5	0,112		13	0,908		21	2,157
6	0,170		14	1,077		22	2,248
7	0,240		15	1,228		23	2,329
8	0,321		16	1,415		24	2,391

Some details about these combined sewer overflows are reported below.

Fig. 8.9: Details of locations of S13 and S14.

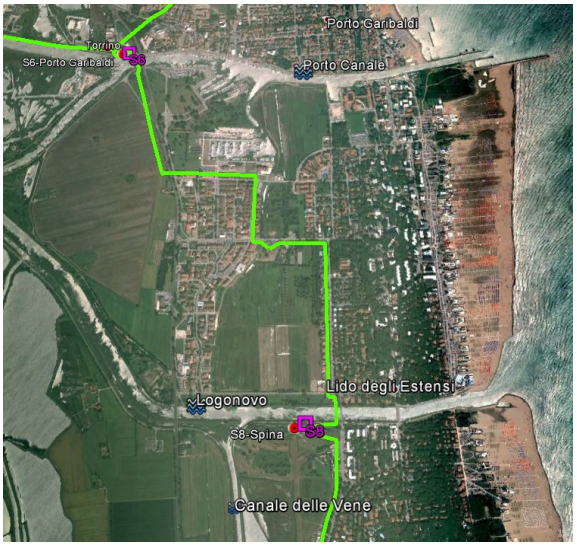


Fig. 8.10: Details of locations of S6 and S8.

S13 is located in the town of Comacchio at the San Pietro's bridge. It collects water from the city located in the northwest sector of the Canale Maggiore. It has two pumps for black sewage/mixed (flow rate of 20 l/s each) which, in dry weather, and whenever possible also in rain periods, carries the waste through the sewer downstream of Canale Maggiore, to the plant S14 in Via Marina; when rainfall is such as to cannot be disposed of by the two pumps, automatically and sequentially, two other electric pumps, with significantly higher flow characteristics (capacity 550 l/s each) come into operation and discharge wastewater directly into the Canale Navigabile.

S14 is located in Via Marina and collects sewage and rainwater from the rest of the town of Comacchio, with the exception of the district "Raibosola" that are conveyed to the WWTP through the plant in Viale Margherita. S14 has three pumps for sewage/mixed (capacity 100 l/sec each), that send their wastewater directly to the WWTP, and three more pumps, flow considerably larger than the previous (2 with capacity 650 l/s each and one with capacity 500 l/s) which drain mixtures of sewage and rainwater surplus directly into Canale Navigabile.
Moreover, other the 3 pumps (flow rate of 1300 l/s each) may intervene. They are used normally to the recirculation of water of the internal channels that, in case of rain, collected from the streets significant amounts of water; the delivery is always the Canale Navigabile.

Fig. 8.11: Satellite image indicating the exit point of CSO S14

S6 raises the black waters of dry weather and rainwater coming from the centre of Porto Garibaldi. It consists of three pumps for black WW/mixed (model Flygt C3152.181/452 HT) and four pumps responsible for the overflow of wastewater mixed that exceed the disposal capacity of the first. The overflow occurs in the Canale Navigabile, near the bridge of the SS 309 Romea, through the discharge line of the same pumps that, working in parallel, can raise highly variable flow rates (1 pump 405 l/s, 2 pumps 754 l/s, 3 pumps 1039 l/s, 4 pumps 1300 l/s).

Fig. 8.12: Satellite image indicating the exit point of CSO S6

S8 consists of two pumps (1 Flygt 3202/615, 1 Flygt 3202/619) that raise towards the WWTP the black WW, mixed in case of rain, derived from the urbanized area of the Lido di Spina. In this area the white waters are collected and conveyed to the pump station by a separate way, therefore, when the these rain flow rates of are such that it cannot be completely sent, together with the black, to WWTP, come into feature two other pumps (Flygt 3300 / 801portata 350 l / s each) that graze only the whitewaste water.

These waters are sent through a long pressure pipe that exceeds the Canale Logonovo, in the “relitto Canale delle Vene”, through which are then discharged into Canale Navigabile, near the Porto Canale that separates the Lido of Estensi from Porto Garibaldi. The discharge of the pressure pipe in the Canale delle Vene is via opening of a cofferdam or with a further lifting depending on the level present in the channel itself.

Fig. 8.13: Satellite image showing the exit S8 in Canale Navigabile

Fig. 8.14: Satellite image showing the exit S8 into Canale delle Vene

The following table shows the exact location of pumping stations, provided by CADF SpA

Table 8.2: Details of pump stations of the urban drainage system under consideration.

Pump station	***Site***	***Address***	***N° of pumps***	***Q max (l/s)***
S0	Lido di Volano	Via Lido Volano, 81		
S1	Lido delle Nazioni	Viale Nazioni Unite, 101	3	170
S2	Lido delle Nazioni	Via casa Garibaldi, 79	3	560
S3	Lido Pomposa	Via Alpi Orientali Sud	5	995
S4	San Giuseppe	Via Eraclea	4	112
S5	Porto Garibaldi	Via dei Mille 249	5	1110
S6	Porto Garibaldi	Via Provinciale, 16		
S7	Lido degli Estensi	Via D. Alighieri, 31		
S8	Lido di Spina	Via Boldini 8		
S9	Lido di Spina	Viale Raffaello, 78		
S10	Lido di Spina	Via Puccini, 2		
S 13	Comacchio	Via Spina, 1		
S 14	Comacchio	Via Marina, 13		
Parco del Sole	Lido degli Scacchi	Via Vega, 9	4	380
Botticelli	Lido di Spina	Via Botticelli		
Margherita	Comacchio	Via Margherita		
Capuccini	Comacchio	Quartiere San Francesco		
Torrino	Porto Garibaldi	Via Marina (ponte Romea)		

The network geometry (plano-elevation of pipes and wells connecting, size and shape of the sections of pipelines, materials) and the characteristics of pump stations (number and type of pumps, quotas and size of the rooms that house the pumps) were provided by CADF through a series of shape files, characterized by descriptive tables, and files in DWG format, viewable through programs ArcGIS ArcMap and AutoCAD, respectively. With such software it has been possible to overlay the route of the drainage system under consideration to the maps relating to the area of the study, both displayed in the correct coordinate system, that is geo-referenced.

Moreover, CADF has provided, for the summer 2014, rainfall data (recorded in some measuring stations located at the pump stations), data on and off-course, and data on flow rate discharged by overflow pumps, data of daily flows treated by WWTP.

They were also provided data on the evolution of tides, which greatly affect the variability of flow and water quality of both the drainage canals that discharge into sea and the groundwater table. Then the infiltration of ground water into the sewer system, in the dry period, are not negligible compared to the flow circulating in the network itself, as noted by the management.

As regards the characteristics of the drainage system, therefore, the information used in this work are represented in the diagram in Fig. 8.13.

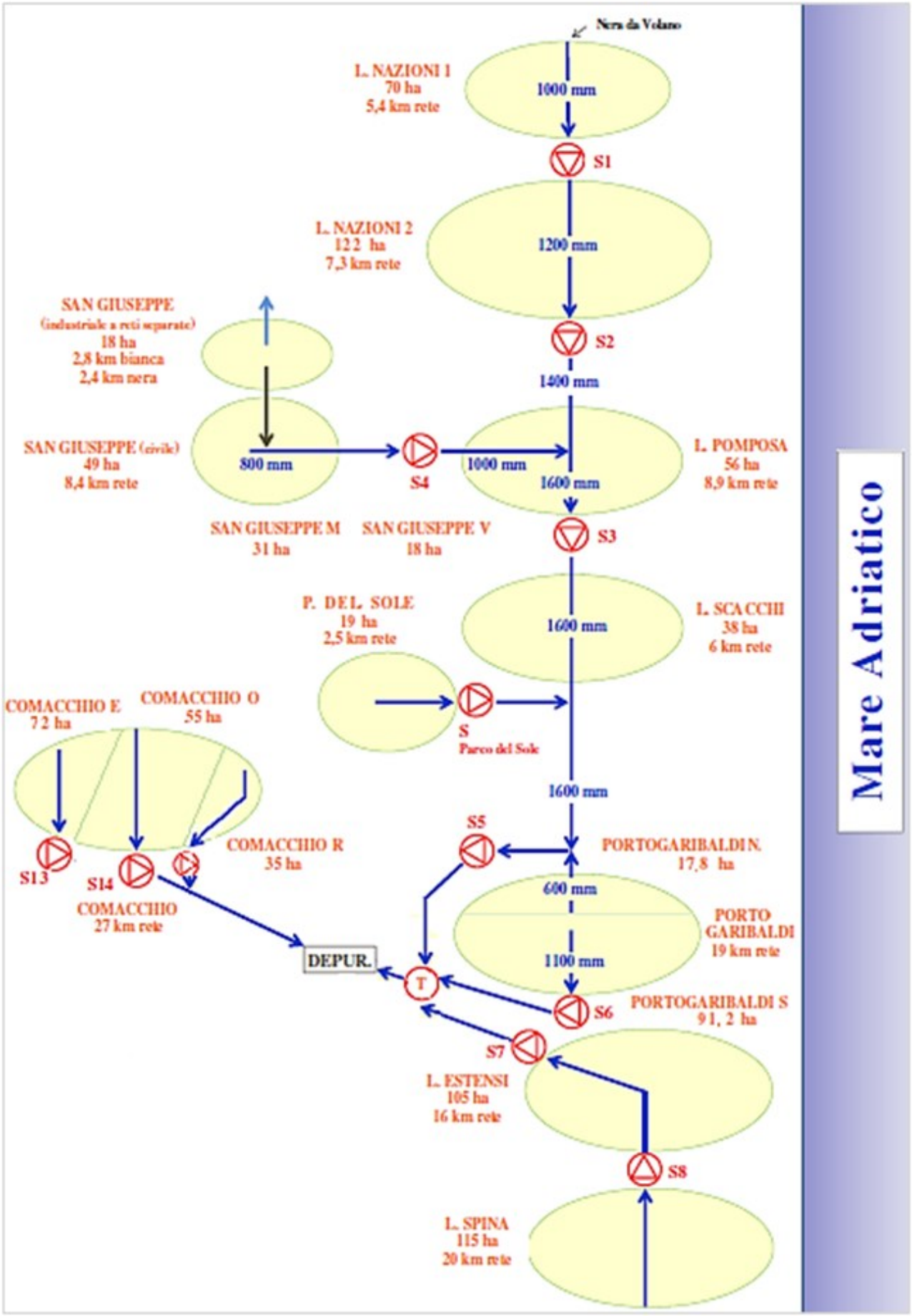


Fig. 8.15: Diagram of the urban drainage system of Comacchio.

This scheme has been drawn, from that provided by CADF, integrating the data provided by the same manager with direct measurements on the cartography in digital format (CTR 1: 5000), on which has been just loaded the route of the network, and other information found in the technical report of the recent "Studio del reticolo fognario del comparto nord dei Lidi di Comacchio" commissioned by CADF SpA to the company HR Wallingford in 2007. This study was also developed within the thesis of two students of the Engineering Course of the University of Ferrara.

From the figure, it can be underlined:

• The three main areas, or sectors, served by the sewerage system and previously described (Comacchio capoluogo, Lidi Nord, Lidi Sud).

• The only route of the main collector with an indication of the main pump stations link to the urban centers, or portions, that belong to the three main areas;

• The number of km of the pipes and their respective diameters are indicated.

• The total surface of the urban areas belonging to each pump station system and the various overflows. In fact, these surfaces are major sub-basins from which the wastewater (black and rain) are sent to the WWTP from different locations within the entire urban basin considered.

The percentages of impervious surfaces (IMP) over the total width of each area were estimated in different ways. Those of sub-basins in the north sector (Lidi Nord) were found in the "Studio del reticolo fognario del comparto nord dei Lidi di Comacchio", taking into account that in the years following the study the urbanized area may be increased. Not having data available for other areas, for the towns of Lidi Sud impermeable percentages similar to those of Lidi Nord were attributed, with higher values for Porto Garibaldi because most built. On the basis of this consideration also for Comacchio it has been estimated an higher value of impervious area.

The evaluation of the urbanized area was performed using cartography as well as satellite photos.

Of course, having the percentage of impermeable area, permeable areas were calculated as PERM = 1 - IMP.

The types of impervious surface (roofs and roads) and permeable (green areas undrained and drained) were characterized by specific coefficients (ψ_{IMP} and ψ_{PERM}) that describe the inflow contribution to the sewage system. In fact, to define the rate of rain water that actually enters the network, it is considered the hydrological methodology of the coefficient of influx ψ, which serves to distribute the rain input flow rate to the basin in the component that gives surface runoff and in one that, instead, infiltrates and is lost through evapotranspiration. Again, the coefficients of influx specific for each type of surface (ψ_{IMP} and ψ_{PERM}) were found by " Studio del reticolo fognario del comparto nord dei Lidi di Comacchio".

The values considered are reported in Table 8.3. From these it was derived an average value for both the impervious surfaces that permeable (ψ_{mIMP} and ψ_{mPERM}), which is reflected with the values reported in the literature.

So, it was possible to apply the formula for the calculation of the coefficient of influx global ψ for urban areas, by using the following equation:

$$\psi = \psi_{mIMP} \cdot IMP + \psi_{mPERM} \cdot (1 - IMP) \qquad \text{(eq. 8.1)}$$

The coefficient of influx was evaluated for each of the sub-basins identified within the overall urban basin. The respective values, with the indication of areas waterproof and permeable and the respective percentages, are given in Table 8.3.

Table 8.3: Values of the coefficients of inflow specific for each type of surface present in the sub-basins urbanized

Type of surface	ψ_{IMP}	ψ_{PERM}	ψ_{mIMP}	ψ_{mPERM}
Well drained road	0.85	-	0.0725	-
Not well drained road	0.65	-		
Connected roofs	0.80	-		
Not connected roofs	0.60	-		
Undrained green	-	0.05	-	0.075
Drained green	-	0.1		

From Table 8.4 it is shows that for the Lido di Volano only black flow rate are sent to the WWTP.

The surfaces of the industrial area of San Giuseppe (San Giuseppe I) are defined but it sends the rain water directly into drainage canals with a separate network. The same applies to the 25% of flow of rain fallen on the town of San Giuseppe upstream of pump station S4 (San Giuseppe M).

Table 8.4: Values of areas (overall and impervious) as well as the percentage of permeable and impermeable areas and the values of the coefficient of the rain net inflow for each of the urbanized sub-basins.

Urban sub-basin	Pump station	Areas [ha]		% Areas [%]		Ψ
		Tot	Imp	IMP	PERM	[-]
Volano	S0	-	-	-	-	-
Nazioni 1	S1	70	27.8	40	60	0.33
Nazioni 2	S2	122	48.2	39	61	0.33
San Giuseppe I	S4	18	10.1	56	44	0.44
San Giuseppe M	S4	30.9	11.3	37	63	0.31
San Giuseppe V	S3	18.3	5.6	31	69	0.27
Pomposa	S3	56	20.9	37	63	0.32
Parco del Sole	S	19.4	13.9	37	63	0.31
Scacchi	S5	38	6.2	32	68	0.28
Porto Garibaldi N	S5	17.8	7.5	42	58	0.35
Porto Garibaldi S	S6	91.2	54.7	60	40	0.47
Spina	S8	115	43.0	37	63	0.32
Estensi	S7	105	39.2	37	63	0.32
Comacchio O	S13	72	39.6	55	45	0.43
Comacchio E	S14	55	33.0	60	40	0.47
Comacchio R	Margherita	35	19.3	55	45	0.43

Finally, by multiplying the net inflow coefficient ψ for the total area of the corresponding sub-urban you get the value of the area contributing to the influx of water in the network.

8.4 Wastewater treatment plant

The WWTP of Comacchio (population equivalent 180 000) is located in the eastern part of Comacchio centre, along via Valle Molino, and discharge its effluent in Canale Navigabile as shown in Fig. 8.16, Fig. 8.17.

Fig. 8.16: View of Comacchio and its WWTP

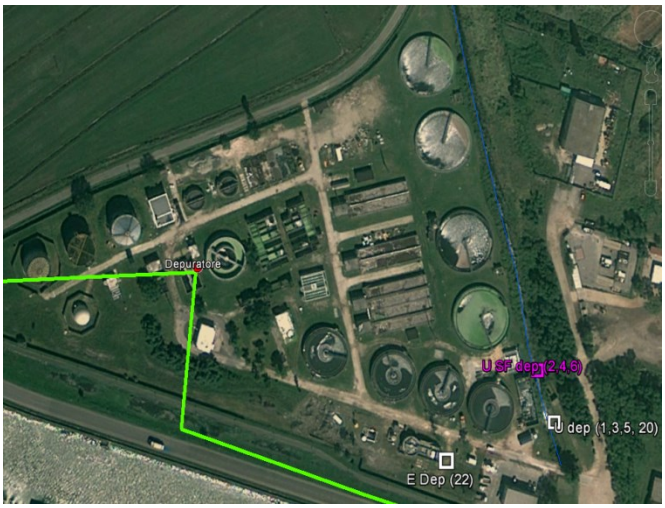


Fig. 8.17: View of the Comacchio WWTP, displaying the entry point (E Dep), the exit point into the canal Adige (U dep) and the upstream WWTP overflow (U SF dep).

The plant treats the wastewater of Comacchio and of the seven seashore: Spina, Estensi, Porto Garibaldi, Scacchi, Pomposa, Nazioni and Volano.

It was built in two phases (1987-2005) and now it consists of primary treatment, activated sludge for carbon removal, denitrification and phoshorus removal and disinfection.

The plant of Comacchio has four lines each of which biological organized into two sub-lines. Originally, each sub-line performs the removal combined nitrogen and carbon through denitrification tank, equipped with a mixer submerged, followed by an oxidation reactor.

From 2010 the biological process that was adopted for the transformation of the existing process is the alternate cycles in a single reactor (AC).

The process ensures both the nitrogen and carbon biological removal and in part also of the phosphorus via a succession of aerobic (for the oxidation of carbon and the nitrification of nitrogen) and anoxic (for denitrification of nitrogen) steps that are realized by a temporal succession in a single basin. By this way there are no dedicated sections, anoxic pre-denitrification and aerobic nitrification, in predefined volumes, nor there is the need to operate the recirculation of the aerated mixture. This entails a considerable simplicity in the creation, saving piping and electromechanical performance.

The energy savings are a immediate consequence of the high performance of biological nitrogen removal, as high denitrification means high recovery of oxygen combined.

To ensure control of the process an automatic control device has been installed.

The final effluent has a concentration of total nitrogen below 10 mg/L. The energetic consumption in March 2011 was dropped below 100 MWh.

Moreover, the sludge products from waters lines are pre-thickened then anaerobically digested under mesophilic and finally post thickened and dewatered mechanically.

The floor plan is reported in Fig. 8.18.

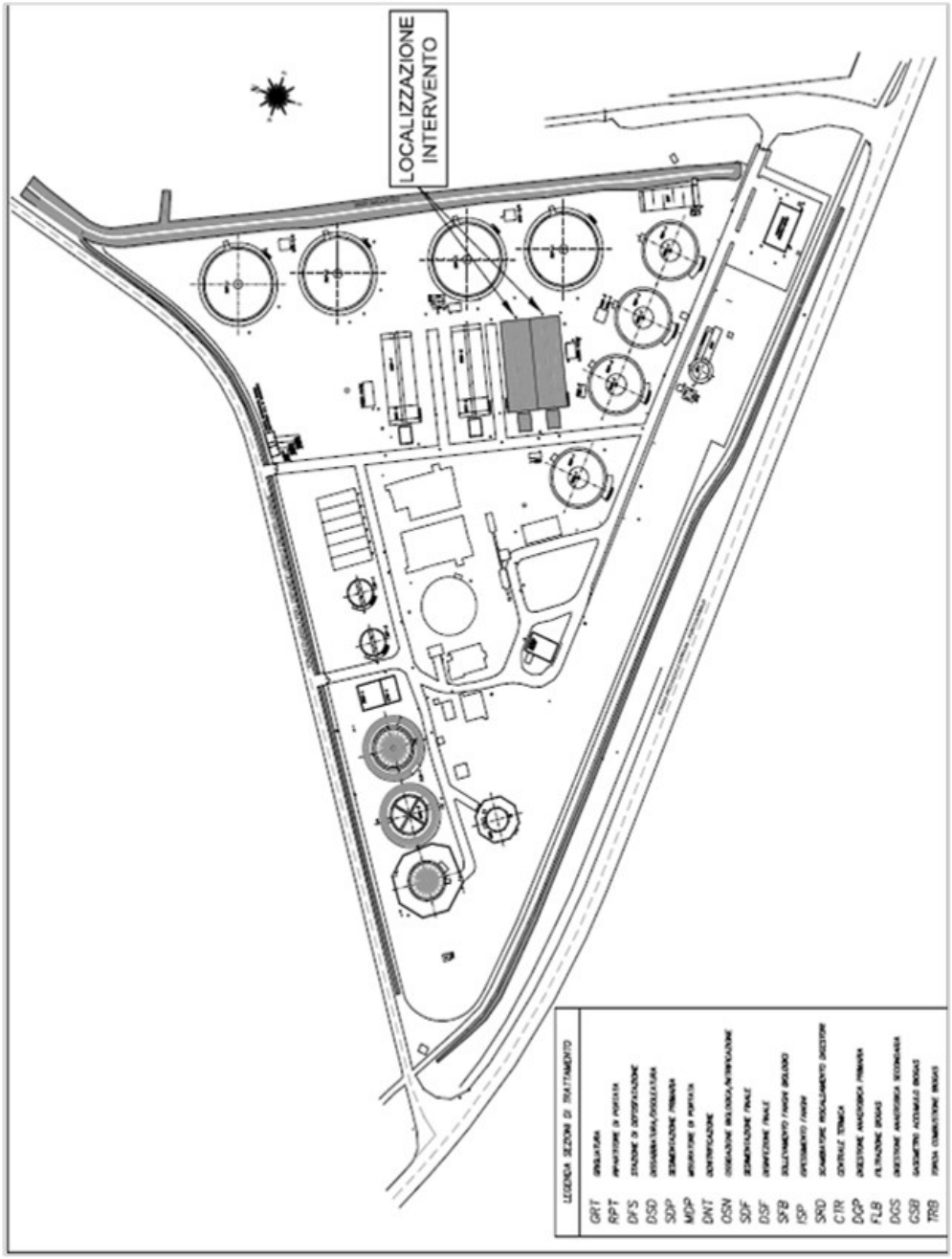


Fig. 8.18: Floor plan of the Comacchio WWTP

The plant treats 6.3 milion of m^3/year with daily flow rate maximum in summer and equal to 31000 m^3/d.

<u>The plant is subject to large load fluctuations during the summer tourist season:</u> this presence is particularly high in the month of August and almost every weekend from June to September. In the summer time, being the greater load due to the presence of tourists, the load is particularly high in nitrogen. In winter the served population drops to about 25 000 units.

The following tables provide design flow and influent concentration data of the most common parameters, considering 180000 e.i. (C.A.D.F., 2012).

	Impianto completo		Singola linea
Potenzialità nominale	AE	180000	45000
dotazione idrica sversata in rete	l/AE d	173	173
Voce	**u.m.**	**Valore**	**Valore**
Carichi idraulici			
Portata media giornaliera	m3/d	31.104	7776
Portata media oraria	m3/h	1296	324
Portata di punta secca	m3/h	1692	423
coefficiente di punta secca		1,31	1,31
Portata massima al biologico (1,7 Qmn)	m3/h	2174	544
Portata massima oraria	m3/h	3600	900
coefficiente di pioggia		2.8	2.8

Fig. 8.19: Design flow values.

Concentrazioni	**u.m.**	**Valore**	**Valore**
BOD5	mg/l	200	200
COD	mg/l	410	410
TSS	mg/l	200	200
TKN	mg/l	55	55
PTOT	mg/l	6	6
Carichi di massa	**u.m.**	**Valore**	**Valore**
BOD5	Kg/d	6.200	1.550
COD	Kg/d	12.710	3.178
TSS	Kg/d	7.500	1.875
TKN	Kg/d	1.705	426
PTOT	Kg/d	186	47

Fig. 8.20: Influent concentrations and mass load.

During rainstorn events the WWTP capacity could be exceded and in this case the CSO upstream the plant discharge the excess in Canale Navigabile.

From the end of the spring 2013, the opening of the valve the controls the upstream overflow at the entrance of the WWTP (MD) is controlled automatically by the remote control system that activates the opening for subsequent steps (24 to full opening) so as to limit the flow rate discharged by the overflow feeding the WWTP with 500 l/s, maximum treatable with four lines. The maximum discharged flow rate is reached by 17 steps corresponding to the maximum flow rate liftable by Porto Garibaldi and Lido of Este pumping stations.

As shown in Fig. 8.21 and in Table 8.5 the mean flow rate treated in tourist season is major than that treated in the rest of the year, while the maximum are substantially equal (±20%).

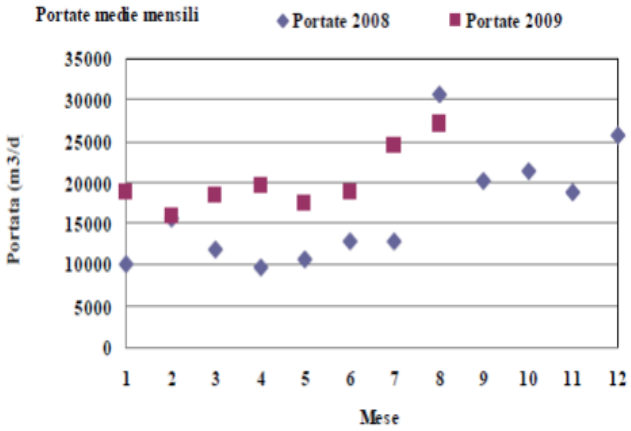


Fig. 8.21: Trend of daily treated flows rate - 2009

Table 8.5: Seasonal flow rate -2008

	Alta stagione	Bassa stagione
	m3/d	m3/d
Qmn	21207	
s.d.	8936	
Q min	12885	
Qmax	30652	
Qmn		15178
s.d.		5620
Q min		9692
Qmax		25613

8.4.1 Influent mass load

The load of macro pollutants are extremely variable. This aspect is not very evident observing data of COD, TSS e BOD_5 during 2008-2009 as there was istantaneous discharges with an elevate concentration of that parameters in October-November 2008 and in June-July 2009. The load that define the e.i. as those related to nitrogen even if this was measured as $N\text{-}NH_4$ and then transformed in N_{tot}. On this basis the e.i. related to the two seasons that characterize the system can be estimated: Summer (June-September) the remaining months (Table 8.7).

COD and N_{tot} are considered, but N_{tot} is assumed more significative as suspended solids may settle in networks of high extension and reduce the COD of the WW incoming the WWTP. Therefore, in summer (August 2008) it has a potential maximum of 184000 p.e. while in the remaining months the mean potential is of 30000 – 38000 p.e.

Table 8.6: Influent mass load during 2008-2009.

Anno	Mese	Portata	LCOD	LBOD5	LTSS	LN-NH4	LNtot	LPtot
		m3/d	Kg/d	Kg/d	Kg/d	Kg/d	Kg/d	Kg/d
2008	Gennaio	10049	1447	613	648	192	260	25
	Febbraio	15651	1753	830	1268	83	112	23
	Marzo	11501	3462	1668	1208	269	363	28
	Aprile	9692	1522	753	485	183	247	18
	Maggio	10693	2828	1283	1230	283	382	30
	Giugno	13315	3162	1531	1691	435	587	53
	Luglio	16773	3531	1879	4646	321	433	61
	Agosto	30652	15939	6146	8000	1635	2208	216
	Settembre	20753	6340	3808	1868	725	978	76
	Ottobre	21329	6015	9673	2837	524	708	206
	Novembre	19429	4838	1447	1574	422	569	101
	Dicembre	25613	2113	1178	4098	172	232	42
2009	Gennaio	18838	3023	1498	1733	996	1345	42
	Febbraio	15885	2121	1247	3392	50	67	19
	Marzo	18327	2337	1301	1998	296	400	37
	Aprile	19575	2594	1556	2838	195	263	32
	Maggio	17332	2227	1257	1317	170	229	36
	Giugno	18774	2279	1367	1085	199	268	215
	Luglio	24439	7438	4463	3542	648	875	198
	Agosto	27085						

Table 8.7: Statistic data of the mass load treated during 2008-2009.

	LCOD	LBOD5	LPtot	LNtot	LTSS	AE COD	AE Ntot
	Kg/d	Kg/d	Kg/d	Kg/d	Kg/d		
2008 non estate							
Media	3036	2052	59	364	1521	25301	30370
Min	1384	613	18	112	485	11532	9367
Max	6590	8527	182	775	2684	54920	64612
2008 estate							
Media	7243	3341	101	1052	4051	60359	87640
Min	3162	1531	53	433	1691	26352	36105
Max	15939	6146	216	2208	8000	132825	183988
2009 non estate							
Media	2460	1372	33	461	2256	20503	38414
Min	2121	1247	19	67	1317	17672	5602
Max	3023	1556	42	1345	3392	25195	112083
2009 estate							
Media	4858	2915	206	572	2313	40486	47630
Min	2279	1367	198	268	1085	18989	22341
Max	7438	4463	215	875	3542	61982	72920

8.4.2 Macro pollutants influent concentrations

The monthly mean values of the concentrations of the main macro pollutants (Table **8.8**) are highly variable with a clear predominance of low concentrations, the influent $N\text{-}NO_x$ are extremely low and are not reported.

Table 8.8: Macro-pollutants influent concentrations

anno	Mese	Portata	pH	COD	BOD5	TSS	N-NH4	Ntot*	Ptot	Cloruri
		m3/d		mgO2/l	mg/l	mg/l	mgN-NH4/l	mgN/l	mg/l	mg/l
2008	gen-08	10049	7,5	144	61	65	19,1	25,8	2,50	1446
	feb-08	15651	7,4	112	53	81	5,3	7,2	1,50	1160
	mar-08	11501	7,6	301	145	105	23,4	31,6	2,40	1342
	apr-08	9692	7,4	157	78	50	18,9	25,5	1,87	1347
	mag-08	10693	7,5	265	120	115	26,4	35,7	2,80	777
	giu-08	13315	7,3	238	115	127	32,7	44,1	4,00	1068
	lug-08	30652	8,1	211	112	277	19,1	25,8	3,65	1980
	ago-08	20084	7,5	520	201	261	53,4	72,0	7,04	117
	set-08	22040	7,7	306	184	90	34,9	47,1	3,65	2353
	ott-08*	18802	8,1	282	169	133	24,6	33,2	9,67	2510
	nov-08*	26467	7,7	249	149	81	21,7	29,3	5,20	4896
	dic-08	16773	7,7	83	46	160	6,7	9,1	1,65	3783
2009	Gennaio	18838	8,0	161	80	92	53	71,4	2	4687
	Febbraio	15885	8,0	134	79	214	3	4,2	1	2794
	Marzo	18327	7,9	128	71	109	16	21,8	2	2025
	Aprile	19575	7,9	133	80	145	10	13,4	2	2540
	Maggio	17332	7,4	129	73	76	10	13,2	2	1981
	Giugno*	18774	7,5	121	73	58	11	14,3	11	9729
	Luglio*	24439	7,3	304	183	145	27	35,8	8	4313

A more detailed assessment allows to observe the following:

- The reference COD/TSS ratio value is 1.7. In the influent that is generally higher than the reference value as the presence of suspended solids is limited. This means that there is a significant sedimentation in the network;

- The characteristic BOD_5/COD ratio is essentially in line with the expected value (0.5-0.6);

- The characteristic COD/N_{tot} ratio, calculated assuming a N_{tot}/N-NH_4 ratio of 1.35, has an average value of 10-11 in the period of non-summer that drops to 7 in the summer period; point values in the report presents strong fluctuations, which means that there could be difficulties in obtaining consistent removal of nitrogen also considering the fact that the influent is subject to primary sedimentation, or to a further decline in carbon that induces a further reduction of the ratio considered.

- The influent has always high concentrations of chlorides (on average 1400-3000 mg/L with peaks at 9000 mg/L); that means that there is a continuous presence of sea water; assuming a concentration of about 35000 mg/L of chlorides in the latter, the average concentration observed in the influent is due to about 4-9% of sea waters (thus 1000 - 2000 m^3/day).

Sea water infiltration is a problem of no small importance because the presence of chloride reduces the reaction kinetics up to cancel them to 6000 mg/L of Cl. Moreover the sudden change of salinity involves alteration of the kinetic behavior of biomass.

8.4.3 Legal requirements

CADF S.p.A. CADF S.p.A. discharges in water bodies has to respect the legal limits posed by Legislative Decree 152/2006. The limit of E.Coli is 5.000 UFC/100 ml.

8.4.4 The energy consumption

The energy consumption in 2008 amounted to 1,600 MWh and do not include consumption for the lifting of the influent. Monthly consumption ranging from 100 to 200 MWh depending on the season: July and August are the months of higher consumption. It is possible to observe that:

- specific consumption per m^3 of treated water are quite low and varies from 0.19 to 0.39 kWh/m^3. The change is largely due to the treated flow rate in the plant;

- it may be noted that specific consumption (Wh/p.e. d) values are very low when the system works at the project data (August 180000 p.e., 40 Wh/p.e. d) - low values up to 40000 p.e. treated (70-90 Wh /p.e. d)- and very high values at lower potential. This means that the system works with a number of lines too high compared to the needs.

8.5 Pollutants under considerations

The substances chosen for the analysis of the loads spilled from the overflow of the sewage system and from the WWTP are one type of bacteria indicators of fecal pollution (FIB), Escherichia coli (E. coli), three pharmaceutical compounds with different characteristics of use: carbamazepine (CBZ), acetaminophen (ACE) and caffeine (CAF).

8.5.1 E.Coli

Fecal pollution of water is a major concern to water quality and human health because it increases the risk of gastroenterites. Agricultural runoff, urban storm water, and streams as well as point sources such as overflows from wastewater treatment plants (WWTPs) have been linked to increases in microbial loads to natural water bodies (McLellan, 2004).The fecal contamination can be estimated by analysing fecal indicator bacteria (FIB), as the search of all types of pathogens is not feasible: pathogens are in the environment with very low densities which can still exert a pathogenic effect and often have an aggregate distribution that makes it difficult to sample. In addition, by an operational point of view, are difficult to put in culture and to monitor all possible pathogens, it is required a number of tests extremely high, with considerable costs, also in relation to the technical expertise required.

Eschericchia Coli are considered the best indicator for predicting the risk associated to the biological contamination by wastewater of the receiving water bodies even if their presence is not always related to the presence of pathogens.

E. coli are faecal coliforms and have a good correlation found between their presence and the various pathologies resulting from exposure to contaminated water. Even faecal streptococci represent a good tracer of fecal pollution because they generally do not reproduce in the environment and their presence tends to become extinct rapidly in the absence of a continuous source of this type of pollution. However, the FIB does not identify the source of humal faecal contamination as a great variety of warm-blooded animals has FIB in their feces. Moreover, although the source of FIB is taken as fecal, scientific evidence based on genetic approaches have pointed out the possible evolution of these populations in various specific environments.

They can be analysed in different way. Plate counts were expressed as colony-forming units (CFU) per 100 mL sample or as Most probable number (MPN).

According to what described, it is noted, however, that in the raw sewage from urban sewage systems, the presence of the FIB is naturally high, with concentrations of E. coli in the order of 10^6-10^7 and even 10^8 MPN/100/ml, in function of the dilution of such waste water.

One very important source of FIB for surface water bodies, over discharges of untreated urban drainage systems, is undoubtedly made up of the runoff of agricultural land and of those used for breeding. This view is confirmed in numerous studies, also the subject of the thesis by students of the Faculty of Engineering of the University of Ferrara, who evaluated how the activities of application of manure and sludge on agricultural land and livestock farming activities can have a significant impact on the presence of bacteria and PhCs in surface water bodies, where carried by runoff water during rainy periods (Jeng et al., 2005). The contribution of these FIB runoff is likely to maintain concentrations in surface water bodies very high throughout the year (see Fig. 8.22).

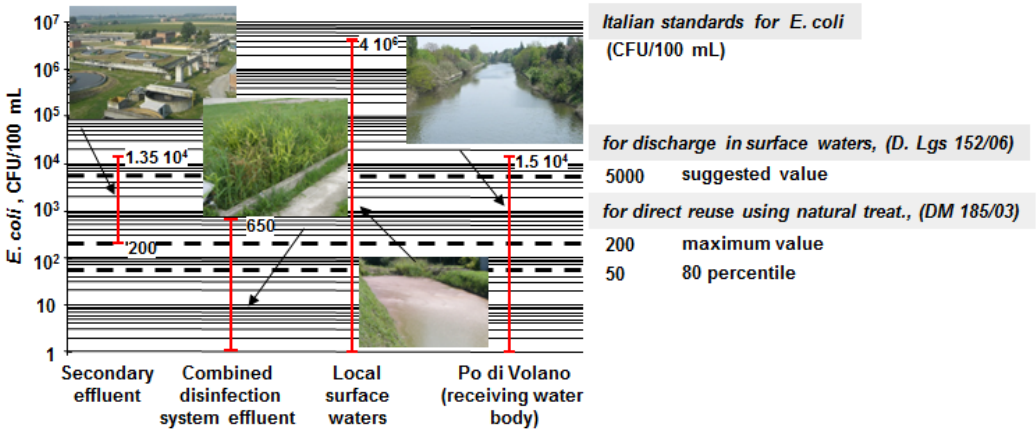


Fig. 8.22: Microbiological content in different samples (secondary and tertiary effluents, local surface waters in Ferrara) together with Italian legal limits. (Verlicchi et al., 2009)

The U.S. Environmental Protection Agency (USEPA) has proposed maximum levels for fecal bacteria in surface water for single sampling events (USEPA, 2000). FIB concentrations for the recreational water quality criterion are 235 CFU/100 mL for E. Coli for single sample maximum. The previous USEPA recommended Fecal Coliform (FC) criterion of 400 CFU/100 mL is used in many parts of the country for total maximum daily load (TMDL) purposes. Table 8.9 provides the FIB limit values posed by Italian regulations.

Table 8.9: Italian limit values for E. Coli.

Sampling point	Parameter	Normative	Limit Value
WWTP effluent	*Eschericchia Coli*	D.Lgs 152/2006	5.000 CFU/100 mL
Inland bathing water	*Eschericchia Coli*	D.Lgs 116/08 - D.M. 30/03/2010 all.A	1.000 CFU /100 mL
Sea bathing water	*Eschericchia Coli*	D.Lgs 116/08 - D.M. 30/03/2010 all.A	500 CFU /100 mL

In particular the D.Lgs 152/2006 transposes the European Directive 2000/60 and the D.Lgs 116/08 transposes the European Directive 2006/07 "Bathing water Directive".

Passerat et al., (2011) determine the fraction of E.Coli attached to the suspended matter. They estimated that by their resuspension, sewer sediments were estimated to contribute to about 75% of the suspended matter and 10-70% of the E. coli that were discharged.

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8.5.2 Pharmaceutical compounds

Due to the high variability of the characteristics of PhCs and to the high cost of their analysis, it is necessary identify some criteria for selecting some representative compounds to be investigated in this study.

Madoux-Humery et al. (2013) said that the selected PhCs should meet these criteria:

- being frequently observed in the WW of the area
- being almost uniquely present in the WW;
- having variable environmental persistence
- being transported mainly by water flow
- being potential threat for the ecosystem
- laboratory effort for their analysis should be reasonable
- having different removal efficiency in the WWTP

Moreover in this phase of the work it is necessary to have:

- data of PhCs consumption
- data of PhCs concentration in the influent, effluent of WWTP
- data of PhCs concentrations on combined sewage overflow

Acetaminophen (ACE), Caffeine (CAF) and Carbamazepine (CBZ) meet all these criteria and were selected also for being investigated for our purposes.

The chemical-physical characteristic of these three PhCs are reported in Appendix A.

Acetaminophen: It is frequently present in WW being largely administered and has a high removal efficiency in WWTP (98-100% Verlicchi et al., 2012c). It is used as a tracer for the presence of raw WW or insufficiently treated water. It could be considered as an indicator of WWTP malfunction or of combined overflow presence, given its reduced half-life (<4 hours). It is the sixth most consumed compound in Itlay.

Caffeine. It is used as a *tracer* of surface water contamination by WWTP effluent and by stormwater outfall due to its large consumption(Sankararamakrishnan and Guo, 2005, Sauvé et al., 2012). It could be present in the runoff in small quantities for its presence in cigarettes and drinks that could be thrown to the ground.

Carbamazepine. Used as an anti epiletic drug it is considered a tracer of antropogenic pollution for its human specific use. It has a low removal efficiency in the traditional WWTP.

8.6 Literature concentrations in WWTP influent, effluent and surface water

For the purposes of this work, the measured concentrations of FIB are reported from the literature referring to urban drainage systems, influent and effluent from WWTPs, surface water and combined sewage overflow discharges.

On the base on these values the loads spilled from the WWTP and CSOs were estimated in our case.

E.Coli

Litterature data of E.Coli concentrations in the sewage network, WWTP influent and effluent were collected in Table 8.10.

Table 8.10: Median value of E.Coli concentration in different studies

Sampling point		Concentration	Reference	Notes
Sewage network (280 000 residents)	2 urban basinsi (SA, SB)	$4.6 \cdot 10^6$ MPN/100mL $6.8 \cdot 10^5$ MPN/100mL	Madoux-Humery et al., 2013	Dry weather, lower values than those found for European WW (10^7 - 10^8 MPN/100mL)
WWTP influent		$1.6 \cdot 10^6$ MPN/100mL	Madoux-Humery et al., 2013	Dry condition
WWTP effluent	WWTP 1 WWTP 2 WWTP 3	$1.7 \cdot 10^4$ CFU/100mL $2.4 \cdot 10^4$ CFU/100mL $3.4 \cdot 10^4$ CFU/100mL	Passerat et al., 2011 Passerat et al., 2011 Passerat et al., 2011	Wet and dry condition

In the WWTP effluent Ibekwe et al. (2011) observed that E.Coli concentrations were similar in storm, recessional (72 h after a storm event) and dry period. The same Authors found that the FIB concentrations in surface water were strongly influenced by spatial location effects showing the importance of the contribution of the urban and agricoltural runoff. The concentration due to agricoltural run off were higher during storm period than those in dry period. During the recessional period the concentrations were even more lower. The high number of FIB found in surface water could indicate the ability of these bacteria to survive and grow outside the intestinal habitat and persist in the environment long after it has been introduced (Ibekwe et al., 2011).
The removal of E. Coli achieved by a WWTP equipped with secondary treatment is included between 93-99.6% (Lucas et al., 2014, Barbaglio et al., 2003)

Pharmaceuticals

Below are shown tables which give the values of the concentration found in some main literature studies for the three selected micropollutants, in correspondence with various types of waters within the urban drainage systems.

On the base on these values the loads spilled from the WWTP and CSOs were estimated in our case.

Table 8.11: Concentrations of CAF, CBZ, ACE in the sewage network together with corresponding references

Compound	Sampling point	Concentration	Reference
ACE	Sewage network	6 730 ng/L (median point A)	Madoux-Humery et al., 2013
	280 000 residents	762 ng/L (median point B)	
	UWW	960-485000 ng/L	Verlicchi et al., 2013b
CAF	Sewage network	7 230 ng/L (median point A)	Madoux-Humery et al., 2013
	280 000 residents	753 ng/L (median point B)	
CBZ	Sewage network	214 ng/L (median point A)	Madoux-Humery et al., 2013
	280 000 residents	50 ng/L (median point B)	
	UWW	20-29300 ng/L	Verlicchi et al., 2013b

Table 8.12: Concentrations of CAF, CBZ, ACE in the WWTP INFLUENT together with corresponding references min - max (mean**)**

Compound	Concentration	Reference
ACE	11 051 ng/L (median)	Madoux-Humery et al., 2013
	13 - 246 000 (38 000) ng/L	Verlicchi et al., 2012c
	39 000 ng/L	Verlicchi and Zambello, 2014
	9000-100000 ng/l (dry and wet weather) 40000 ng/l (mean) 20000 ng/l (median)	Margot et al., 2011
CAF	> 10000 ng/l (dry and wet weather)	Margot et al., 2011
	7 482 ng/L (median wet weather)	Madoux-Humery et al., 2013
	25 000 ng/L (mean)	Verlicchi and Zambello 2014
CBZ	100 – 2000 ng/l (dry and wet weather) 600 ng/l (mean) 300 ng/l (median)	Margot et al., 2011
	229 ng/L (median)	Madoux-Humery et al., 2013
	1 200 ng/L	Verlicchi and Zambello, 2014
	<loq - 3 780 (1 200) ng/L	Verlicchi et al., 2012c
	570 ng/L measured	Verlicchi et al., 2014

Table 8.13: Concentrations of CAF, CBZ, ACE in the WWTP EFFLUENT together with corresponding references min - max (mean)

Compound	Concentration	Reference
ACE	<loq - 20 000 (1 040) ng/L (mean)	Verlicchi et al., 2012c
CAF	200 - 980 ng/l (dry and wet weather)	Margot et al., 2011;
CBZ	<loq - 19 800 (890) ng/L	Verlicchi et al., 2012c

The removal of these PhCs achieved by a WWTP equipped with secondary treatment is around. 98 % for acetaminophen, 30% for carbamazepine (Verlicchi et al., 2012c) and about 50% for caffeine (Martin et al., 2012b).

8.7 Literature concentrations in Overflow events

It is not easy to understand which is the typical behaviour of these events because two antagonistic effects occur: on one hand the increasing of sources of suspended matter and FIB for the resuspension of sewer and river bed sediments, and on the other the dilution of the contaminants by the larger amount of stormwater in CSO discharges and in the river (Passerat et al., 2011)

E.Coli

Many studies observed a decay in the microbiological water quality in different receiving water bodies due to CSO events (Hall et al., 1998; Ham et al., 2009, Donovan et al., 2008).
Table 8.14 reports literature concentrations of E. Coli.

Table 8.14: E. Coli concentrations [MPN/100ml] for CSO events in different studies

Sampling point	Concentration		Reference	Notes
CSO (OA) CSO (OB)	$1.7*10^6$ $2.0*10^5$	EMC	Madoux-Humery et al., 2013	8 CSO events (n=120) 2 CSO events (n=10)
OA	$2.2\cdot10^6$ $3.0*10^6$ $6.4*10^5$	EMC	Madoux-Humery et al., 2013	Summer Snowmelt Fall
CSO	$2*10^4$	Mean	Arnone and Walling, 2006	USA
CSO	$1.4*10^5$	Mean	Soonthornnonda and Christensen, 2008	USA
CSO	$9.7*10^5$	Mean	Marsalek et al., 1994 i	Canada
CSO	$2.8*10^4$-$1.1*10^6$	Mean	Marsalek et al., 1996	Canada
CSO	$1.4*10^6$	Mean	Marsalek and Rochfort, 2004	Canada
CSO	$3.9*10^5$	Mean	Kim et al., 2005	South Corea
CSO	$105\text{-}10^8$	Max	Ellis, 1989	UK
CSO	$3.8*10^5$	Min	Passerat et al., 2011	FR
CSO	$6.4*10^6$	Max	Passerat et al., 2011	Max during the first 30 min of discharge
CSO	$1.5\cdot10^6$	Mean	Passerat et al., 2011	

The studies reported in the literature have analysed samples of the composite type (flow proportional), to better understand the impact of variability of the flow and the quantity of substance (FIB) fed into the grid on the concentration value determined in the laboratory. In fact, for a given quantity of a substance present in the water discharged the concentration value may be greater or lesser depending on the degree of dilution brought about by the portion of water that does not contain microorganisms or compounds investigated. While at the same flow rate is necessary to consider the trend (pattern) with which the FIB and the substances investigated are typically introduced into the network, depending on the sources that produce them.
These studies included both overflow events in summer, as in our case, and events that occurred in other seasons of the year, for different types of urban basin with regard to population, use and extent of the area drained, network length and characteristics thereof. The presence of E. coli and

is still mainly associated with fecal material and, therefore, in an urban drainage system, sanitary discharges of civil users.

Pharmaceuticals

The following table reports the concentrations of the selected PhCs in CSOs but data of more compounds are necessary Madoux-Humery et al. (2013).

Table 8.15: Concentrations of CAF, CBZ, PAR in CSO water together with corresponding references.

Compounds	Sampling point	Concentration		References	Notes
ACE	CSO	3591 ng/l	EMC (median)	Madoux-Humery et al., 2013	8 events 3 in summer (n=125)
ACE	CSO	7150 ng/l	Higher median	Madoux-Humery et al., 2013	winter
ACE	CSO	9052 ng/l	Max	Madoux-Humery et al., 2013	winter
ACE	CSO	1245 ng/l	EMC (mean)	Del Rio et al., 2013	10 events summer and winter
CAF	CSO	3248 ng/l	EMC (median)	Madoux-Humery et al., 2013	8 events 3 estivi (n=125)
CAF	CSO	4172 ng/l	Higher median	Madoux-Humery et al., 2013	winter
CAF	CSO	12176 ng/l	Max	Madoux-Humery et al., 2013	winter
CAF	CSO	7720 ng/l	EMC (mean)	Del Rio et al., 2013	10 events summer and winter
CAF	CSO bypass, only primary treatment and disinfection	11000-12000 ng/l	range	Phillips et al., 2012	1 year
CBZ	CSO	184 ng/l	EMC(median)	Madoux-Humery et al., 2013	8 events 3 in summer (n=125)
CBZ	CSO	240 ng/l	Higher median	Madoux-Humery et al., 2013	winter
CBZ	CSO	623 ng/l	Max	Madoux-Humery et al., 2013	winter

8.8 Variability in literature

It is important to keep in mind the daily variability with which these substances are consumed and expelled in order to predict the variation of presence on the network and set up an appropriate campaign of sampling and analysis. In fact, the daily variation of the concentration in the network, and consequently also in the overflow, in input to the WWTP and to a lesser extent in the effluent, reflects precisely the variability of consumption and of the pattern of excretion of various substances, and the variability of the water flow in the network. In this regard, please refer to the work of Ort et al., (2010c) who has extensively studied the uncertainties related to the sample as a function of these characteristics. Indeed, the analysis of the daily variation is also important to set up an appropriate experimental campaign.

8.8.1 WWTP influent

Pharmaceuticals

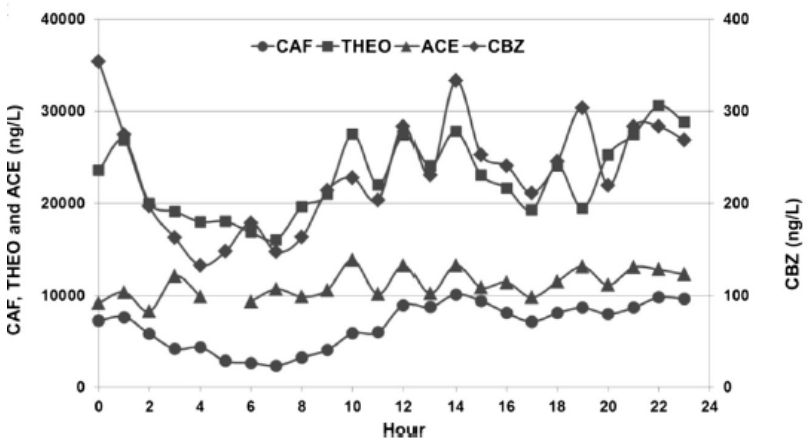


Fig. 8.23: Daily pattern concentrations in the WWTP influent for four substances (Madoux-Humery et al., 2013)

Looking at the graph of Fig. 8.23, relating to the daily patterns of concentrations of the substances investigated in input to the WWTP investigated by Madoux-Humery et al., (2013), it is clear that these substances can be divided into two categories: highly variable and approximately constant.

The three selected PhCs can be ranked as follow:

a) highly variable: CBZ and CAF. Trend similar to E. coli with a minimum at 7.00, 1 peak in the afternoon and another around 22.00. CBZ is taken in the evening but then left in the body for 25 to 65 h depending on the dose taken.

   The variations may reflect:

   - intermittent consumption,
   - different half-lives
   - different pattern of excretion and absorption in the body,
   - different residence times in the network based on the persistence of a substance.

b) Approximately constant: ACE

E.Coli

The daily graph of concentrations of FIB (E.Coli) in dry weather at the entrance of the treatment plants is characterized by a growth in the morning, a peak in the afternoon and then a gradual decrease, in accordance with the pattern of excretion by the population, which provides a peak into drains morning, and the residence time of the drainage system.

In the following figure by Heaton et al., 1992 it is clear that the majority of defecations occurred in the early morning. Because they are associated mainly with the fecal matter, the peak of excretion of E.Coli occurs in the sewage network between 7.00 and 8.00 a.m.

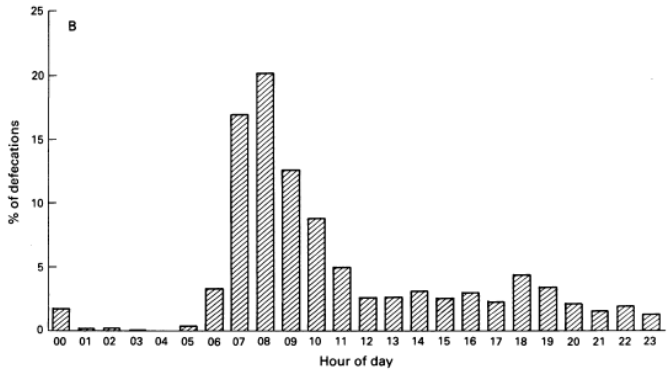


Fig. 8.24: Defecation pattern (Heaton et al., 1992)

In accordance with the residence time of the sewage system investigated by Madoux-Humery et al., 2013, the concentrations in the influent of the WWTP increase in the morning and there is a peak in the afternoon after that they decrease. The daily trend of E.Coli concentrations in the WWTP influent observed by the same authors during dry weather conditions is reported in Fig. 8.25.

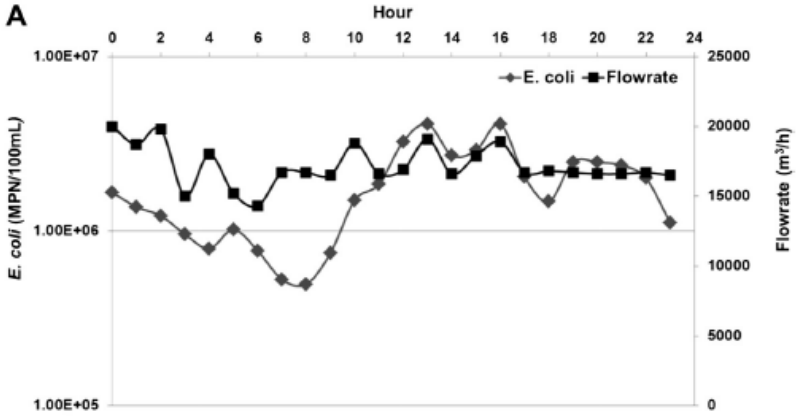


Fig. 8.25: E.Coli concentration pattern in the WWTP influent (Madoux-Humery et al., 2013).

8.8.2 WWTP effluent

Pharmaceuticals

An in-depth analysis of daily variability of PhCs was carried out by Nelson et al., 2010 that identify three caterogies based on characteristics of daily cycles: (1) intense pulses (rapid cycle); (2) broad daily cycle (slower cycle); (3) constant concentration (no cycle). Compounds that exhibit these different cycles are listed in Tab. 8.16. These aspect should be considered when a sampling mode is to be selected.

Table 8.16: Categories on the basis of concentrations variability in the WWTP effluent

	1° Group	**2° Group**	**3° Group**
Compounds	Estone Triclosan Naproxen Sulfamethoxazole Diclofenac Furosemide Trimethoprim	Azithtomycin Iopromide Gemfibrozil Propanolol Atenolol Erythromycin	**Carbamazepine** Primidone Triclocarban Fluoxetine Metoprolol Phenytoin
Characteristic of daily cycle	Intense Pulse (rapic cycle)	Broad daily cycle (slower cycle)	Constant Concentrations (no cycle)
	High relative standard deviation		
Peak duration	3-6 h	6-15 h	
Highest concentrations Time	4-8 p.m.	4-7 p.m.	
Lowest concentrations Time		8-11 a.m.	10-11.30
Description of pattern	Background concentration at or below the analytical reporting limit, a rapid incerase to a daily maximum, and then a rapid drop back down to the background concentration.		

8.8.3 Combined sewage overflow

Pharmaceuticals

As regards CSO, variability in exposure is certainly influenced by the amount of rain water that enters into the sewer system and does not contain such substances, but also of the concentration present in the raw sewage, both in the dry period preceding the meteoric event and during the event itself. Also in this case a resuspension of sedimented material can lead to an increase in the concentrations mobilized.

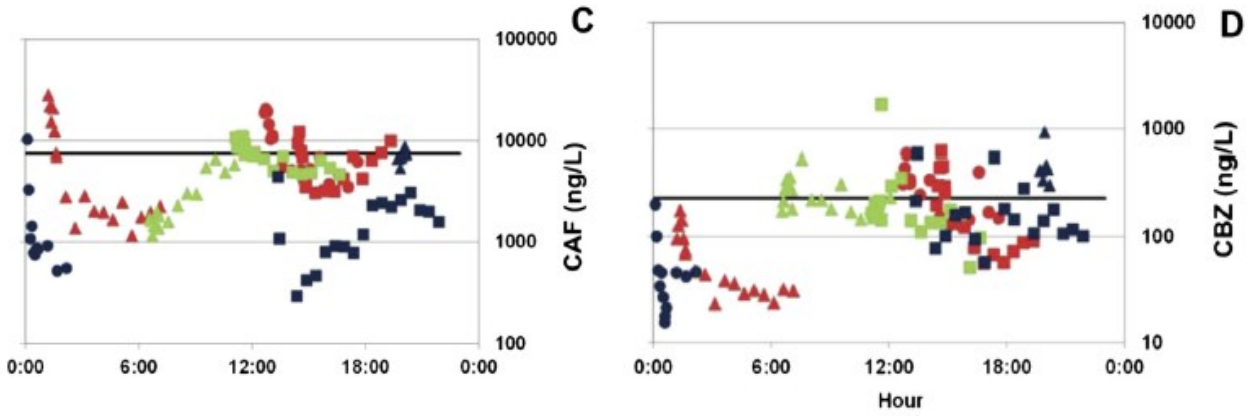


Fig. 8.26: Daily pattern of CSOs concentrations of CAF and CBZ (Madoux-Humery et al., 2013). In red shows the events autumn, in summer green ones, blue ones in the winter

By way of example, Fig. 8.26 shows the daily pattern of the concentration of CBZ and CAF in the water overflow during the events analysed by Madoux-Humery et al. (2013). The pattern is reconstructed by placing in succession for the various events happening now in relief, also belonging to different seasons.

From the comparison between concentrations in the overflows and those on the network and input to the sewage plant is noted that the waters discharged by CSOs will have lower values than those in sewer but, despite the dilution water of rain remain, quite high.

During *rainfalls* there is thus greater dilution in water discharged by CSOs, designed for just this purpose. From the data of the literature it is noted that the concentration in the inlet to the WWTPs is very variable, presenting similar values, or at least of the same order of magnitude, between conditions in time of rain and in dry weather. Then, as in the case of the FIB, for some PhCs, it is thus recognized a seasonal variability of the concentrations in the waters of CSOs.

It's also important to consider that a greater dilution of the water in input to the WWTP can result in a lower removal of micropollutants due to the lower residence time within the plant. Output from WWTP substances *less efficiently removed* (such as CBZ) may present then the concentrations similar to those output from the spillways, thanks to the fact that dilution is a factor more important than the lack of treatment for these waters. Conversely, the concentrations of the outgoing waters from the spillways, despite dilution, are certainly greater than those output by the purifier for substances with *high removal efficiency* in the treatment process, for the same compound considered. This is naturally reflected in the value of the load spilled and the *different contribution* from spillways and WWTP, as demonstrated by the results obtained in this work and reported in the following sections, obtained from concentrations taken from the literature and from consumption.

E.Coli

In time of rain, and then, the concentration of FIB in the waters of CSOs can be very variable depending on the concentration present in the raw sewage entered by the users, both in the dry period preceding the meteoric event that during the event itself, but also in function of the degree of dilution brought by water from rain and from the resuspension of sedimented material in the network which is caused by the higher flow outflowing during the rains. These aspects are related to the characteristics of rainfall events themselves (duration, intensity). Even time survival of these bacteria and their ability to affect the playback detectable concentrations.

It is therefore plausible to expect even in water discharged by CSOs, in relation to the time of day, concentrations of E. Coli close to those found in raw sewage during dry weather (10^6-10^7 MPN/100ml) for 3-4 hours whether the overflow event is limited (up to) whether it extends beyond; for the next time for discharges longer than 3-4 hours lower concentrations (10^5 to 10^4 MPN/100 ml) can be considered due to the dilution of sewage by rain water and clean the network made by them. Values of the order of 10^5-10^4 MPN/100 ml may still result in overflows of short duration (1-2 hours or less) if the period of dry weather that precedes them turns out to be short (of the order of a few days or less). The values given are just typically reduce an order of magnitude for Enterococci. Minimal concentrations were at least one order of magnitude higher than concentrations usually observed in the treated effluents of the WWTPs from Paris agglomeration (Passerat et al., 2011).

The concentration of FIB were the highest in the first 30 min of discharge and then dropped to reach a minimun when the proportion of runoff water peak in the CSO discharge, then they increase progressively until the end of the CSO. The increase was pronounced for E. coli, leading to a final E. coli concentration close to what was observed in the first sample.

These considerations are precisely reflected in both the literature data that the values detected by CADF. In addition, it should be noted that the sampling on the overflowed water, made by the management of the sewage system in question, was generally carried out fairly distributed over the duration of the relief.

From the data of the literature also it recognizes a seasonal variability. In autumn the concentrations of E.Coli are 3-5 times smaller than those of summer/winter which, however, are of the same order of magnitude. The largest concentrations you have in winter. This contradicts the common belief that, in winter, the concentrations are lower because of the dilution. This season occur less rainfall events and has reduced infiltration so you have more storage on the pipelines. Under these conditions, even events with a small precipitation height and long life, such as winter, can lead more frequently to the discharge of highly contaminated water for a long period. It is noted again that the concentration of E.coli in the course touched falls more and more quickly in summer compared to winter precisely because of meteorological events shorter and more intense, causing in turn peak flow advances, and by a larger amount .

To confirm that the values of concentration of E. coli in the waters of CSOs are slightly lower than those of water into the sewer in dry weather is also shown a graph, always taken from one of the studies cited, in which are also observed variability seasonal and daily concentrations.

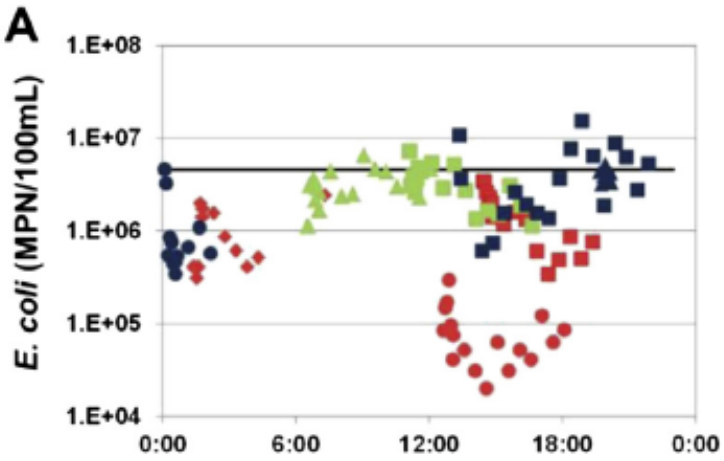


Fig. 8.27: Daily pattern of CSOs concentration (Madoux-Humery et al., 2013). Autunal events (red), summer events (green), winter events (blue).

Microbiological characterization upstream the CSO outfall

For estimating the microbiological quality of the channel before being impacted by the CSOs, the channels should be sampled directly upstream from the CSO once the CSO event had begun.

The mean concentration ($4*10^4$ E.Coli) in river before being impacted by the CSO was sampled upstream from outfall once the CSO had begun. These values in wet weather conditions were compared with data in dry weather ($2*10^3$ E.Coli). These values are mainly constant in time and space.

The upstream concentrations in wet weather conditions are more than one order of magnitude higher than those observed in dry period. Therefore the microbiological quality of the river was already impacted by the rainstorm upstream from the CSO outfall (Passerat et al., 2011).

Microbiological characterization downstream the CSO outfall

This aspect was investigated by Passerat et al. (2011). In their case the water mass impacted by CSO outfall reached the limit of the study area in only 20 h (30 h in dry weather). They did three series of samples downstream in 3 different points and at different time considering the transition time. In the point directly downstream (B) the concentration is 1 log higher than those measured upstream. They subsequently decrease in C and D representing the 34% of the concentration in B for E.Coli.

Vertical distribution

Passerat et al. (2011) found that there are weak differences between surface and bottom samples. Where difference where significant the FIB concentration was higher in the bottom samples.

Correlation with other substances

Some studies have investigated the relationship between physicochemical parameters and metals, the most studied, and concentrations of FIB, leading to a number of correlations that can be interpreted to estimate the contribution of sewage, rainwater, groundwater, CSO or deposits in sewer to FIB occurrence in the waters. Other studies have shown a link also between presence of micropollutants and FIB (Madoux-Humery et al., 2013).

FIB concentrations were positively correlated to conductivity, suggesting that they are mainly by the WW.

Passerat et al. (2011) found that the first 30 minutes of the CSO carried out a very high load of suspended matter (830 mg/L) when the concentration in the entrance of WWTP was 264 mg/L. After 1 h the concentration dropped to 290 and finally reaches 110 mg/L. They estimated that 86% of SM load was due to other sources than wastewater. Two source can be proposed for it: Stormwater runoff on urban areas or resuspension of sewer sediments.

77% of the E.Coli discharged during the whole CSO were attached to SM. The highest quantity was 91% in the first sample while it was on average 68% in other samples.

During CSO events the contribution of WW, runoff and sewer deposit resuspension should be determined. Gasperi et al. (2010) analysed these three contributions to the suspended matter load in function of rain intensity. They estimated a contribution of 22-44% of wastewater, 7-12% runoff and (47-69%) sewer deposit resuspension. The sewer resuspension tent to increase with the intensity of rain event while WW contribution tents to decrease. Runoff appears to be less related to rainfall intensity. The contribution due to resuspension is evident in the first 30 minutes.

8.9 Concentrations in the study of Comacchio

Also for the purposes of this paper the knowledge of daily patterns in network is important, in order to estimate more realistic loads and better interpret results related both to the treatment plant and to overflows. However, referring to PhCs, not yet been carried out a proper campaign measures, it was decided to assume, as a first approximation, constant values throughout the day using average concentration values obtained from the *literature studies* first reported. This is also justified by the fact that, while for CSO data of the trend of discharged flow rate with time for the duration of the event are available, for the WWTP will have only the values of the overall daily flow rate, from which the values of total flow circulating in the network in a generic day are defined. Furthermore, assuming that the variability of the flow and excretion affects in the same way all the compounds, it may take the variability of the concentration as a result of the different consumption of one or the other substance.
Assuming that the flow and excretion variability affects all the compounds in the same manner, also the variability of the measured concentrations may be considered as the results of the different consumption.

E.Coli were determined by the management authority for the sewage network with the Colilert method that is able to provide data of concentration in only 24 hours (http://www.idexx.it/water/products). This method approved by EPA (Environmental Protection Agency) and included in the Standard Methods for Examination of Water and Wastewate, is able to quantify both E. Coli and the Total Coliform at the same time.

The concentration of E.Coli were not measured in the sewage network.

For considering the concentration of E.Coli in the effluent of WWTP under study, the legal limit of 5000 MPN/100 mL was assumed in the calculation. This is a great source of uncertenties because in this point the concentration can be much lower than the legal limit.

Autumn concentrations of E. coli are 3-5 times smaller than those of summer/winter (which are of the same order of magnitude). The largest concentrations are during the snowmelt period. It contradicts the belief that winter concentrations are more diluted. Actually, in winter I have less rainfall events, reduced infiltration and increased deposit on the pipes. Moreover, in winter it may get less rainfall height for a longer period (even lower frequency) and therefore have contaminated water for longer periods. The concentration of E. Coli falls more and more quickly in summer than in winter because I have more intense peaks (CADF SpA, 2013).

8.10 Hydraulic characterization of Comacchio's sewage network

8.10.1 Wastewater

To evaluate the black flow coming to the WWTP in the months considered for the study, both in dry weather conditions and time of rain, we proceeded by finding, first of all, the data of the resident population and tourists for the villages of the Municipality of Comacchio served by drainage system under consideration.

Data of resident population provided by Demographic Office and of tourist population provided by Statistical Office of the Province of Ferrara are reported in the degree thesis of Malavasi (2015).

It is important to note that tourist numbers are almost entirely concentrated in the summer.

Therefore, for the calculation of black flow rate, recognize two time periods during the year:

• the summer season, divided in the months of June, July, August and September, and for which we must consider, in addition to the resident population, also the number of tourists;
• the other seasons of the year, a period called, for short, winter season, and for which it is considered the only resident population.

Once collected the data of population of summer and winter seasons, it was decided to further divide them by relating to each sub-basin of the drainage system, according to the scheme of the drainage system previously developed (Fig. 8.15) and considering the area of competence of each pump station as a percentage of the total area.

The resident population of each sub-basin was given a water availability per capita, according to the number of inhabitants and the socioeconomic level of the area of interest, namely the existing services and industrial input inside the urban fabric. It was in the range of 130-180 l/(ab d).

In a first approximation it is assumed a coefficient of influx into the drainage system equal to 0.85 to take account of loss and waste in the distribution network, leak into the sewage system and the portion of water used for different purposes which does not enter into sewer (washing cars, watering gardens, etc.).

Thanks to the parameters defined so far has been possible to estimate the medium black flow produced during the day from each sub-basin, referring both to an average day of the months of the winter season to an average day of each of the four months of summer $Q_{nm,i,m}$. In addition, they are also calculated flow black averages for each of the days of the summer $Q_{nm,i,d}$ taking into account the weights attributed to the number of tourists as a function of time and the sub-urban considered. In fact, the daily average black course which goes to the purifier by a generic sub-basin i, is calculated as:

$$Q_{nm,i,j} = \varphi \cdot \frac{q_r \cdot N_r + q_t \cdot N_t}{86400}$$

where:

- $Q_{nm,i,\ j}$ is the scope black daily average of the sub-basin, evaluated for an average day of the month (j = month) or for each specific day of the four summer months (j = day) [l/s];

- Φ is the coefficient of influx in the network assumed equal to 0.85;

- q_r is the water availability per capita for residents in l/ab.d, different to the city centre (Table 8.17);

- q_t is the water availability per capita for tourists, assumed for each sub-basin of 130 l / ab.d;
- N_r is the number of residents of various urban centres (fixed);
- N_t is the number of tourists to the resort (variable in the summer season, zero in the winter);
- 86400 are the seconds of a day.

In Fig. 8.28 the daily pattern of discharges is reported in Wallingford (2007).

Table 8.17: Breakdown of sub-basins, its permanent residents and per capita water availability assigned

Urban sub-basins	Sollevamento ricevente	N_r	q_r
Volano	S0	210	150
Nazioni 1	S1	329	150
Nazioni 2	S2	620	150
San Giuseppe M	S4	2049	160
San Giuseppe V	S3	1214	160
Pomposa	S3	579	150
Parco del Sole	S	204	150
Scacchi	S5	619	150
Porto Garibaldi N	S5	294	150
Porto Garibaldi S	S6	4537	150
Spina	S8	662	150
Estensi	S7	1672	150
Comacchio O	S13	2793	180
Comacchio E	S14	3269	180
Comacchio R	Margherita	2080	180

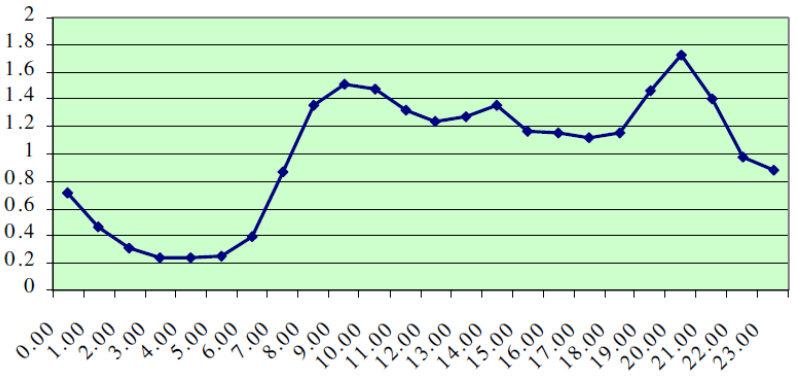


Fig. 8.28: Daily pattern of discharges

Applying a peak coefficient (K_{max} equal to 1.7), it is possible to determine the maximum black flow rate from the media, according to the formula:

$$Q_{n\max,i,j} = K_{\max} \cdot Q_{nm,i,j}$$

From the values of average daily black course relative to each specific day of 2014 and each sub-basin, it is possible to calculate the total black flow rate reaching the treatment plant in the day from each of its sub basin ($Q_{n,i,d)}$. These flow values are also the amount of water input to each of the pump stations of the network. The total daily value $Q_{n,d}$ input to the system is the sum of these individual values coming from the various sub-basins. So we have the following relationships that express the flow in m^3 per day (m^3/d):

$$Q_{n,i,d} = 86.4 \cdot Q_{nm,i,d}$$

$$Q_{n,d} = \sum_{i} Q_{n,i,d}$$

The contribution of groundwater infiltration and drainage canals was taken into account as it was reported by the management as significant in dry weather. it was estimated to be about 50-200 l/s in dry period while in during rain events this amount is replaced by rainwater entering the network.

Comparing the data of various years from 2008 to 2014 it is evident that the population has remained almost similar in recent years and tourism has not had major changes.

8.10.2 CSO events

Data of rain flow are reported in table in the thesis of Margherita Malavasi (2015).

The flow of the receiving channels (mainly Canale Navigabile) is strongly impacted by the rainstorm derived from the CSOs.

The overflow events considered in this work were characterized first of all in function of the point where the overflow occurs. Then following points: **S13**, **S14**, **S8**, **S6** and **MD** (overflow upstream the WWTP) are to be considered. In addition, it is also considered the overflow after primary treatment of WWTP that send water directly to disinfection and then exit, jumping biological treatment (abbreviated **BY**).

This subdivision is important because each of the four pumping systems, that act as overflow, are connected to different portions of the network, each of which presents, therefore, a different width of the area drained, different amplitudes and characteristics of permeable and impermeable surfaces, different number and type of connected users, and different characteristics of rainfall events that stress the basin. These differences, together with the peculiarities of each of the lifting stations (number and type of pumps, flow rate raised), result in a different volume values of the water discharged from each point, in response to the same event of rain. Consequently, also the loads spilled and the concentrations of the selected substances appear to be specific as a function of the point of overflow.

To the overflow upstream the WWTP come, instead, the flow that come from all over the urban basin as a whole, net of those overflowed by the CSOs placed along the network. So, it is noted that in addition to entering into operation in conjunction of the other CSOs, this discharge is operated more often and typically longer, to make up to the overcoming of the hydraulic capacity of the WWTP and to the fact that the whole area of the compartment Lidi Nord is drained by a portion of the network that does not have CSOs for the flow rates in excess.

Other than the subdivision of overflow events according to their location, it was made a classification according to the day in which they have occurred, thus characterizing them as a function of the different rain events that caused them (see Malavasi, 2015).

For each of these meteorological events, moreover, has been considered the spatial variability within the basin, which diversifies them in terms of time of the event, total duration, height and intensity of rain fall in the time interval at which the event takes place. To take account of what, rainfall data were considered (heights of cumulative rainfall provided by CADF) for three tracking stations, each representing an area of the urban basin overall.

Data refer to all rain events that occurred during the four summer months of 2014 and reported the total height of rain falls of up to that moment, according to a very precise sampling interval (usually 9 minutes).

After 3 hours from the end of the rain the height measurement of each cumulative pluviograph is reset, to consider the occurrence of a new event separate from the previous one. Therefore, for the purposes of the definition of the rain events that cause CSO events, they are seen as separate events those separated by a period of at least 3 hours, even found in the same day, while they are part of a single event values of height of rain recorded at time intervals of less than 3 hours, occurring even riding on two different days. The pluviographs considered are placed at:

• pump station S14 site in Comacchio (P14);

• pump station S2, located in Lido Nations (P2);

• pump station S7, located in Lido of Este (called P7).

The area of expertise of each of these pluviographs was evaluated by Thissen's polygons. A detailed description of the procedure is reported in Malavasi (2015).

It is to be noted that the overall urban basin is divided into three areas to which associate the different characteristics of each rainfall event, and that these areas are almost totally the three main areas of division of the basin, as a function of location of the areas in the municipality and the position of WWTP. The only difference is that the entire town of Porto Garibaldi falls entirely in the area of influence of pluviograph P2.

In Malavasi (2015) tables report, for the month, the overflowed daily flows divided by the point of discharge, compared also with WWTP effluent flow. Therefore, by observing tables it is possible to notice how at the same rain event occurred or not in different discharge points, with different volumes depending on the extent and duration of the precipitation.

From the same data it was possible to draw the following graph, reported from Malavasi (2015). They show how great is the percentage of flow exiting from the various points of discharge in the network during the various days in which CSO events occur.

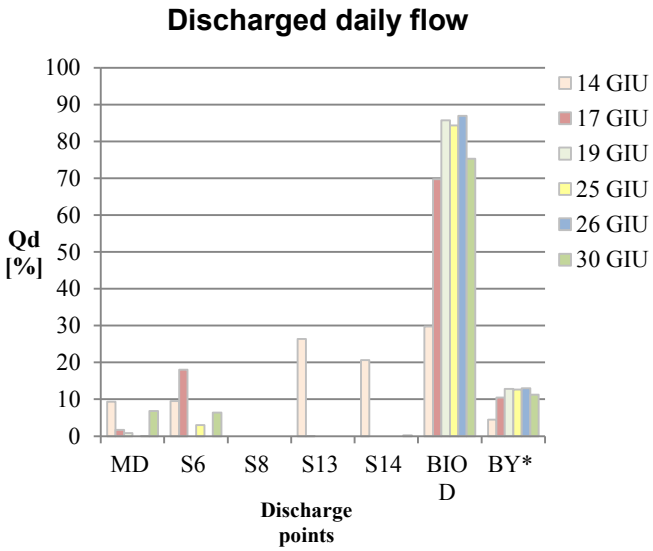


Fig. 8.29: percentage of discharged flow (Qd) on the total flow entering the sewage system (QCSOs + Qtreated by the WWTP) for the month of June

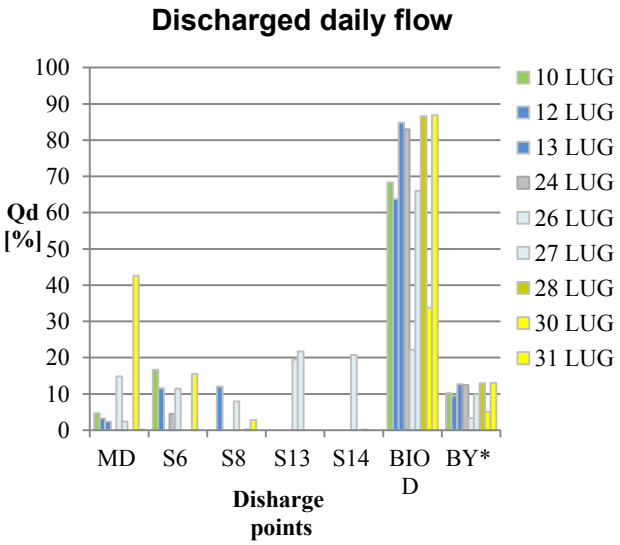


Fig. 8.30: percentage of discharged flow (Qd) on the total flow entering the sewage system (QCSOs + Q treated by the WWTP) for the month of July.

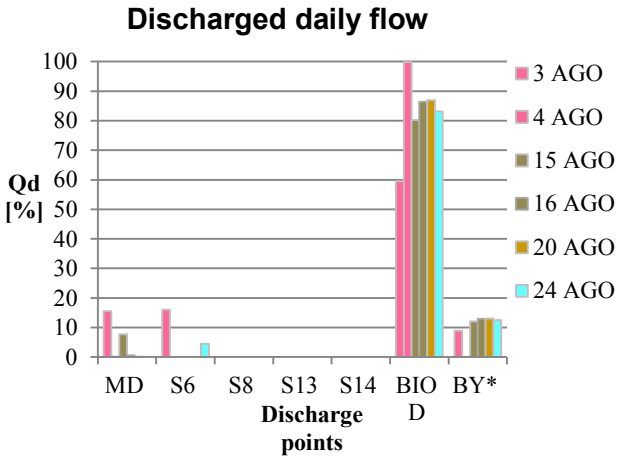


Fig. 8.31: percentage of discharged flow (Qd) on the total flow entering the sewage system (QCSOs + Q treated by the WWTP) for the month of August

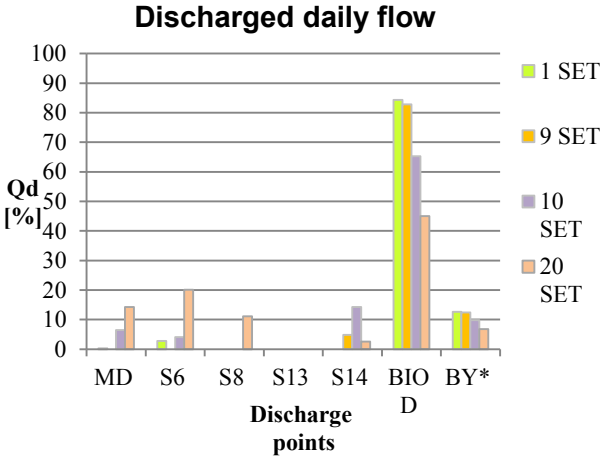


Fig. 8.32: percentage of discharged flow (Qd) on the total flow entering the sewage system (QCSOs + Q treated by the WWTP) for the month of September

Table 8.18: CSO and rain events characteristics

CSO events characteristics						Rain events characteristics						
Point (number)	Event	T_{eff} [min]	$Q_{d,\,tot}$ [m^3/d]	Q_m [l/s]	Q_{max} [l/s]	T_{tot} [min]	$h_{cum,tot}$ [mm]	$i_{m,tot}$ [mm/h]	$i_{m\,eff,tot}$ [mm/h]	$h_{cum,asf}$ [mm]	$i_{m,asf}$ [mm/h]	$i_{m\,eff,asf}$ [mm/h]
S13	14 June	367.9	16299	738	1100	189.0	32.0	10.2	10.2	7.3	7.1	7.1
	17 June	0.4	24	1075	1100	153.0	6.6	2.6	4.4	6.6	2.6	4.4
	26-27 July	461.9	15243	550	550	308.0	36.4	9.0	12.4	11.4	4.3	25.2
		271.5	8961	550	550							
S14	14 June	240.0	12748	885	1800	189.0	32.0	10.2	10.2	9.1	8.5	8.5
	30 June	1.9	73	650	650	269.0	14.6	3.1	6.1	8.1	2.7	6.4
	26 July	199.8	16247	1355	1800	308.0	36.4	9.0	12.4	8.5	3.3	20.9
	28 July	0.7	41	917	1150	-	-	-	-	-	-	-
	9 Sept	15.3	581	634	1300	-	-	-	-	-	-	-
	10 Sept	94.4	3681	650	650	252.0	22.8	5.4	6.6	11.4	11.5	21.2
	20 Sept	19.3	753	650	650	279.0	16.8	3.6	4.5	6.6	4.5	7.6
S8	12 July	134.1	3737	464	700	279.3	15.0	3.2	6.2	8.8	12.0	12.0
	26 July	226.0	6218	459	700	164.6	41.4	16.3	16.3	27.6	63.6	63.6
	28 July	3.2	68	350	350	-	-	-	-	-	-	-
	30 July	96.4	2025	350	350	511.1	35.8	4.2	5.4	32.4	4.4	5.7
	20 Sept	154.9	3253	350	350	306.2	25.8	5.1	6.1	13.6	7.0	12.5
S6	14 June	167.4	5550.3	553	754	202.7	16.2	4.9	5.2	2.5	4.5	4.5
	17 June	115.8	5418.2	780	1300	117.6	14.8	7.5	8.1	9.5	9.2	11.1
	25 June	19.2	505.5	440	754	90.5	4.2	1.0	2.8	4.2	1.0	2.8
	30 June	75.3	1871.4	414	754	232.4	10.6	3.6	6.2	6.8	3.4	6.1
	10 July	207.6	6760	543	754	733.0	25.8	2.1	4.9	9.4	13.2	13.2
	12 July	117.5	3595	510	754	279.3	15.0	3.2	6.2	5.5	13.7	13.7
	24 July	34.8	845	405	405	126.6	3.01	1.4	3.2	2.36	4.2	5.7
	26 July	179.2	8967	834	1300	164.6	41.4	16.3	16.3	12.0	67.8	67.8
	30 July	253.0	11078	730	1300	511.1	35.8	4.2	5.4	11.4	2.0	2.9
	3 Aug	140.0	6943	826	1300	186.0	15.4	8.1	20.2	7.3	54.7	54.7
	24 Aug	47.8	1161	405	405	35.8	4.0	6.7	6.7	4.0	6.7	6.7
	1 Sept	24.4	593	405	405	108.3	6.4	3.5	4.3	5.9	4.2	4.7
	10 Sept	43.8	1063	405	405	207.0	9.0	2.6	2.9	3.4	3.8	3.8
	20 Sept	202.2	5866	484	754	306.2	25.8	5.1	6.1	12.7	7.3	14.9

A further investigation of the relationship between CSOs and rain events is reported in Malavasi (2015).

Of the total rainfall events that occurred in the area of interest, broken down by area of competence of each of the three pluviographs considered (46 events in total), only those of a certain size in terms of height and intensity of rainfall led to CSO events that occur, generally, after a dry period (antecedent dry period, ADP) of a few days (average 2 days). The degree of saturation of the soil prior to the events that caused the CSO events does not seem to significantly affect the value of total CSO flow rate.

It is also noted that in correspondence of a same meteoric event not all the CSOs are activated in function of the spatial variability associated with the event, but also based on the surface of the urban basin afferent to the pump station and on the capacity of the pumps that must send the water to the WWTP.

Table 8.19: CSO events characteristics

CSO events characteristics					
Point (number)	Event	T_{eff} [min]	$V_{d,\,tot}$ [m^3/d]	Q_m [l/s]	Q_{max} [l/s]
MD	14 June	675	5805	143	449
	17 June	180	509	47	112
	19 June	150	239	27	65
	26 June	30	18	10	16
	30 June	345	2020	98	321
	10 July	930	1900	34	170
	12 -13 July	165	1002	101	170
		240	633	44	112
	26-27 July	450	11618	430	651
		135	966	119	321
	30-31 July	735	30383	689	2023
		150	36	4	4
	3-4 Aug	420	6710	266	775
		30	29	16	16
	15-16 Aug	450	2415	89	170
		90	168	31	65
	20 Aug	30	18	10	16
	1 Sept	75	61	14	16
	10 Sept	450	1669	62	240
	20 Sept	450	4164	154	321

Thus, CSOs S13 and S8 are actuated for a number of times lower compared to S14 and S6, respectively placed further downstream along the same line of the drainage system. In this therefore seems to also affect the degree of saturation that the soil reaches during such events of rain.

The CSO placed immediately upstream of the WWTP is then the one that operates the greater number of times, because to it all the WW comes from the entire urban basin by the various lines, including those of the compartment Lidi Nord that prior to this point does not possess CSOs needed to discharge the excess water. Generally, overflows events of longer duration are related to longer rain events, depending to the total cumulative rain height. In addition, it is noted that the rainfall event of *26th July* has caused significant flooding throughout the city, in particular in the Lidi Sud, for its high intensity and short duration (few hours).

In fact, comparing this exceptional event with the next one on 30th July, while noting little difference in the cumulated height of rain fall, there is, however, a great disparity in the intensity in respect of which the event on 30th July has values on a longer duration very lower than those of 26th July. Then, evaluations related to the occurrence of overflows according to rain events can be made also considering other characteristics of the rain events themselves, as the average intensity on the effective total duration of the event and the one evaluated on the time that elapses between the start of the raining event and the beginning of the overflow; or considering the variability in the time that each specific CSO event takes in correspondence of the respective event rain. By way of example, the rain hyetograph and corresponding CSO hydrograph are reported in the figure below.

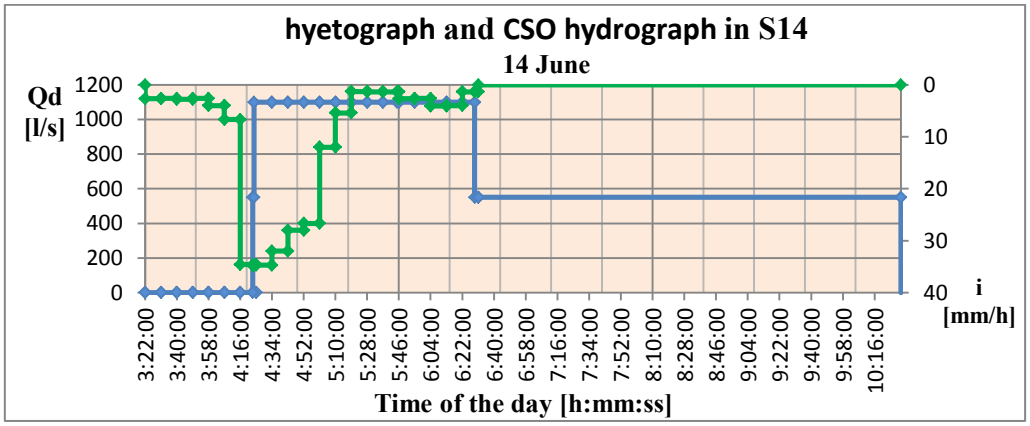


Fig. 8.33: Hyetograph of rain and CSO hydrograph (S14)

The characteristics of rainfall events were drawn from the rainfall data mentioned above; while the daily flows discharged by CSOs (or total volumes discharged on the duration of the various events day by day), the time of occurrence and duration of each discharge were calculated from data of switching on and off of the appropriate pumps and the degree of opening of the valve for the CSO upstream of the WWTP.

It worth notice that the flow rate treated into the WWTP, always provided by CADF, for Sundays and public holidays are determined from the first data available the next day making a division into equal parts between the day of detection of the data and the days for which data are not available.

As regards the flow rate that sometimes bypasses the biological treatment, not having data available for the period considered in the study, an estimation was made with reference to the data provided for the months of September and October 2013. From these, in fact, it was possible to note that the by-pass action is always in correspondence of the rain events, even for those that do not cause overflow, and that, for those which give overflow in the other points, the flow rate of by-pass tends greater the higher the flow rate is discharged just upstream from other CSOs. So, based on the data analysed, it was possible to determine that the daily flow by-pass, in the days in which they occur other overflow events, is around an average value equal to about 14% of the daily flow rate of the treated to the biological treatment at WWTP, with minimum values around 3% and maximum values comprised between 20% and 24%.

Variability in this range cannot be blamed, for the little amount of information available, to the duration and extent of the overall height of the rainy rainfall events that cause the various overflow, although the higher values are found at of precipitation events, isolated and not, with average duration (4-8 hours) and long (> 8 hours, very long> 15 hours) and heights from intermediate values (10-20 mm / 20-35 mm) to high values (35-50 mm) and very high (> 50 mm). The lower values occur for rain events that are isolated or preceded by a considerable period of dry weather, despite the long duration and intermediate values of rainfall height; or occurring in the case where the heights of rain are low (<5 mm), even for long durations of the event, or even for events characterized by both durations and heights low.

It worth noticing, also, that even on days when there is no rain, you experience episodes of bypass of the biological treatment that are modest during significant periods of dry weather (average volumes of 1-3% of the biological treated volume), while they can abut to the average value found during rain events, in the case of days that follow considerable rainfall events. In the first case this may be due to the presence of a high flow rate of infiltration in the network that, according to what reported by the managing entity, can reach high values, also because the evaluation was carried out for two months in which tourist numbers are low (September) or minimal (October). In the second case, more than for the infiltration flow rate, the by-pass of the biological treatment may enter in operation due to the high flow arriving to the WWTP after a conspicuous meteoric event that occurred the day before, depending on the time of the event and to time of concentration of the various areas drained.

Even the by-pass daily flow are reported for the days when the other overflow events occurred by Malavasi (2015).

8.11 Sampling collection of CSO discharges

Few studies monitored the CSO compositions and its impact on the receiving water bodies over the time in order to explore the contamination due to fecal bacteria and micropollutants. The difficulty in doing this is to develop a precise sampling campaign for monitoring in parallel the CSO and the river, in order to monitoring the pollutant dynamics in the river during several hours (Passerat et al., 2011).

The overflow events considered, as well as the WWTP effluent, were also characterized as a function of the concentrations of FIB selected, measured by the management of the sewer system. These concentrations were determined using samples collected during a sampling campaign aimed at assessing the impact on the quality of marine waters near the coast of Comacchio's Lidi of CSOs and WWTP effluent against the runoff of the plots agricultural, brought to the sea by the drainage canals when the CSOs of the urban drainage system discharge their flow rate. The sampling plan and analysis just mentioned monitored several significant points in water bodies near the coast, both in the months of September and October 2013, and 2014. The points of interest for to the summer months of 2014 are the points of exit of the CSOs waters and of those treated by the WWTP (see images in paragraph 8.3).

The samples, taken from internal staff, were analysed at the laboratory of CADF S.p.A. located at the Central Water Management of Ro. The laboratory is accredited ISO 9001. Concentrations of Escherichia coli are detected by the laboratory.

The collected samples in all the selected points are "grab", that is to say instantaneous, and were taken with different operational procedures in function of two distinct scenarios:

• periods without rain (dry weather): a sample was collected daily. As regards the present study, the only point of interest monitored in this period is that of the output of the WWTP effluent, which comprises the WWTP effluent and the water that have bypassed the biological treatment passing directly from the primary treatments to disinfection. FIB concentrations detected in this point were not, however, provided by CADF for the 4 summer months being evaluated, however, as stated by CADF, this value can always be considered less than or equal to the legal limits placed on discharges from WWTP effluents (5000 MPN / 100ml for E.coli), if the by-pass is not present flow. Instead, in the case where the latter is present, CADF found in general concentrations less than or equal to 10,000 MPN / 100ml on that point.

• rainy periods characterized by significant rains (rain significant): the number and frequency of samples collected will vary depending on certain conditions evaluated by the management of the sewer system. Specifically, it is assumed that the rain event is relevant when disposing of rainwater requires application of the CSOs of the WWTP for at least 25% of the theoretical capacity (6-step opening of the remote-controlled valve). Once this threshold is reached and the rain was continuing to fall, it is enabled the procedure of sampling in correspondence of all the overflows present at the pump stations and at the WWTP and in correspondence of the treated effluent, according to different requirements.

In particular, the samples taken to the treatment plant are the following:

• WWTP effluent, an hour from the opening of the weir

• CSO output upstream WWTP, an hour from the opening

• WWTP effluent, two hours of opening

• CSO output upstream treatment plant, two hours of opening

• WWTP effluent after three hours the opening of the CSO

• CSO output upstream WWTP three hours after opening

For the other points the number of samples varies according to the duration of overflow and to the number of pumps that are switched on; that is based on the amount of the discharged flow. In general, more samples are taken for long durations and higher flow rates in relation to the maximum flow rate liftable by the plant.

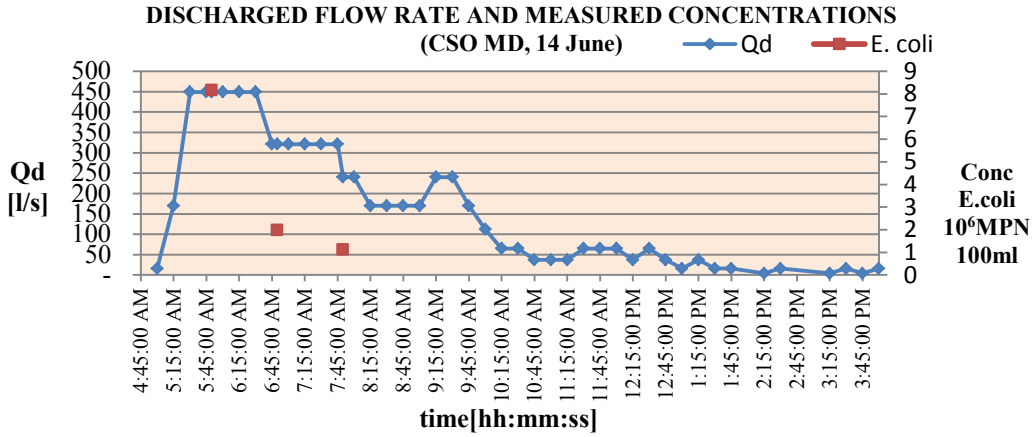


Fig. 8.34: CSO hydrograph and measured concentrations of E.Coli (CSO upstream WWTP, MD)

8.11.1 Different samples and sampling campaign

The water sampling is the collection of representative water quantities for doing analyses. The quality of the measurements is strictly connected to the sampling mode, influencing the uncertainty of the results. Developing an adequate sampling campaign is the first step of an environmental analysis.

Many studies state that the sampling mode can be one of the major source of uncertainty (Ramsey et al., 2007; Ort et al., 2010c). They estimate that the uncertainty correlated to the sampling mode contributes up to the 30-50% of the total uncertainty of the analytical result, being so much higher than that associated with the analytical phase of the laboratory (about 5%).

This phase should be composed by the following phases:

- pianification
- collections of samples
- trasportation and storage
- analysis

The type of sampling that can be collected depends on the flow variability, water quality variability (concentration of the different types of compounds), on the required accuracy and the availability of funds to carry out the sampling campaign and the subsequent phase of analysis in the laboratory.

Mainly, two main category can be identify:

- "grab sample" is a single discrete sample taken over a short time interval (usually not more than 15 minutes). It can be collected manually or using special automatic samplers.

- "composite simple" is a sample obtained from mixing or combining several individual discrete samples taken at well-defined points in time (time proportional) or in proportion to flow (flow proportional) or in proportion to volume (volume proportional).

The frequency, as the frequency to obtain one composite sample, is determined by the concentration variations.

In the following lines, some references about various type of sampling are listed:

- EPA (1992): Storm Water Sampling guidance document. Separate sewage network. In this case the parameters are sampled in the first 3 hours because then the concentrations are lower.
- EPA (1982): procedures for sampling of micro and macro parameters.
- USGS (2005): (http://water.usgs.gov/owq/FieldManual/) information on procedures, handling preservation, but for lake, groundwater not for overflow.
- Ort et al. (2010b): information on sampling frequency (based on pulses) and method

8.11.2 Variability consideration on sampling

Observing the figure reported in Ort et al., (2010c) it is clear that tha sampling mode is to be different depending on the analyzed substance (frequently or rarely discharged). The observed variations can be attributed to intermittent “wastewater pulses” which compose the nonhomogeneous wastewater stream in sewers.

Fig. 8.35: Concentrations of substances with different consumption and excretion patterns

The value of the concentration, at a certain instant depends on the flow rate of that instant, as well as the value of the concentration of a period of time is obtained as the weighted average of the concentrations taken as frequently as possible with respect to their corresponding flow rates in that period. Therefore, if in an hour the total flow rate has negligible variations, one sample per hour can be taken because the weight of that flow rate for the calculation of the concentration of the total composite sample is about the same on all that hour; however, if during the time major changes in flow are registered (because consumption varies or it starts to rain and then stops or both) in order to have an average concentration of that hour, and then throughout the composite sample, take samples in smaller time intervals are to be taken in order to take into account the different weights of the different flow rate. And this (increase of the sampling frequency compared to an hourly frequency) has to be done also in the case of substances infrequently discharged: the frequency must be higher in relation to their low number of pulses in the day.

The variability of the flow in the fractions of time in a day occurs especially because of the events of rain, as the daily trend due to consumption of water taken from the aqueduct is more or less the same (urban consumption is greater in the hours of the day than the night and is highest in the morning (8-12 h) where there is a peak and then there's another peak but lower in the evening (19 -20).

In general, from an hour to another, flow rates may be significantly different at different times of the day, but in dry days you should not record large fluctuations around an average value of time in the hour and then you can use a single value as the average concentration of that hour.

Instead, when it rains for calculating a mean value on the duration of the event of rain a sample every hour weighted on the corresponding flow rate is not sufficient but samples of smaller time intervals are to be preferred in order to consider different weight.

That is: if *it does not rain* make the average concentration over the duration of 24 hours (concentration of the sample composite samples over 24 hours with 1 hour or even less than 1 hour if over every hour the Q is very variable) level the variability of flow between the periods of the day that have higher consumption and lower consumption. In *the case of rain*, the average value of the concentration done only on the period of rain (with a frequency commensurate with the variability of the flow) is certainly lower than the average value made on the same period, however, in the case where it might not rain, because at equal flow containing the substance originating from consumption but is instead more share of water without the substance.

So, Ort et al. (2010c) said that if a suitable tools available it is better to make composite samples of 1 to 2 hours, (of course sampling frequency increased to take account of the variability of Q at that time) for have a weighted average concentration of small durations (1 - 2 hours); then from these average values of small durations you see if there is a lot or little variability during the day and then consider (analyze) a mean concentration on day sense. In fact, the value of the concentration of the entire composite sample also depends on the total time it takes to obtain the composite sample itself, because if this period covers the hours that have very different consumptions of water and substance, the average value will inevitably change.

So, to sample overflow events downstream of CSOs it is good to sample them for the duration of the relief. To take into account the high variability of flow within the single hour (in relation to the variability that the flow rates have into the sewer before the overflow, due to the amount of rain water that turns into flow in a network) higher frequency has to be used for sampling, that is, several times in one hour, to obtain a composite sample with an average concentration representative of the entire CSO event. See Phillips et al (2012).

Moreover, Coutu et al., 2013 recommend an hourly sampling frequency for assessing the intra-day dynamics of PhCs (antibiotics).

The samples for sampling CSO events could be taken in the channel downstream the overflow.

Moreover:

- In rain events the concentrations discharged could not be representative of the massimum load discharged through the overflow because the rain event could be happened when there were a small amount of pulses contributing to the load.
- For substances rarely discharged, we have to choose a representative rain event but also different moment of the day for not over or under - estimating the contribution and the load.

8.12 Model for substance flow analysis

It was decided to develop a simplified model of the urban drainage system under consideration, in order to carry out an analysis of the flows of substance entering and exiting from the system itself for the pollutants concerned (substance flow analysis). In fact, one of the approaches that you chose to apply to quantify loads of pharmaceutical compounds spilled from the wastewater treatment plant and the sewage overflows, is the one that is based on the principle of mass balance, according to which we evaluate all the sources from which the polluted object of study can be entered into the system and the processes that characterize the transfer of such substances along the path of the network and to surface water, and also the processes of transformation, accumulation and removal.

This model is also useful in assessing the possible dilution, with reference to the substances under consideration, that the mixed flow rates sent to WWTP and that CSO flow rates may suffer as a result of the mixing of sewage and rain water during rainfall events (time of rain).

The analysis of the flows of substance, then, is a process that considers the urban drainage system as a closed system, defining the spatial and temporal limits.

The time limit is defined by the period on which the analysis is carried out, that is to say the four summer months of 2014.

The spatial boundary, instead, is defined by the development of the same urban drainage system and is therefore defined by the scheme already presented in Chapter 8.3.

For an analysis of the substance flows, the main compartments of the system so defined may be divided as follows:

• sub-urban;
• drainage system, including lifting equipment, touches and purification;
• surface water receivers (channels and sea).

On the basis of this scheme can then be drawn up the simplified model for the substance flows analysis, which provides to identify inside the compartments of the main system components that characterize the urban metabolism of the selected substances, with reference to the objective treated in this work: the quantification of the load discharged by WWTP effluent and CSOs in surface water.

Such components can therefore be classified by the logic block of belonging in:

• "source processes" that are the sources from which the selected pollutants are entered into the defined system; can therefore be considered as "flow" components being input to the other components "process" of the system, described below.
In our case the sources are constituted by wastewater coming from the sanitation services of civil users connected to the urban drainage system.
Instead, the runoff of agricultural land is not considered for now, although it is certainly important especially for the intake of bacteria as well as pathogenic FIB. This view is confirmed in numerous studies, also the subject of the thesis by students of the Faculty of Engineering of the University of Ferrara, who evaluated how the activities of application of manure and sludge on agricultural land and livestock farming activities in the selected area, can greatly affect the presence of bacteria and pharmaceutical substances in surface water bodies. Even the sampling campaign conducted by

CADF SpA, following the forced closure of bathing in the coastal area of Comacchio during summer of 2013, and other campaigns of surface water analysis in the Province of Ferrara confirm this thesis. As part of this study, for evaluating the only contribution of the urban drainage system, comparing the part relating to sewage overflows with that discharged from the treatment plant, this source input has been placed outside the space boundaries of the model.

• "additional processes", i.e. all parts of the system in which it can verify the transfer, processing, accumulation and removal of the substances considered. In our case they are divided: in pipes and lifting of the drainage system which transports substances from the sources to the sewage treatment plant; in mechanical CSOs transferring wastewater directly to surface water bodies; in the WWTP which eliminates, in whole or in part, the various pollutants through different mechanisms to remove, by entering the load in surface water remained. Even within the network and water bodies receiving can occur mechanisms of accumulation and degradation of pollutants, as well as of reproduction for microbiological ones. The only output from the spatial boundaries of the system is represented by the removal carried out in the WWTP and the accumulation of substances in the sludge, which is then appropriately disposed.

• "flows", that is to say all the quantity of the substance, expressed as loads, input and output from different "processes."

It worth noticing that discharges of sanitary facilities in residential areas in the municipality of Comacchio, during the summer, significantly increase due to the tourist area, while can be considered negligible the contributions of selected micro pollutants from washing out of the urban areas during all year.

The diagram of the simplified model for the substance flow analysis, which is based on the reduced scheme of urban drainage system previously developed (Fig. 8.15), is shown in Fig. 8.36. As it is known, to estimate the overflowed pharmaceutical load in the period under investigation, it is necessary to characterize the model with a number of parameters:

- The flows into the system from sources. This will be done for two selected pharmaceuticals from the consumption of the same;

- The transfer coefficients k and removal of the quantity of substance between the various components of the process system. In particular for CSOs, including the by-pass of the biological treatment, they are transfer coefficients, while for the WWTP the coefficient is the removal efficiency of the WWTP.

The transfer coefficients are evaluated on the basis of the hydraulic balance within the drainage system, carried out with reference to the daily flows mobilized in the selected period. These flow are provided by CADF S.p.A. as the amount of water leaving the WWTP for each day of the reporting period and are thus overall values. So, in order to determine these coefficients k, it is necessary to assess the fraction of the flow consists of black water, that contain PhCs, compared to the fractions of rain water, during rainfall events that lead to CSO events.

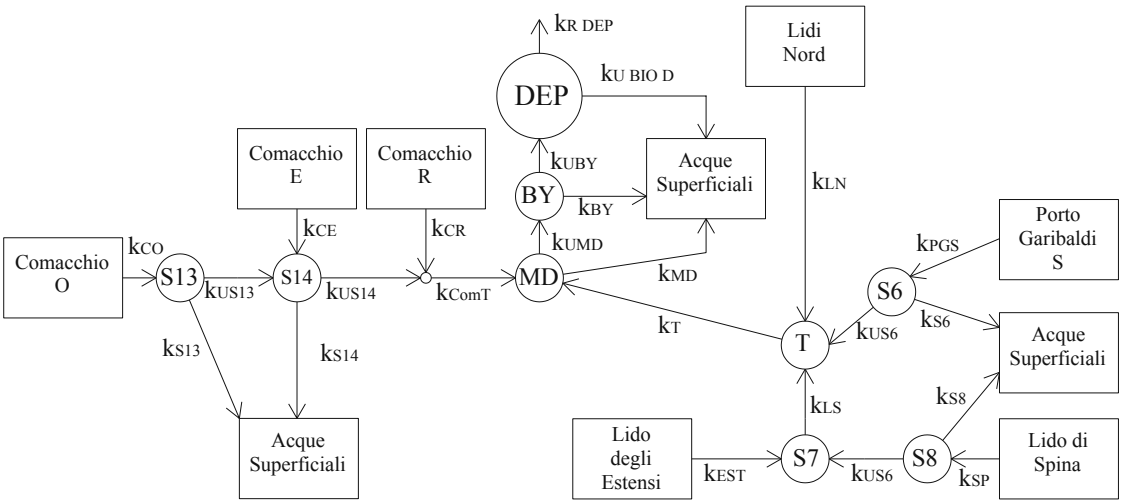


Fig. 8.36: Model diagram for the substance flows analysis.

8.12.1 Estimation of the rate of rain water that reaches the WWTP and determination of transfer coefficients

The rate of rain water in the flow which goes to the WWTP, on each day of the period considered, and from the various sub-basins, is estimated in accordance with the principle of conservation of mass, according to which the flow into the drainage system must be equal to the sum of that exit from the various discharge points (CSOs + WWTP effluent). See Fig. 8.36. Data of rain was provided for June, July, August and September 2014.

Therefore, by summing the total daily flow treated by the WWTP (Q BIO D), provided by CADF, with bypass flow rate (Q BY), estimated, and with the flow exiting the valve located immediately upstream of the treatment plant (Q MD) for the same day, you get the flow entering the plant that comes from the set of urban centres drained from the sewer system (Q IN D). This of course is evaluated for rainy days as CSO events occur. Subtracting the mixed flow thus obtained (Q IN D), the black course overall in that day arrives plant $Q_{n,d}$ (previously defined), it is possible to determine the portion of rain water that remains on the network and arrives upstream of the WWTP during that day $Q_{p,d}$ (Q IN D = $Q_{n,d}$ + $Q_{p,d}$).

The rain flow, estimated as explained in paragraph 8.10.2, must now be broken down to determine the rates that come to each of lifting installations, in order to separately evaluate the contribution of each of the sub-basins to the total rain flow that is sent to the WWTP.

The allocation is performed by multiplying the flow rate to be split ($Q_{p,d}$) for two types of weight:

- The weight due to the amount of rain that fell in the macro-area that comprises the sub-basins; This weight is measured as the ratio between the height of rainfall in each of the three main areas of the considered pluviographs and the total height of rain fall in three main areas, the same day. Therefore, three distinct weights are obtained for each day.

- The relative weight of the contributing area to the rain inflow; this weight is measured as the ratio of the contributing area of the generic sub-basin (previously defined as the product of the coefficient of influx to the total area of the sub-basin) and the overall contributing area of the sub-basins in the same macro-area subdivision on the basis of rainfall data. So you get eight different weights, considering that the area north of Porto Garibaldi, while falling in the area of the northern beaches, however, belongs to the competence of pluviograph P7. In addition, all other towns in Lidi

Nord are rated as overall contribution regarding the flow sent to the WWTP, since there are no overflows.

So, *first of all by multiplying the flow rate $Q_{p,d}$ for the first type of weight is obtained a breakdown on the three macro-areas for diversified characteristics of rain events. Consequently they determine three values of flow rate. Subsequently, by multiplying each of these three values for the corresponding weight of the second type, the values of the rain flow rate coming out of pump stations places on four different lines leading to the WWTP are obtained*. For "Lidi Nord" the final value that comes from the plant S5 is considered.

The total flow rate at the input of each pump station in the generic day is then obtained by the sum of that rate of white water just calculated, with the CSO flow rate and with the black flow rate of the urban sub-basin, also referred to the same day.

The mass transfer coefficients for the substance flow from the CSO to surface water (k_{sfioro}) are defined simply by the ratio between the overflowed flow rate and the total flow rate input to the same. These, according to the outline of the pattern, are therefore: K_{S6}, k_{S8}, K_{S1}, k_{S14}, K_{MD} and also K_{BY}.

The transfer coefficient of the flow from the urban centers to lifting they are obviously all equal to 1 because they represent the inputs to the system (KCO, KCE, KCR, KLN, Kest, KSP, kPGS); the same is true for the coefficients for the set of flow rate from different lines downstream the overflows (KLS, kT, kComT). Therefore the transfer coefficients k of output flow to the WWTP by CSO pumping stations are given by 1- k_{sfioro}, and are kU_{S13}, kU_{S14}, kU_{S6}, kU_{S8}, kU_{MD} and Ku_{by}.

The transfer coefficient of the WWTP effluent with the surface water kU BIO D is determined as the complement to 1 of the removal efficiency of the plant, which represents the transfer/degradation k (kR DEP), which carry the depurative processes depending on the substance.

Below the coefficients relating to CSOs are reported, from which all others can be defined, made for the days when CSO events occur. With these parameters you can determine the loads of substances based on their consumption by urban residents.

Table 8.20: Transfer coefficients

June						
DATE	kS6	kS8	kS13	kS14	kMD	kBY
14/06/2014	0.744	0.000	0.829	0.670	0.215	0.130
17/06/2014	0.508	0.000	0.852	0.706	0.009	0.053
19/06/2014	0.000	0.000	0.000	0.000	0.015	0.129
25/06/2014	0.208	0.000	0.000	0.000	0.000	0.126
26/06/2014	0.000	0.000	0.000	0.000	0.001	0.130
29/06/2014	0.000	0.000	0.000	0.000	0.000	0.130
30/06/2014	0.400	0.000	0.000	0.010	0.057	0.113

July						
DATE	kS6	kS8	kS13	kS14	kMD	kBY
10/07/2014	0.593	0.000	0.000	0.000	0.047	0.103
12/07/2014	0.508	0.504	0.000	0.000	0.032	0.096
13/07/2014	0.000	0.000	0.000	0.000	0.024	0.127
24/07/2014	0.247	0.000	0.000	0.000	0.000	0.125
26/07/2014	0.702	0.622	0.813	0.713	0.149	0.033
27/07/2014	0.000	0.000	0.713	0.000	0.023	0.099
28/07/2014	0.000	0.018	0.000	0.006	0.000	0.130
30/07/2014	0.609	0.245	0.000	0.000	0.427	0.051
31/07/2014	0.000	0.000	0.000	0.000	0.001	0.130

August						
DATE	kS6	kS8	kS13	kS14	kMD	kBY
03/08/2014	0.613	0.000	0.000	0.000	0.155	0.089
04/08/2014	0.000	0.000	0.000	0.000	0.001	0.130
15/08/2014	0.000	0.000	0.000	0.000	0.078	0.120
16/08/2014	0.000	0.000	0.000	0.000	0.006	0.130
20/08/2014	0.000	0.000	0.000	0.000	0.001	0.130
24/08/2014	0.282	0.000	0.000	0.000	0.000	0.125

September						
DATE	kS6	kS8	kS13	kS14	kMD	kBY
01/09/2014	0.205	0.000	0.000	0.000	0.003	0.126
09/09/2014	0.000	0.000	0.000	0.234	0.000	0.124
10/09/2014	0.376	0.000	0.000	0.363	0.065	0.098
20/09/2014	0.656	0.583	0.000	0.147	0.143	0.068

8.12.2 PhC predicted environmental load (PEL) from PhC consumption

The estimate of the loads spilled into surface waters from various discharge points of the drainage system can be accomplished by means of substance flow analysis already described. The substance flow entering the system is to be described. These flows are originated from discharges of sanitary services of civil users connected to the sewerage, and are therefore linked to the consumption of these substances by the population and tourists in the area of interest.

The consumption of the selected compounds, namely **carbamazepine** and **acetaminophen**, has been defined from the national consumption of these substances, retrieved from annual reports OsMed (National Observatory on the Use of Medicines). Specifically have been considered the report of 2013 and 2011, because in the others carbamazepine is considered along with other drugs in a single category. This latest report has also been used in a recent study (Verlicchi et al., 2014) to define the consumption of many substances in the Municipality of Ferrara in order to determine the concentrations PEC input and in output to the WWTP.

Consumption from the report are expressed in DDD/1000ab die (Defined Daily Dose or Defined Daily Dose per 1000 inhabitants per day) on a national basis, it is therefore provided to determine a daily consumption per inhabitant (in mg/ab die) in order to apply it only to the population of the area of interest in the selected period. In this regard it was decided to attribute to tourists the same substance consumption of residents. This is justified by the fact that consumption provided by OsMed report is recognized on the basis of drugs prescription but even on the use of drugs for self-medication made in Italy during the year and, therefore, take into account all possible consumptions (in public structure and for private use).

Furthermore, as the two substances are commonly used for their properties even outside the hospital administration, throughout the year both in Italy and abroad, it can be assumed that they are also used by Italian and foreign tourists during the summer.

Of course the value of consumption is affected by a uncertainty that must be evaluated.

First of all, consumption of these substances has been estimated, expressed in DDD/ab die:

$$Co_{ab,DDD,k} = \frac{Co_{1000ab,DDD,k}}{1000} \qquad \text{(eq. 8.2)}$$

on which:

- $Co_{ab,DDD,k}$ is the consumption of the substance *k* expressed in DDD/ab die
- $Co_{1000ab,DDD,k}$ is the consumption of the substance *k* expressed in DDD/1000ab die

The obtained value, indicated in Table 8.21, was multiplied by the conversion factor of DDD in mg (Conversion Factor CF: active compound mg/DDD), specific for each substance. This value is established by the World Health Organization according to the magnitude of the effects that different substances can have if administered at the same dose. It therefore is no more than the definition of DDD expressed in milligrams. The mass of substance consumed per day per inhabitant is then obtained by the following relation:

$$Co_{ab,k} = Co_{ab,DDD,k} \cdot CF_k \qquad \text{(eq. 8.3)}$$

on which:

- $Co_{ab,k}$ is the consumption of the substance *k* expressed in mg/ab die
- $Co_{ab,DDD,k}$ is the consumption of the substance *k* expressed in DDD/ab die
- CF_K is the conversion factor for the substance *k* to switch from DDD to mg

The amount of consumed drug is partly absorbed by the body and partly excreted ($Co_{ab,k,E}$) or in the form of the same active principle or as metabolites. Every substance, therefore, is characterized by a factor of excretion E_k. This factor must be applied to the consumption in order to determine the quantity of substance that daily and per inhabitant enters the sewage system. The application is given by the following simple relationship:

$$Co_{ab,k,E} = Co_{ab,k} \cdot E_k \qquad \text{(eq. 8.4)}$$

From the literature it is noted that the mean factor of excretion for the acetaminophen is 0.8, while for the carbamazepine is reported a fairly wide range variable from 0.01 to 0.30. In this case it was decided to use a factor of excretion for this second substance equal to 0.08, as using a E_k 0.30 in the concentration of carbamazepine was overestimated, both in water input and output of WWTP Verlicchi et al. 2014). This study was conducted also in an area (the Municipality of Ferrara) close to that considered and inhabited by many of the people who go to vacation during the summer in the seaside resorts of Comacchio.

Table 8.21: PhC consumption and excretion factor

Composto	$Co_{ab,DDD,k}$ **[DDD/ab·die]**	CF_k **[mg/DDD]**	$Co_{ab,k}$ **[mg/ab·die]**	E_k [-]	$Co_{ab,k,E}$ **[mg/ab·die]**
Carbamazepine	0.00146	1000	1.46	0.08	0.12
Acetaminophen	0.0061	3000	18.30	0.80	14.64

Now, just by multiplying the amount calculated for the resident and tourist population of each of the urban areas, considered separately according to the scheme of the model developed (Fig. 8.36), you can calculate the input load to the urban drainage system, from various points of entry, for the two substances of interest.

So, starting from these input loads, applying the model for the substance flow analysis, (that imply the transfer coefficients k and removal), the corresponding load spilled in receiver water bodies by the WWTP effluent and by CSOs in period was determined. In this regard it worth noticing that the removal efficiency of WWTP of carbamazepine, in a plant that carries out the treatment of denitrification-nitrification (as that considered), it can be taken equal to 30%, while for the acetaminophen may be assumed equal to 98%, as confirmed by literature data.

The results of discharged load (g/d) are shown in Malavasi (2015) by means of tables and graphs. The CSOs load from various points was also expressed in percentage of the total entered the surface water, for each of the days in which overflow occurs and for each of the months in total.

In the following graphs the discharged load id reported in percentage of the total entered the surface water for each month and for each discharge point.

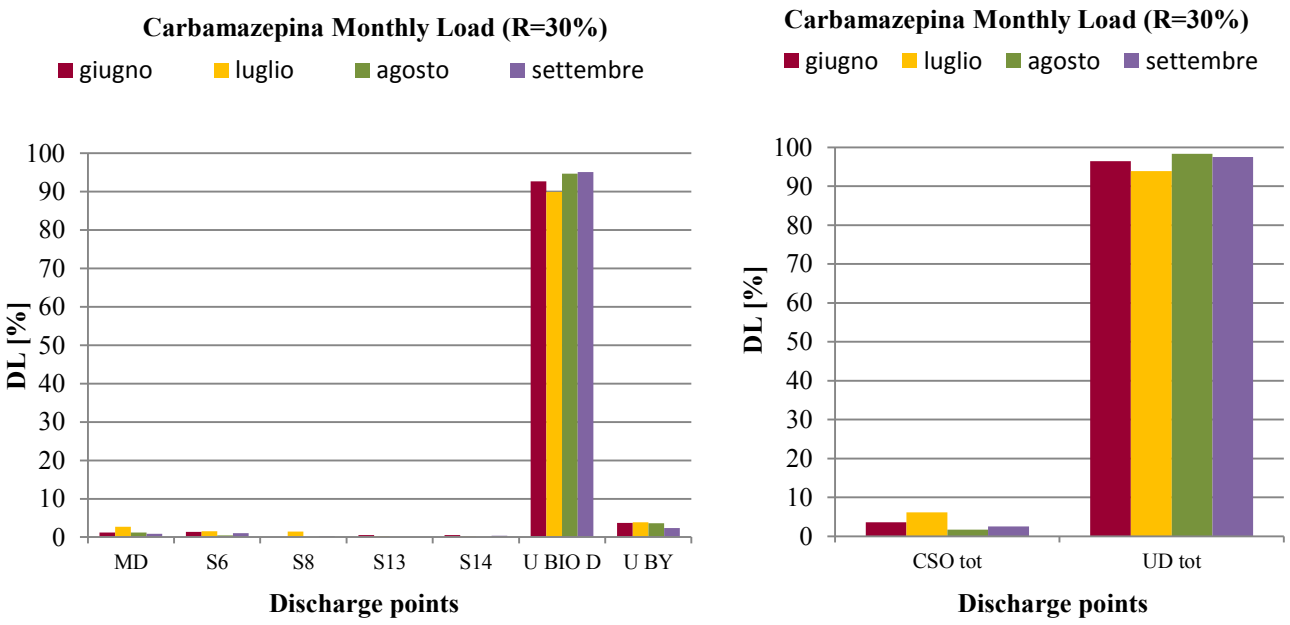


Fig. 8.37: Monthly discharged load for carbamazepine. R is the removal efficiency

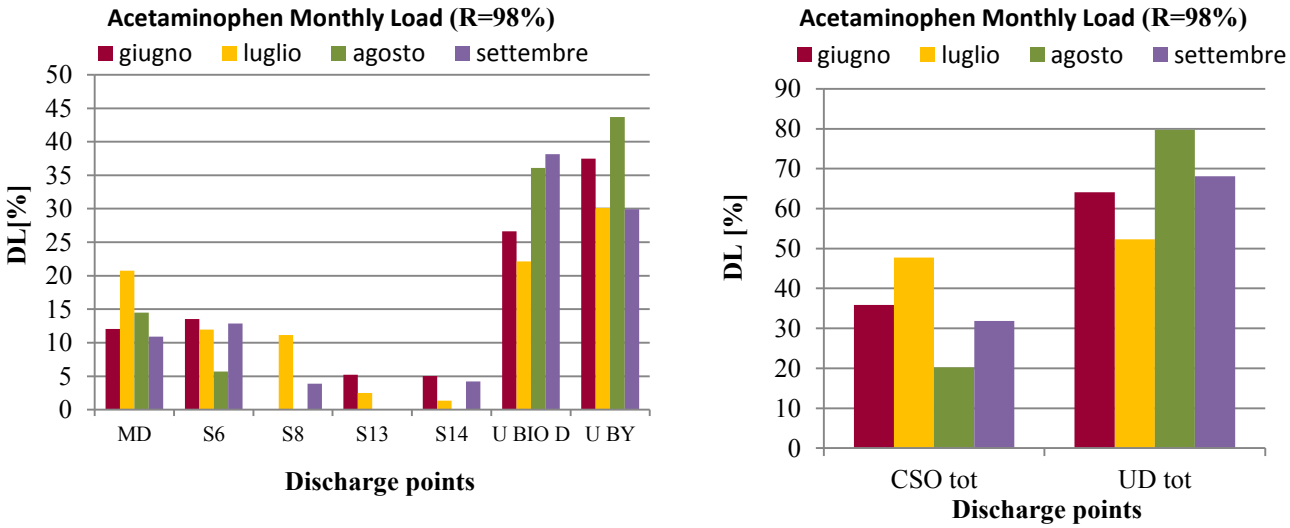


Fig. 8.38: Monthly discharged load for acetaminophen. R is the removal efficiency

8.12.3 PhC predicted environmental load (PEL) from literature concentration data

Being not yet performed a suitable measurement campaign for the concentrations of selected drugs or other medications tracers of anthropogenic pollution, it was decided to assume, as a first approximation, concentration values constant over the day, both in the waters overflowed that in those exiting the WWTP and equal for all the period considered. This is also justified by the fact that, while for CSO events the trend of discharged flow is available with time for all the duration of the event, for the WWTP only the values of the overall daily flow rate are available, from which the values of total flow circulating in the network in a generic day are also defined. For these flow there is not variability related to the time, from which understanding the real trend of the concentration (higher or lower dilution). The fact of considering the same concentrations in every day of the summer, despite the different number of inhabitants in the urban basin, is due to fate that the greater consumption and excretion the greater the flow entering the sewer system.

The concentration values used were derived from those previously seen in literature, which can be considered plausible given the characteristics of urban basins investigated (higher use of residential than business, craft and open area; ratio between population and urbanized area, ratio between number of inhabitants, flow and consumption) and also the seasonality of rainfall events and CSOs events considered in summer months.

The general criterion was to consider, even during the days when you have overflow events, concentrations in the WWTP influent almost identical to those that occur in the same place in dry weather. This is justified by the fact that, in our case, rainfall events are of short duration and generally do not affect the whole day, therefore, the total daily flow treated by the plant is not all the time mixed with rain water that does not contain such substances. Furthermore, in our case, it was found that, in dry weather, there is a considerable infiltration flow rate from groundwater and drainage canals. Its entity was often equal to that due to the rain water that remain within the urban drainage system reaching the treatment plant, without being overflowed.

This fact can lead, thus, maintaining similar concentrations in the two different conditions (dry and rainy) for the flow coming to the treatment plant, since it is believed that in the flow rate that infiltrate nil or very small concentrations of the investigated pharmaceuticals are present. Even in literature data the range of concentrations that enter the WWTP in dry and wet weather is extremely variable. Therefore, the values chosen in our case for the calculation of the loads, can still reflect a dilution of the flow incoming the system during rain periods.

As regards the concentrations in the water discharged by spillways, it has been considered a lower value of concentration, even only slightly (same order of magnitude) if compared to flow rates simultaneously sent to the WWTP. It is therefore considered less dilution of water remaining in sewer and arriving at the WWTP than those placed in surface water by CSOs. This is confirmed in the same literature data and by the fact that, while the flow rate which reaches the WWTP during the day always includes the majority portion of the black water (only black water in dry weather), the flow exiting from the CSOs starts to be discharged when the fraction of the rain water is considerable compared to black water. In fact, CSOs must operate when the amount of white water in arrival is such that the mixed flow, at which CSO begin to operate, is diluted in order to be discharged in the receiving water bodies at least in respect of the legal limits provided for the typical macro-pollutants.

The calculation of loads for the three substances was carried out by simply applying its definition, bearing in mind that the concentration is assumed constant for the all duration of the discharge:

$$\boldsymbol{DL_k = \frac{M_k}{t} = C_k \cdot Q \cdot 10^{-9}} \qquad \text{(eq. 8.5)}$$

in which:

• DL_K is the load of substance (k), defined as the ratio between the mass of the substance (MK) and the total time t in which it is quantified, expressed in g/d
• C_k is the concentration in water of a substance in ng/l (converted to g/l with the factor 10^{-9})
• Q_k is the flow rate which corresponds to a determined concentration value C in l/d (that is, the total number of liters in a day).

The results obtained are shown by means of tables and graphs reported in Malavasi (2015). The table report the load, expressed in grams per day (g/d), output from the CSOs and from the WWTP in the days of the four months when there were overflows. The same tables also show the load spilled through the same points on the total number of days of each month. In another table, instead, it is indicated the value of the load spilled from various points in percentage of the total entered in surface water, and this always for each of the days in which overflow occurs and for each of the months in total. Herein, the main results about carbamazepine, acetaminophen and caffeine are reported.

The following graphs report the monthly flow rate discharged in surface water from various points as a percentage of the total discharged flow.

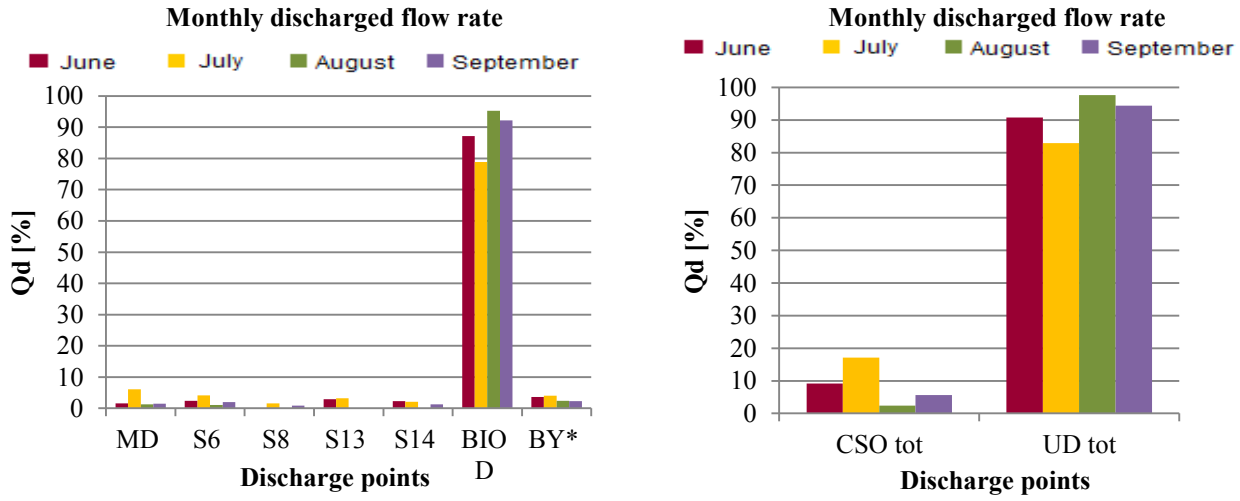


Fig. 8.39: Monthly discharged flow rate as a percentage of the total discharged flow

Table 8.22: Literature data considered in this analysis

	Carbamazepine	Caffeine	Acetaminophene
Selected concentration for WWTP (BID) and Bypass (BY)	229 ng/l	7482 ng/	20000 ng/l
Removal efficiency considered in WWTP (BIO D) (biological and nitrification)	30%	92 %	98 %
Concentration calculated in WWTP effluent (BIO D)	160 ng/l	599 ng/	40 ng/l
Concentration output bypass (BY)	229 ng/l	7482 ng/	20000 ng/l
Concentration in the various CSOs	184 ng/l	3248 ng/l	7150 ng/l

RESULTS CARBAMAZEPINE

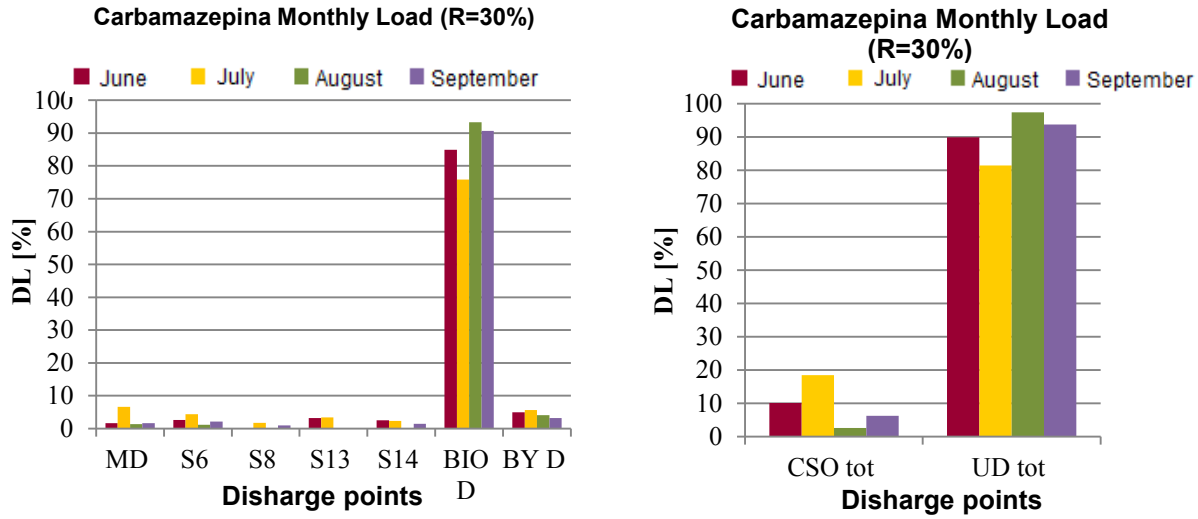


Fig. 8.40: Discharged load of carbamazepine as percentage on the total discharged flow

RESULTS CAFFEINE

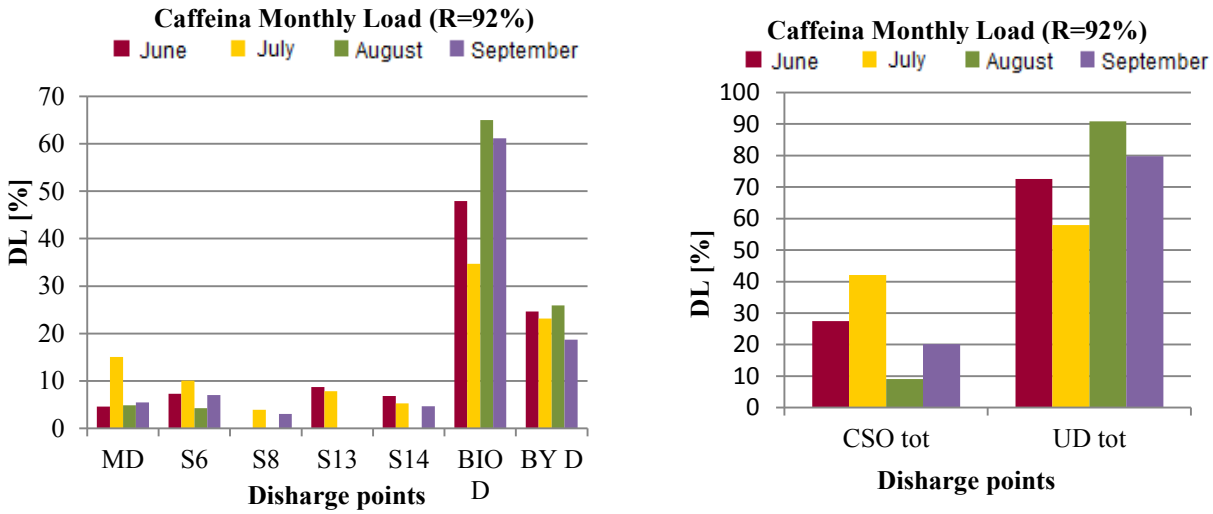


Fig. 8.41: Discharged load of caffeine as percentage on the total discharged flow

RESULTS ACETAMINOPHENE

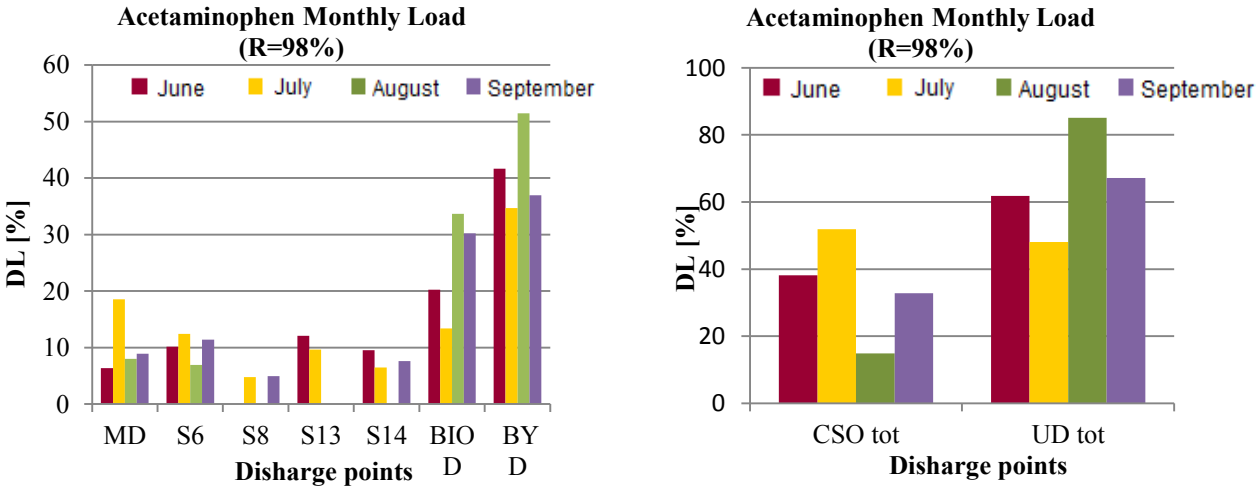


Fig. 8.42: Discharged load of caffeine as percentage on the total discharged flow

8.12.4 Load estimation of FIB from measured concentration

The approach used to quantify the E. coli load, spilled by the CSOs and by WWTP effluent onto surface water, is essentially based on the elaboration of data of discharged flow rate and values of the corresponding detected concentration.

The measured concentration for overflow events, in fact, are to be considered realistic and representative even if assessed from grab samples, as are in agreement with the values reported in literature that, instead, derived from composite samples used for better understanding the impact of variability of the flow and of the quantity of substance (FIB) present into the network on the concentration values determined by laboratory.

In fact, for a given quantity of a substance present in discharged water the concentration value may be greater or lesser depending on the degree of dilution brought about by the portion of water that does not contain microorganisms or investigated compounds. While at the same, flow rate is necessary to consider the trend (pattern) with which the FIB and the investigated substances are typically introduced into the network, depending on the sources that produce them.

Studies of literature considered overflow events in the summer, as in our case, and events that occurred in other seasons of the year, for different types of urban basin with respect to the number of inhabitants, use and extent of the drained area, network length and characteristics of the same. The presence of E. coli is still mainly associated with fecal material and, therefore, in an urban drainage system, sanitary discharges of civil users. These discharges can be considered homogeneously distributed in space and over the day. However, the daily graph of concentrations of FIB in dry weather at the entrance of the WWTP is characterized by a growth in the morning, a peak in the afternoon and then a gradual decrease, in accordance with the pattern of excretion by the population, which provides a peak during morning, and the residence time of the drainage system.

Based also on the considerations reported in paragraph 8.8.3, it has been possible to assess the CSOs contribution to the load of FIB, it was decided to use the measured concentrations from CADF on the overflowed water, assigning each instantaneous value detected to flow out until that moment. The CSO event is thus divided into concentration ranges with values decreasing over time. The same value is still assigned for a further duration to discharges which extend beyond the last detected concentration value. The duration is function of the same discharge event duration and based on the just exposed assessments on the variability of concentrations. In accordance with these assessments, the concentrations for the remaining overflow time has been reduced. This was also applied to events for which only one value for the whole duration of the discharge is available (only a few cases of reduced duration). For events in which instead was not detected any concentration, because of the brevity of the overflow event and/or of its modest entity, they are applied values equal to those of events which occurred in the other discharge points as a result of the same weather event, or occurred at the same point during other rain events with similar characteristics. In these cases it is also taking into account the different tourist flow. It should be recognized, however, that these overflows affect very little and can be neglected. Similar reasoning were applied to events that have not concentration values, but which have considerable flow and duration (two in July, one in August and one in September). It should also be noted that on some CSO events has been activated a disinfection with peracetic acid, determined according to the capacity of the pumps switched on, and then the concentrations are extremely low.

In order to evaluate, the contribution arising from the WWTP, it was decided to assign at the effluent which has undergone all treatments a value of 5000 MPN/100ml, to take account of the fact that, even if the legal limits are always respected daily, leak out of the activated sludge may occur and that in any case the managing body, as provided by law, consider the concentration once a day.

The precise value of these concentrations was not provided. Also for these reasons, and to take account of the lower treatment accorded to flow bypassing the biological, are attributed to these values precautionary 10000 MPN/100ml (upper limit generally detected by CADF); in fact during some weather events of 2013 the effluent concentrations were well above the limit of 5000 MPN/100ml.

The FIB load has been estimated basing on assumptions described so far and simply applying the definition of load for a substance:

The following tables show so load values of FIB estimates based on assumptions described so far and simply applying the definition of load for a substance:

$$\boldsymbol{DL_k = \frac{M_k}{t} = \sum_i C_{i,k} \cdot Q_i \cdot 10 \cdot \Delta t_i} \quad \text{(eq. 8.6)}$$

on which:

• DL_K is the load of substance (k), defined as the ratio between the mass of the substance M_K and the total time t in which it is quantified; in this case the substance *k* is made once by the bacteria E. Coli (from which $DL_{E.Coli}$). It is expressed MPN/d that day, but for spillways is related to the length of the weir for every day.
• C_k is the concentration in water of a substance, in this case the two FIB considered valid for a particular time interval i in which there is the corresponding flow rate, and therefore expressed in MPN/100ml;
• Q_i is the flow rate which occurs for a certain time interval Δt_i and to which corresponds a certain concentration value C_i, is expressed in l/s, to be multiplied by 10 to convert it into ml/s;
• Δt_i is the time interval in seconds in which occurs a certain flow rate Q_i and are responsible for a certain concentration

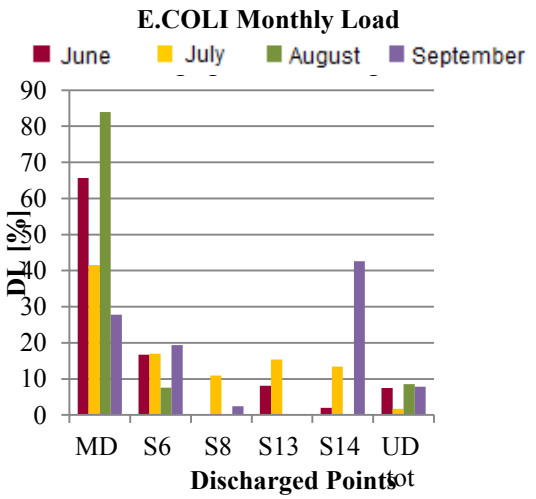
In Malavasi (2015) the loads estimations for E. Coli, in correspondence of the individual overflow events and divided by the point of discharge (overflows and WWTP) has been reported. Since this division the monthly contribution is obtained, and for the whole summer period, the total load spilled into surface waters from each of the discharge point of the urban drainage system under consideration. All estimates are provided both in absolute terms, as the amount of substance from any point, and in relative terms, as a percentage of the load discharged by each input in receiving water bodies out of the total discharged load.

It worth noting that, according to the characterization of overflow events previously made, the different incidence of each discharge depends on the type, the location on the network, the duration, extent and characteristics of the raised meteorological events.

A comparison between the sources of bacteria during overflow events is possible. As you would expect spillways affect much more than the output of the WWTP, even when considerable amounts of water bypassing the biological treatment. The overflow upstream the WWTP, in all the cases when present, is found to be the point of greatest impact.

Table 8.23: E.Coli load dicharged in different points

	ESCHERICHIA COLI							
	June							
	DATA	DL UD tot [MPN/d]	DL MD [MPN/d]	DL S6 [MPN/d]	DL S8 [MPN/d]	DL S13 [MPN/d]	DL S14 [MPN/d]	DL CSO tot [MPN/d]
June	14/06/2014	1.2E+12	1.41E+14	3.61E+12		2.81E+13	6.32E+12	**1.79E+14**
	17/06/2014	1.4E+12	1.02E+12	3.32E+13		1.45E+11		**3.44E+13**
	19/06/2014	7.0E+11	3.96E+11					**3.96E+11**
	25/06/2014	9.1E+11		3.10E+12				**3.10E+12**
	26/06/2014	1.4E+12	5.54E+10					**5.54E+10**
	29/06/2014	1.4E+12						**0.00E+00**
	30/06/2014	1.4E+12	8.61E+13	1.83E+13			7.20E+11	**1.05E+14**
	tot month [MPN/month]	**2.61E+13**	**2.29E+14**	**5.83E+13**	**0.00E+00**	**2.82E+13**	**7.04E+12**	3.22E+14
July	10/07/2014	1.8E+12	2.39E+13	9.62E+13				**1.20E+14**
	12/07/2014	1.3E+12	9.71E+13	7.57E+13	7.26E+13			**1.73E+14**
	13/07/2014	1.5E+12	2.58E+13					**2.58E+13**
	24/07/2014	1.0E+12		2.10E+13				**2.10E+13**
	26/07/2014	1.1E+12	3.86E+14	1.18E+14	1.26E+14	1.99E+14	2.45E+14	**9.48E+14**
	27/07/2014	1.8E+12	7.03E+12			8.25E+13		**8.95E+13**
	28/07/2014	1.8E+12			8.42E+11		5.12E+11	**5.12E+11**
	30/07/2014	1.6E+12	2.19E+14	6.42E+10	4.15E+10			**2.19E+14**
	31/07/2014	1.7E+12	1.80E+10					**1.80E+10**
	tot mese [MPN/ month]	**3.1E+13**	**7.6E+14**	**3.1E+14**	**2.0E+14**	**2.8E+14**	**2.5E+14**	1.60E+15
August	03/08/2014	1.7E+12	2.55E+14	2.68E+12				**2.58E+14**
	04/08/2014	1.3E+12	1.44E+11					**1.44E+11**
	15/08/2014	1.2E+12	8.30E+13					**8.30E+13**
	16/08/2014	1.2E+12	7.70E+11					**7.70E+11**
	20/08/2014	1.0E+12	4.36E+11					**4.36E+11**
	24/08/2014	1.4E+12		2.81E+13				**2.81E+13**
	tot mese [MPN/ month]	**3.4E+13**	**3.4E+14**	**3.1E+13**	**0.0E+00**	**0.0E+00**	**0.0E+00**	3.71E+14
September	01/09/2014	1.2E+12	1.46E+12	1.41E+13				**1.56E+13**
	09/09/2014	6.5E+11					1.01E+13	**1.01E+13**
	10/09/2014	1.1E+12	3.29E+13	7.38E+12			8.03E+13	**1.21E+14**
	20/09/2014	8.5E+11	3.18E+13	2.47E+13	5.77E+12		1.12E+13	**6.78E+13**
	tot mese [MPN/ month]	**1.9E+13**	**6.6E+13**	**4.6E+13**	**5.8E+12**	**0.0E+00**	**1.0E+14**	2.14E+14



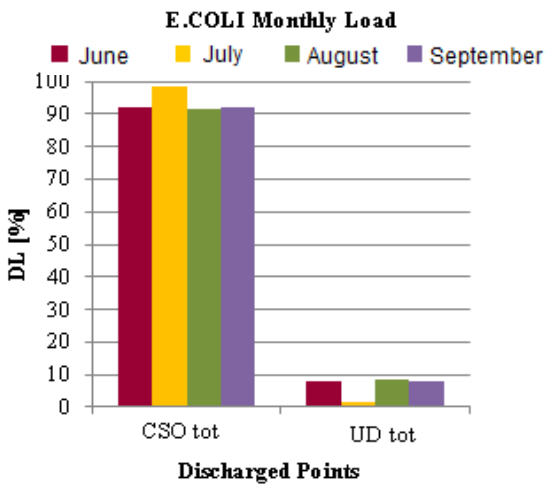


Fig. 8.43: Monthy load of E.Coli discharged in different points as percentage out of the total discharged load

Passerat et al. (2011) estimated that the total load of E.Coli discharged during the CSO event (6 h) was $8.7*10^{15}$ CFU. This value was compared with those discharged by the WWTP effluents.

It was estimated that the CSO discharged 79 more E.Coli than WWTP eeffluent. The total load discharge by the three analysed WWTP was $1.1*10^{14}$ CFU for E.Coli.

The major contribution of the increased concentration is due to the resuspension of suspended matter present at the interface between water and the gross of the bed. This layer is favorable for the survival of fecal bacteria and part of E.Coli is expected to be in an attached form in these sediments.

8.13 Conclusions

Although the flow discharged from the CSOs of the urban drainage system in question is, for the period under consideration, much less than that entered in surface water bodies from the WWTP (about 9% for June, 15% for July , 2% to 5% for August and September on the total flow entering the network), the load of bacteria made from these water is much higher in all months analyzed. This happens even when considerable amounts of water bypassing the biological treatment and are subjected only to the primary treatment and disinfection. These results confirm what was expected; these treatments are able to efficiently remove the bacterial load.

Instead, the loads of selected pharmaceutical compounds, estimated both with the model for the substance flows analysis that using the concentrations taken from the literature, confirm the fact that for substances more efficiently removed in the WWTP (ACE and less CAF), the contribution of the CSOs is greater than that of the substances less efficiently removed (CBZ). For the first type of substances, the values on a monthly basis show that the percentage of load spilled from the totality of the spillway, with respect to the overall quantity injected by all the points of discharge together (WWTP + overflows), is always largely increased to 10% with peaks at over 50% for Acetaminophene in July, which is the wettest month with the greater overflowed flow rate. Similar values are also found for Caffeine. For the second type of pharmaceutical compounds, however, the percentage of load spilled from the CSO overall stood at levels well below the 10% for August and September, joining him in June, and they were still below 20% in July.

For the same reason, from the comparison in equal substance between load percentage spilled from the WWTP (including by-pass) and percent made by CSOs, it is clear that, for the substances more efficiently removed by WWTP, the contribution of the plant is smaller than the more readily degradable substances.

It is noted that the load estimations obtained with the model of the substance flows analysis are in agreement, even if they show some small difference, with those obtained starting from the concentrations.

It is therefore considered that the prediction of the loads spilled based on consumption is to be considered a useful and valuable tool, with the possibility to adopt, software that can carry out the substance flows analysis in order to have much more precision and slenderness of calculation. This is also confirmed by studies in the literature.

Therefore, in terms of strategies for pollution control in this time of year, it is clear that to reduce the load of bacteria entering the surface water bodies from urban drainage system, we need to act

at overflow level, at least on those most abundant and prolonged, performing even a simple disinfection with peracetic acid, such it has already been tested by the same CADF SpA in some cases (such dosage hypochlorite 10 ppm). Especially we note that it would be better to act on the CSO upstream of the treatment plant, the more frequently in operation. Also, note that in this study only compared the contribution to the load of bacteria made from sewer overflows and WWTP effluent, without considering that due to the runoff of agricultural land which, on the basis of the studies cited in the thesis, was found to be high for the bodies of the Province of Ferrara. The waters of these latter provide, in fact, concentrations of FIB very high throughout the course of the year.

For pharmaceutical substances, however, it is recommended those additional treatments in the WWTP, rather than just on CSOs, considering that they may work only in this time of year, when the increase in population leads to higher inputs substances into the sewer system. Moreover, in this regard, it must keep in mind that caffeine and especially acetaminophen, are substances that, even in the aquatic environment without treatment, undergo a fast decay with greater degradation than carbamazepine. Then it is also a substance more dangerous for its effects on living organisms than the first two.

On the basis of these considerations, additional treatments to be included in the WWTP may take into account the fact of increasing the efficiency in removing only Carbamazepine, since as seen for acetaminophen and caffeine, the efficiency is already high. However, these treatments should further degrade even these two substances.

Treatments that have shown large increase in removal efficiency for the Carbamazepine are the use of ozone (95%) or powdered activated carbon combined with ultrafiltration (90%). It should be noted, however, that these processes are very expensive and, for the case in question, given the seasonal nature of the application, may be uneconomic. The use of activated carbon powder to be mixed only during peak periods could therefore be the best solution.

In addition, it should be stated that it is necessary to analyse a greater number of PhCs having low removal efficiencies, over a period of observation even greater and repeated over time, in order to determine which further treatments perform in the system, and whether it is really necessary to introduce them considering together with the aspect of environmental protection and health of living things also the economical one.

CONCLUSIONS

As it has been demonstrated that PPCPs may show toxic effects in the environment, it is very important to investigate the presence of these compounds in urban and hospital wastewater and also to analyze different strategies in order to reduce their discharge into surface water. This work has this clear aim.

First of all, the overview of the PhC concentrations in WWTP influent and effluent, carried out collecting literature data referred to conventional activated sludge systems, identify the compounds that exhibit the highest concentrations. Moreover, analyzing PhC removal efficiencies, it was possible to identify the most recalcitrant compounds. PhCs chemical and physical properties, (namely solubility, volatility, adsorbability, absorbability, biodegradability, polarity) have been discussed considering their repercussions on PhC behavior during the treatments. Among the many factors governing the complex interactions in treatment systems, lipophilicity was found to influence the sorption of pollutants onto sludge. In fact sludge originates during biological processes in WWTPs contains a wide spectrum of PCPs which are separated from the liquid phase during treatments.

For this reason, an in-depth analysis of the occurrence of PCPs in untreated and treated sewage sludge was carried out basing on literature data. There is an ongoing debate within the scientific community in order to evaluate potential (environmental) risks in this kind of practice, due to the occurrence of toxic and persistent substances in sludge. In this work an environmental risk assessment has been performed in order to identify the compounds that pose the highest risk and the critical aspects of the method.

Not only conventional treatments but also natural ones are investigated in the thesis.

Constructed wetlands (CWs) are systems where different micro-environments contribute to the removal of PPCPs. The occurrence of these compounds in influent and effluent of different kinds of CWs, as well as the corresponding removal efficiency, were investigated collecting data from literature works. The main factors (design parameters and operational conditions) that affect the removal were discussed in order to identify the potentiality of this system.

After the analysis of literature data, in order to evaluate the removal efficiency achieved by a conventional activate sludge system followed by a CW and their respective contribution, an experimental campaign was performed at a large municipal WWTP in the Po Valley, northern Italy, where a pilot CW station, directly fed by the WWTP secondary effluent, has been in operation. The PhC mass load in the raw WWTP influent and secondary effluent, as well as in the polished effluent, was analysed. The mass loads discharged in the presence and absence of this polishing treatment were compared, underling that CWs may efficiently contribute to the removal of most of the selected compounds.

Moving the attention to hospital effluent, literature data were collected in order to review and discuss lessons learned from previous investigations and studies carried out on dedicated treatment of hospital WW in different countries worldwide. It offers a critical analysis of data collected from lab, pilot and full scale treatment plants acting as primary, secondary and tertiary steps, underling that in Europe dedicated treatment for hospital WW are equipped with a tertiary step. Attention is paid to the removal efficiencies observed for contaminants, including conventional parameters but in particular emerging ones: mainly PhCs, detergents and disinfectants.

Hospital effluent concentrations could be difficult due to not only to high analysis costs of the investigations, but also due to the difficulties in organizing water sampling campaigns inside health facilities, a model for predicting concentration based on PhCs consumption was evaluated. Predicted and measured concentrations in the effluent of a hospital located in North Italy were compared. The different results between the two models were discussed considering the potential factors that could affect both the methods. The parameter of the model that most influence the results were investigated through a sensitivity analysis.

Finally, the attention was posed to combined sewage overflows (CSOs) present along the sewage network. In fact, PhCs cannot get treatment and may be discharged directly into the water body through CSOs during rainfall events of particular entities. The study quantify the contribution of the load of faecal indicator bacteria (FIB) and of three selected PhCs discharged by CSOs in the area of Comacchio, Italy. This work give indications about the management and treatment of the water flow discharged by CSOs. In order to further reduce the load discharged in surface water, a treatment at CSO would be useful for the pollutants efficiently removed by WWTP.

This work provides the knowledge of PPCP concentrations in municipal and hospital wastewater and removals obtainable with the most common treatment systems. This knowledge will allow you to consciously manage the wastewater and environmental risk by applying various strategies to reduce the pollution load spilled into the surface water body.

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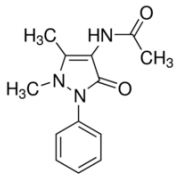
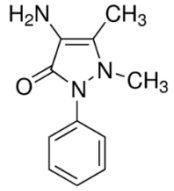
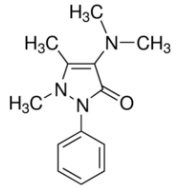
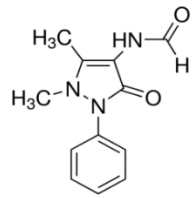
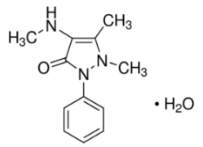
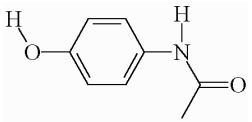
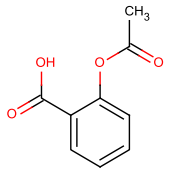
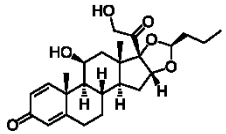
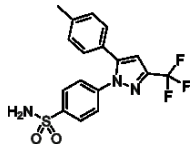
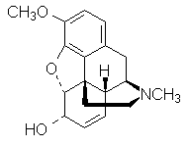
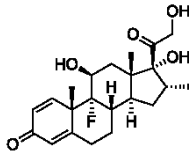
APPENDIX A

Physico-Chemical Properties

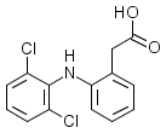
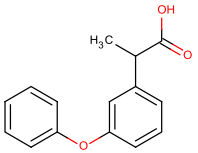
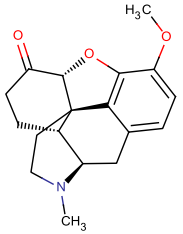
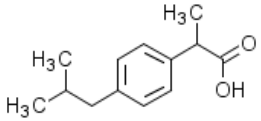
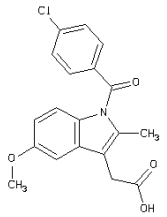
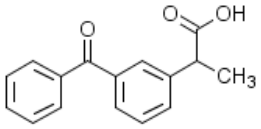
Appendix A

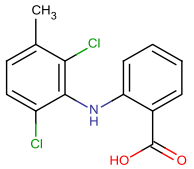
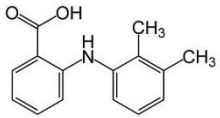
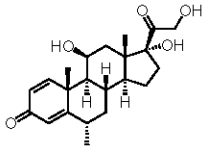
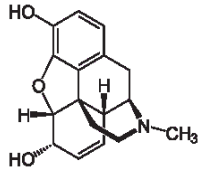
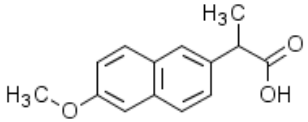
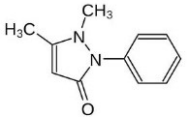
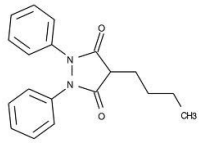
Table A 1: List of PhCs reviewed in this thesis, grouped according to their therapeutic class, with the corresponding physico-chemical properties. Kbiol data with a star as apex refer to MBR systems while others refers to CAS systems. [$PNEC_{water}$ indicated with a star (*)derive from Orias and Perrodin, 2013; (^) from Santos et al., 2013]

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	$PNEC_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Analgesics/Anti-inflammatories	4-acetamidoantipyrine CAS # 83-15-8						Negative		Chapter 6	Verlicchi et al., 2015
	4-aminoantipyrine CAS #83-07-8						Neutral		Chapter 6	Verlicchi et al., 2015
	4-Dimethylaminoantipyrine CAS # 58-15-1						Neutral		Chapter 6	Verlicchi et al., 2015
	4-formylaminoantipyrine CAS #1672-58-8						Negative		Chapter 6	Verlicchi et al., 2015

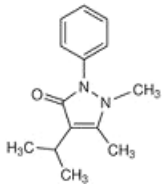
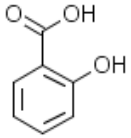
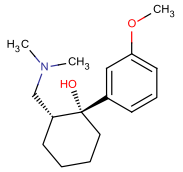
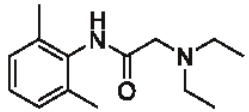
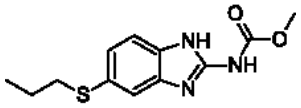
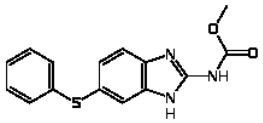
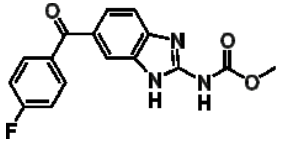
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	4-methylaminoantipyrine CAS # 1619981-09-7						Negative		Chapter 6	Verlicchi et al., 2015
Analgesics/Anti-inflammatories	Acetaminophen CAS # 103-90-2	9.38	0.46	3.06[t]	58-80 106*-240*	1	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7 Chapter 8	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015; Verlicchi et al., 2015;
	Acetylsalicylic acid CAS # 50-78-2	3.5[h]	1.13			61	Negative		Chapter 1 Chapter 6	Verlicchi et al., 2013b; Verlicchi et al., 2015
	Budenoside CAS # 51333-22-3								Chapter 4	Verlicchi and Zambello, 2014
	Celecoxib CAS # 169590-42-5						Negative		Chapter 4	Verlicchi and Zambello, 2014
	Codeine CAS # 76-57-3	8.21	1.19	1.15[j]	4.7-4.8 [j]	16	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Dexamethasone CAS # 50-02-2					1	Negative		Chapter 6	Verlicchi et al., 2015

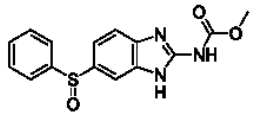
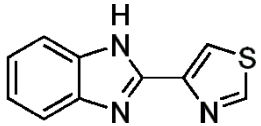
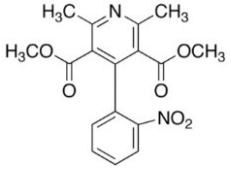
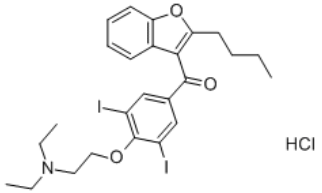
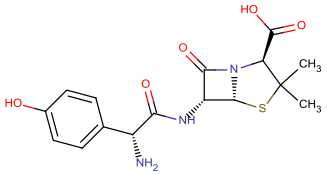
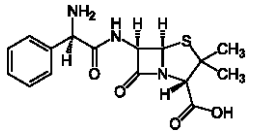
Appendix A

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Diclofenac CAS # 15307-86-5	4.15[a]	4.51/0.7	1.2[l]	<0.04-1.2 [o] ≤0.1 ≤0.1* <0.002*-<0.1* [s]	9,7	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015; Verlicchi et al., 2015
	Fenoprofen CAS # 31879-05-7	7.3	3.9		10-14 3.3*-5.9*		Negative		Chapter 1 Chapter 4	Verlicchi et al., 2013b; Verlicchi and Zambello, 2014
Analgesics/Anti-inflammatories	Hydrocodone CAS # 125-29-1	8.48	2.16	1.23[j]			Positive		Chapter 2	Verlicchi and Zambello, 2015
	Ibuprofen CAS # 15687-27-1	4.51[e]	3.97/0.45	0.9[l]	1.5-20 [o] 21-35 9*-22* 1.33*->3* [s]	1,65	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015; Verlicchi et al., 2015
	Indomethacin CAS # 53-86-1	4.5	4.27		≤0.3 ≤0.21*	3,9	Negative		Chapter 1 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014
	Ketoprofen CAS # 22071-15-4	4.45[f]	3.12/-0.44	1.2[t]		15,6	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Meclofenamic acid CAS # 644-62-2		6.02				Negative		Chapter 1	Verlicchi et al., 2013b;
	Mefenamic acid CAS # 61-68-7	4.2	5.12	2.6[t]		0,43	Negative		Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
Analgesics/Anti-inflammatories	Methylprednisolone CAS # 83-43-2					0,026*	Negative		Chapter 6	Verlicchi et al., 2015
	Morphine CAS #57-27-2						Positive		Chapter 6	Verlicchi et al., 2015
	Naproxen CAS # 22204-53-1	4.2[b]	3.18/-0.34	1.1[o]	<0.2-9 [o] 1.0-1.9 0.4*-0.8* 0.08*-0.4* [s]	2,62	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Phenazone CAS # 60-80-0	1.4	0.38			1,1	Neutral		Chapter 1 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlcchi and Zambello, 2014
	Phenylbutazone CAS # 50-33-9	4.5	3.16			0,026*	Negative		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014

Appendix A

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Propyphenazone CAS # 479-92-5	---	1.96			0,8	Neutral		Chapter 1 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014
	Salicylic acid CAS # 69-72-7	3.5 [b]	2.26/-2.42			1,28	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Tramadol CAS # 27203-92-5		3.01	1.11[j]	≤0.11-≤0.13 [j]		Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
Anesthetics	Lidocaine CAS # 137-58-6					0,00261			Chapter 2 Chapter 6	Verlicchi et al., 2015 Verlicchi and Zambello, 2015
	Albendazole CAS # 54965-21-8					0,0428^	Negative		Chapter 6	Verlicchi et al., 2015
	Fenbendazole CAS #43210-67-9						Negative		Chapter 6	Verlicchi et al., 2015
	Flubendazole CAS # 31430-15-6						Negative		Chapter 6	Verlicchi et al., 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Oxfendazole CAS # 53716-50-0					3,596*	Negative		Chapter 6	Verlicchi et al., 2015
	Thiabendazole CAS # 148-79-8	4.64	2.47			0,309^	Neutral		Chapter 6	Verlicchi et al., 2015
Antianginal	Dehydronifedipine CAS # 67035-22-7						Neutral		Chapter 2	Verlicchi and Zambello, 2015
Antiarrhythmics	Amiodarone CAS # 19774-82-4		7.57				Positive		Chapter 2	Verlicchi and Zambello, 2015
Antibiotics	Amoxicillin CAS # 26787-78-0	2.4[d]	0.87[b]			0,0037	Neut./Neg.		Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014
	Ampicillin CAS # 69-53-4					0,00001*	Negative		Chapter 4	Verlicchi and Zambello, 2014

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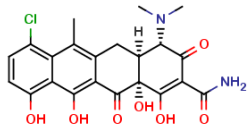
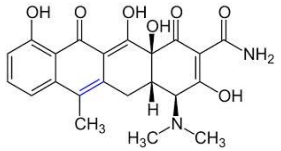
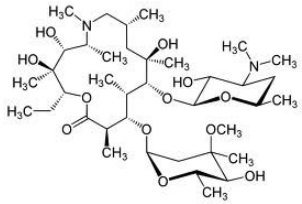
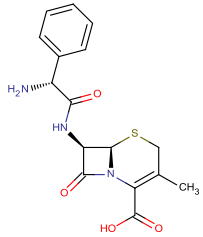
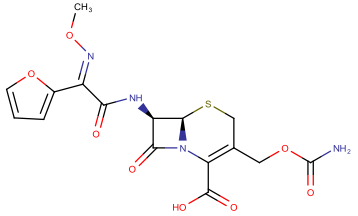
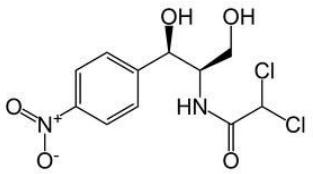
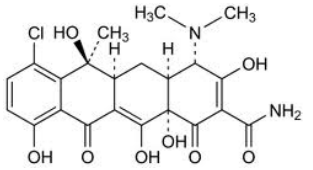
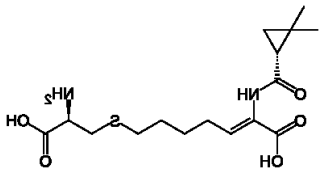
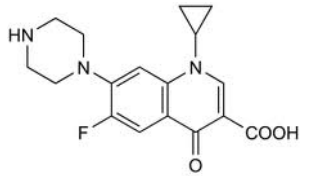
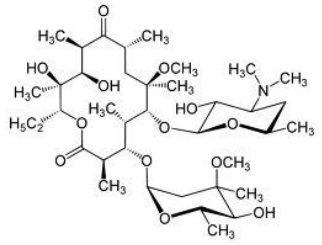
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Anhydro chlortetracycline CAS#65490-24-6								Chapter 2	Verlicchi and Zambello, 2015
	Anhydrotetracycline CAS #13803-65-1						Negative		Chapter 2	Verlicchi and Zambello, 2015
	Azithromycin CAS # 83905-01-5	pK_1 =8.7 pK_2= 9.5	4.02	2.5-2.7[k]	≤0.1 ≤1.2* 0.17* [s]	0,15	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Cefalexin CAS # 15686-71-2					2,5	Neut./Neg.		Chapter 1	Verlicchi et al., 2013b;
	Cefuroxime CAS # 55268-75-2					91*	Negative		Chapter 6	Verlicchi et al., 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Chloramphenicol CAS # 56-75-7	5.5	1.14			1,6	Neut./Neg.		Chapter 1 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014
	Chlortetracycline CAS # 57-62-5	pK_1= 3.3 pK_2= 7.4 pK_3= 9.3	-0.62			0,1*	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Cilastatin CAS # 82009-34-5						Negative		Chapter 6	Verlicchi et al., 2015
	Ciprofloxacin CAS # 85721-33-1	6.38[g]	0.4[j]	4.3[k]		938	Pos./Neut.		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Clarithromycin CAS # 81103-11-9	8.99	3.16	2.5-2.6[k]	≤0.4 ≤1.7* 0.034*-0.2*[s]	0,07	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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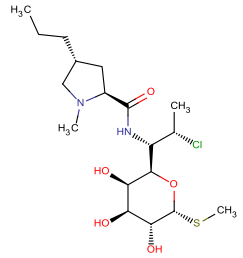
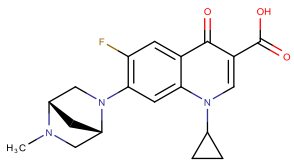
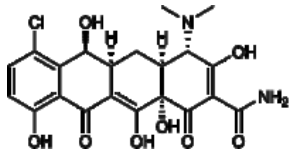
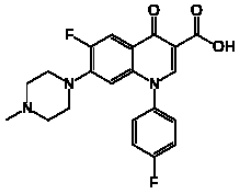
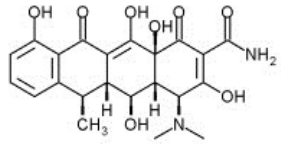
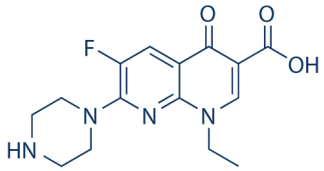
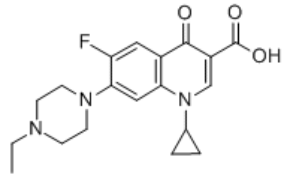
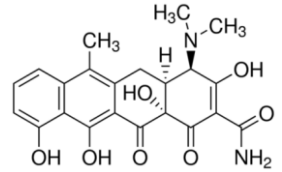
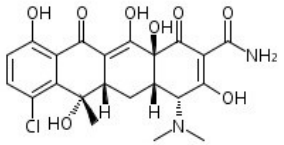
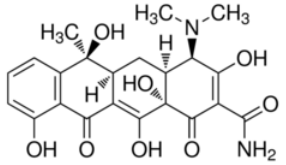
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Clindamycin CAS # 18323-44-9		2.01			0,5	Pos./Neut.		Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Danofloxacin CAS # 112398-08-0						Negative		Chapter 4 Chapter 5	Verlicchi and Zambello, 2014; Verlicchi et al., 2013a;
	Demeclocycline CAS # 127-33-3		-1.14				Negative		Chapter 2	Verlicchi and Zambello, 2015
	Difloxacin CAS # 98106-17-3		.28;0.89				Negative		Chapter 2	Verlicchi and Zambello, 2015
Antibiotics	Doxycycline CAS # 564-25-0	pK_1= 3.5 pK_2= 7.7 pK_3= 9.5	-0.02			0,3	Neut./Neg.		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Enoxacin CAS # 74011-58-8	pK_1= 6.3 pK_2= 8.7	-0.2			316	Neutral		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014;
	Enrofloxacin CAS # 93106-60-6	6.27[g]	1.1[h]	4.5[u]			Neut./Neg.		Chapter 1 Chapetr 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Epianhydrotetracycline CAS # 4465-65-0						Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Epichlortetracycline CAS # 101342-45-4						Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Epitetracycline CAS # 23313-80-6						Neut./Neg.		Chapetr 2	Verlicchi and Zambello, 2015

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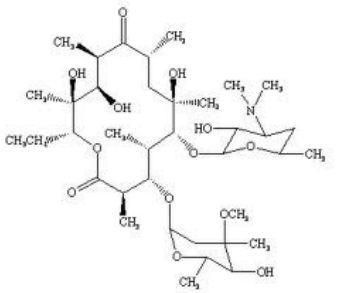
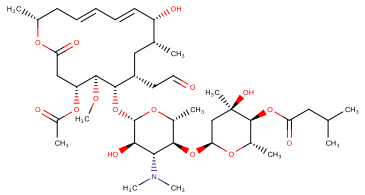
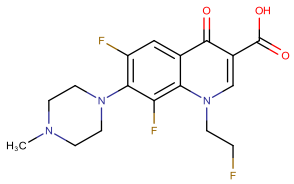
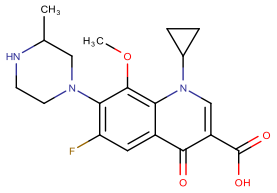
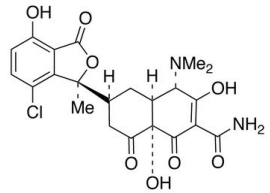
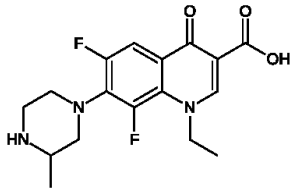
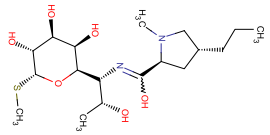
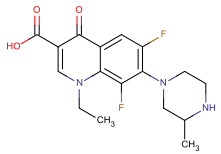
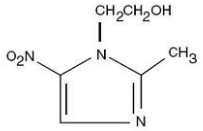
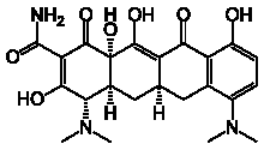
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Erythromycin CAS # 114-07-8	8.8-8.9 [b]	3.06	2.2[l]	0.15-6 [o]	0,02	Positive		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015: Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Josamycin CAS # 16846-24-5					0,91*	Positive		Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Fleroxacin CAS # 79660-72-3						Negative		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Gatifloxacin CAS # 112811-59-3						Positive		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Isochlortetracycline CAS # 514-53-4						Negative		Chapetr 2	Verlicchi and Zambello, 2015
Antibiotics	Levofloxacin CAS # 98079-51-7						Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Lincomycin CAS # 154-21-2		0.29			82	Pos./Neut.		Chapetr 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Lomefloxacin CAS # 98079-51-7		0.31	4.16[u]		0,002*	Neutral		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Metronidazole CAS # 443-48-1	2.5	-0.1; -0.02			2,5	Neutral		Chapter 1 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014
	Minocycline CAS # 10118-90-8		0.05			0,178	Negative		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015

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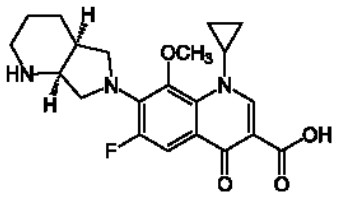
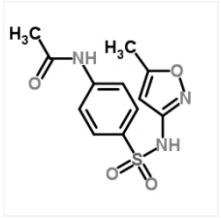
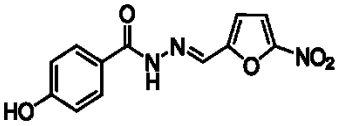
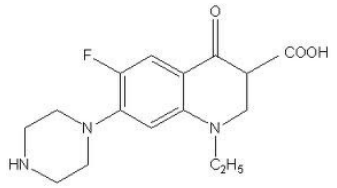
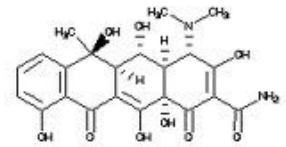
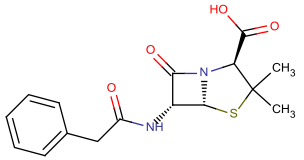
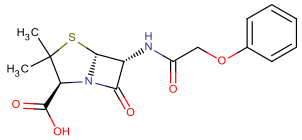
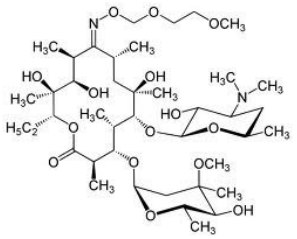
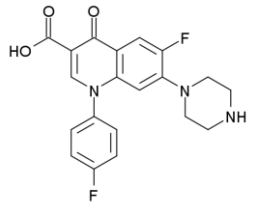
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Moxifloxacin CAS # 354812-41-2						Positive		Chapter 1 Chapetr 2 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	N4-Acetylsulfamethoxazole CAS # 21312-10-7								Chapter 6	Verlicchi et al., 2015
	Nifuroxazide CAS # 965-52-6						0,328*		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Norfloxacin CAS # 70458-96-7	pK_1= 6.3, pK_2= 8.4	-1.03	4.2[k]		15	Positive		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Ofloxacin CAS # 82419-36-1	5.97	0.35	4.2[u]		0,016	Neut./Neg.		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Oxytetracycline CAS # 79-57-2	pK_1= 3.27 pK_2= 7.3 pK_3= 9.1	-0.90; -1.6 (pH 7.5) 1.22			0,207	Negative		Chapter 1 Chapetr 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Penicillin G CAS # 61-33-6	2.74				0,006	Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Penicillin V CAS # 87-08-1	2.79	1.87			177	Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Roxithromycin CAS # 80214-83-1	8.8[c]	2.75	2.2-2.7[k] 2.3-2.6[l]	0.2-9 [o] ≤0.2 ≤0.3* 0.022*-0.023*[s]	4	Positive		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sarafloxacin CAS # 98105-99-8		1.07				Positive		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015

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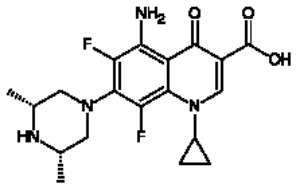
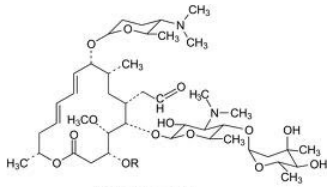
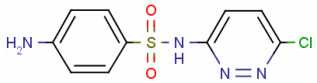
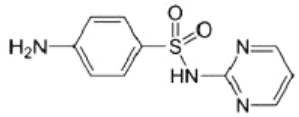
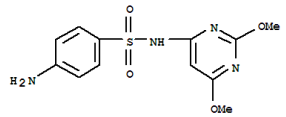
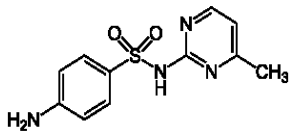
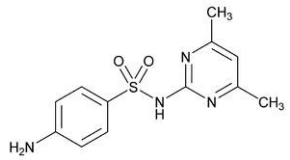
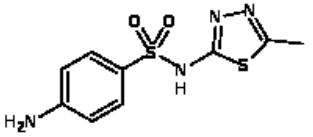
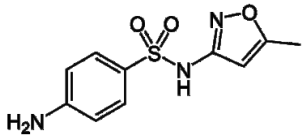
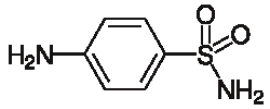
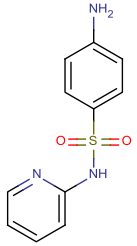
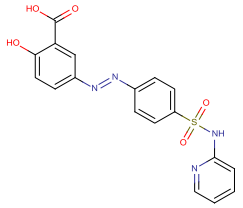
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Spafloxacin CAS # 110871-86-8						Positive		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Spiramycin CAS # 8025-81-8	8.0				0,005*	Positive		Chapetr 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sulfachloropyridazine CAS # 80-32-0		0.31			26,4	Neut./Neg.		Chapter 1	Verlicchi et al., 2013b;
	Sulfadiazine CAS # 68-35-9	pK$_1$= 6.36 pK$_2$= 2.1	-0.09			0,135	Neut./Neg.		Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sulfadimethoxine CAS # 122-11-2		1.17			3,5	Neut./Neg.		Chapter 1 Chapetr 2 Chapter 4	Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sulfamerazin CAS # 127-79-7		0.21 0.14				Negative		Chapetr 2	Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Sulfamethazine CAS # 57-68-1	2.65[q]	0.89[h]			12,77*	Neut./Neg.		Chapetr 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2013a; Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sulfamethizole CAS # 144-82-1					2,54*	Negative		Chapter 6	Verlicchi et al., 2015
	Sulfamethoxazole CAS # 723-46-6	5.7[c]	0.89[i]	2.1-2.7[k] 2.3-2.6[l]	0.3 [o]	0,027	Neut./Neg.		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sulfanilamide CAS # 63-74-1	10.58	-062			0,206*	Neut./Neg.		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Sulfapyridine CAS # 144-83-2	Pk1= 8043 Pk2=2. 3	0.35	2.3-2.6[k]		21,61	Neut./Neg.		Chapter 1 Chapetr 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Sulfasalazine CAS # 599-79-1		3.81				Negative		Chapter 1	Verlicchi et al., 2013b;

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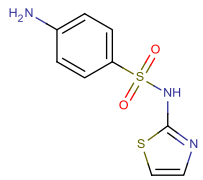
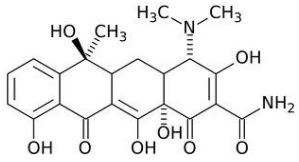
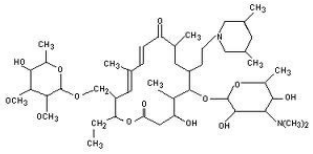
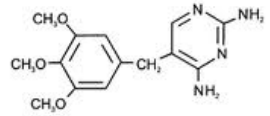
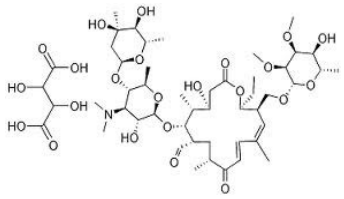
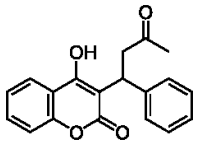
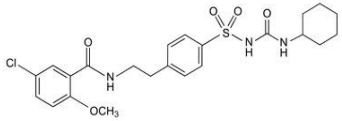
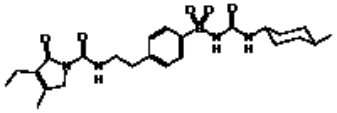
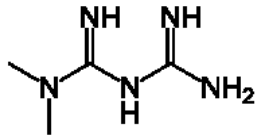
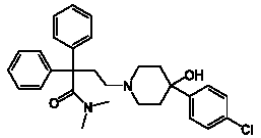
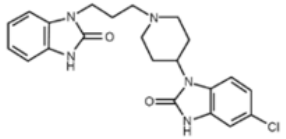
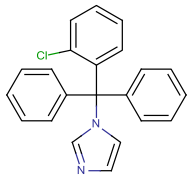
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antibiotics	Sulfathiazole CAS # 72-14-0		0.72			85,4	Negative		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello 2015
	Tetracycline CAS # 60-54-8	pK_1= 3.3 pK_2= 7.7 pK_3= 9.7	-1.30	3.9[k]		0,09	Negative		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello 2015
	Tilmicosin CAS # 108050-54-0	8.18	3.80			0,665*	Positive		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Trimethoprim CAS # 738-70-5	7.2	0.91	2.2-2.6[k] 2.3[l]	0.15 [o]	2,6	Pos./Neut.		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Tylosin CAS # 1401-69-0	7.1[p]	1.63			1,28*	Pos./Neut.		Chapetr 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello 2015
Anticoagulant	Warfarin CAS # 81-81-2		2.7				Negative		Chapetr 2	Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antidiabetics	Glibenclamide CAS # 10238-21-8	5.3	4.8	2.4[t]		0,176	Negative		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Glimepiride CAS # 93479-97-1						Negative		Chapter 4	Verlicchi and Zambello, 2014
	Metformin CAS # 657-24-9					60^	Positive		Chapter 1 Chapetr 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
Anti diarrhoea	Loperamide CAS #53179-11-6								Chapter 4	Verlicchi and Zambello, 2014
Antiemetic	Domperidone CAS # 57808-66-9	7.9	3.9				Positive		Chapetr 2	Verlicchi and Zambello, 2015
Antifungals	Clotrimazole CAS # 23593-75-1		6.26			10^{-8}*	Pos./Neut.		Chapetr 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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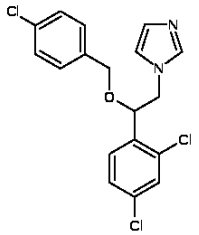
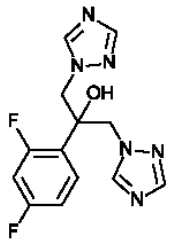
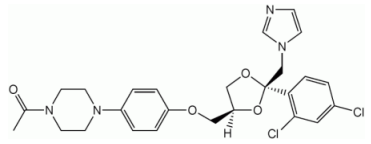
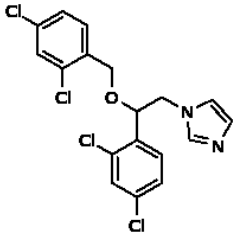
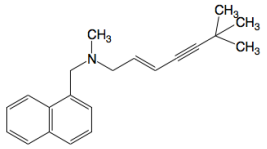
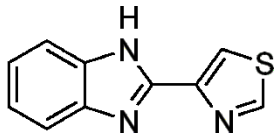
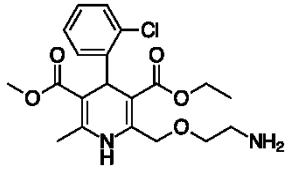
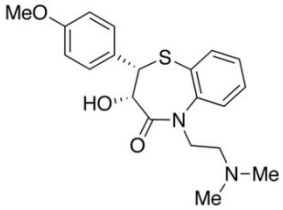
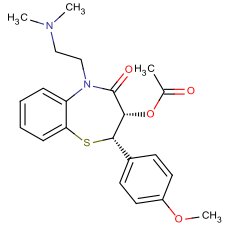
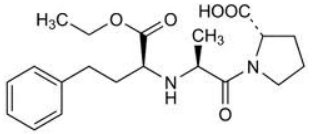
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antifungal	Econazole CAS # 27220-47-9						Positive		Chapetr 2	Verlicchi and Zambello, 2015
	Fluconazole CAS # 86386-73-4						Neutral		Chapetr 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Ketoconazole CAS #65277-42-1		4.45;4.35				Positive		Chapetr 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Miconazole CAS # 22916-47-8		6.25				Positive		Chapetr 2	Verlicchi and Zambello, 2015
	Terbinafine CAS # 91161-71-6						Positive		Chapetr 2	Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Thiabendazole CAS # 148-79-8	4.64	2.47				Neutral		Chapetr 2	Verlicchi and Zambello, 2015
Antihypertensives	Amlodipine CAS # 88150-42-9		3.00				Positive		Chapetr 2	Verlicchi and Zambello, 2015
	Desacetyl diltiazem CAS # 42399-40-6						Positive		Chapetr 2	Verlicchi and Zambello, 2015
	Diltiazem CAS # 42399-41-7		2.79			1,9	Positive		Chapter 1 Chapetr 2 Chapter 4	Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Enalapril CAS # 75847-73-3	---	2.45			21*	Negative		Chapetr 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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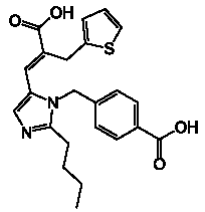
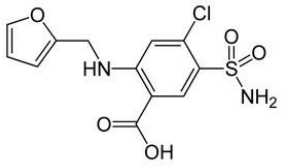
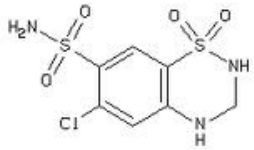
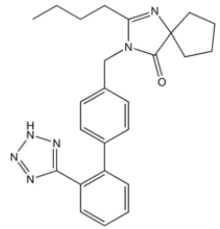
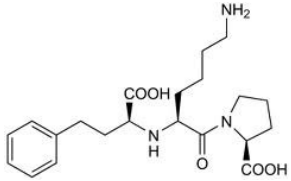
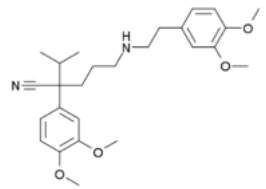
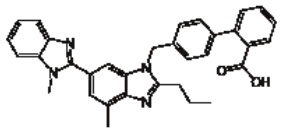
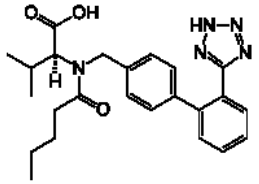
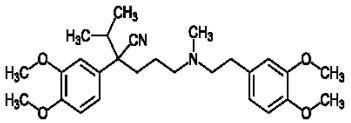
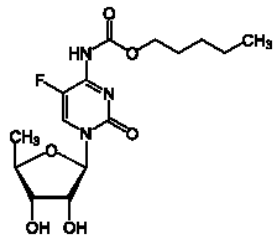
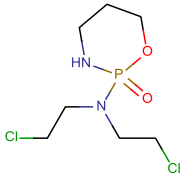
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Eprosartan CAS # 133040-01-4						Negative		Chapter 4	Verlicchi and Zambello, 2014
	Furosemide CAS # 54-31-9	3.9	2.03			1*	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Hydrochlorothiazide CAS # 58-93-5	7.9	-0.07	1.8[t]		317*	Negative		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015; Verlicchi et al., 2015
	Irbesartan CAS # 138402-11-6						Negative		Chapter 4 Chapter 6	Verlicchi and Zambello, 2014; Verlicchi et al., 2015
	Lisinopril CAS #83915-83-7	2.5	-1.22			333*	Negative		Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Norverapamil CAS # 67018-85-3		4.59				Positive		Chapetr 2	Verlicchi and Zambello, 2015
	Telmisartan CAS #144701-48-4						Negative		Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014
	Valsartan CAS #137862-53-4					580^	Negative		Chapter 6	Verlicchi et al., 2015
	Verapamil CAS # 34245-14-2	9.68; 8.92	3.79; 4.8			0,6*	Positive		Chapetr 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
Antineoplastic	Capecitabine CAS # 154361-50-9						Negative		Chapter 6	Verlicchi et al., 2015;
	Cyclophosphamide CAS # 50-18-0		0.97			11	Neutral		Chapetr 2 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2015

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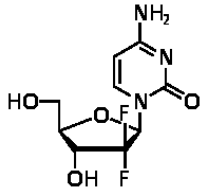
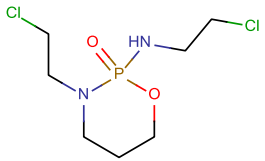
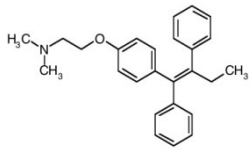
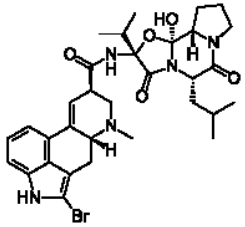
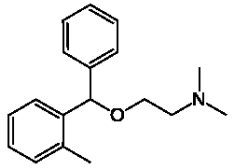
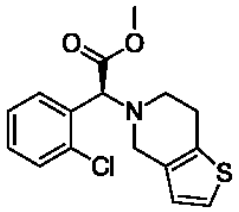
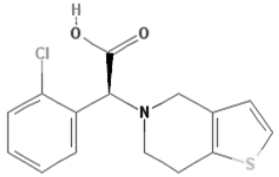
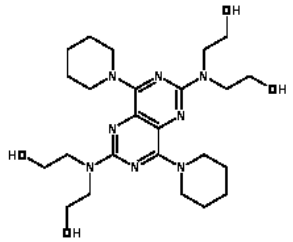
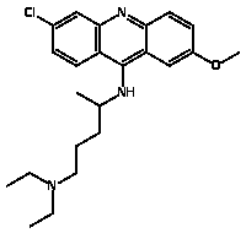
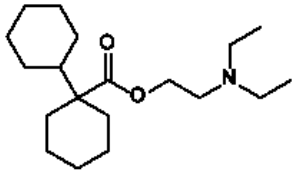
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Gemcitabine CAS # 95058-81-4					200*	Negative		Chapter 6	Verlicchi et al., 2015
	Ifosfamide CAS # 3778-73-2		0.97			11	Neutral		Chapter 1 Chapetr 2 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Tamoxifen CAS # 10540-29-1		6.30			0,00038*	Positive		Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2015; Verlicchi and Zambello, 2014
Anti parkinson	Bromocriptine CAS #25614-03-3						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Orphenadrine CAS #83-98-7						Positive		Chapter 4	Verlicchi and Zambello, 2014

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antiplatelets	Clopidogrel CAS # 113665-84-2		3.82				Neutral		Chapetr 2	Verlicchi and Zambello, 2015
	Clopidogrel carboylic CAS # 144457-28-3						Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Dipyridamole CAS # 58-32-2		2.74				Positive		Chapetr 2	Verlicchi and Zambello, 2015
Antiprotozoal	Quinacrine CAS # 83-89-6		5.75				Positive		Chapetr 2	Verlicchi and Zambello, 2015
Anti spasmodic	Dicycloverine CAS #77-19-0						Positive		Chapter 4	Verlicchi and Zambello, 2014

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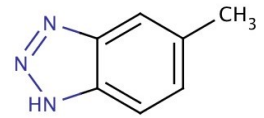
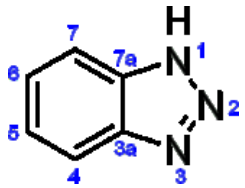
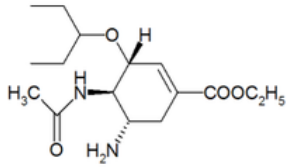
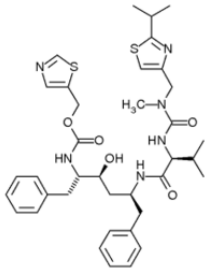
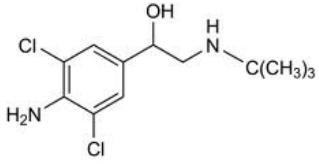
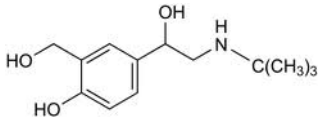
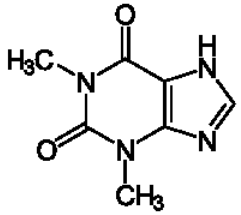
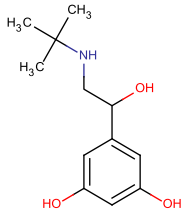
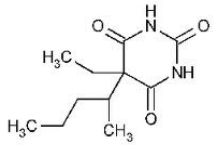
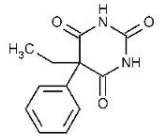
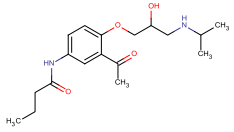
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Antivirals	4/5-methylbenzotriazole CAS # 136-85-6								Chapter 6	Verlicchi et al., 2015
	Benzotriazole CAS #95-14-7						Negative		Chapter 6	Verlicchi et al., 2015
	Oseltamivir CAS # 196618-13-0						Positive		Chapter 6	Verlicchi et al., 2015
	Ritonavir CAS # 155213-67-5						Neutral		Chapter 6	Verlicchi et al., 2015
Beta-agonists	Clenbuterol CAS # 037148-27-9	---	2.00			2	Positive		Chapetr 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Salbutamol CAS # 35763-26-9	pK_1= 9.3, pK_2=10.3	0.6, 0.01			1,158*	Positive		Chapter 1 Chapetr 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Theophylline CAS # 58-55-9	8.81	-0.02				Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Terbutaline CAS # 23031-25-6		0.67			1,05	Positive		Chapter 4	Verlicchi and Zambello, 2014
Barbiturates	Pentobarbital CAS # 76-74-4	8.11	2.10				Negative		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Phenobarbital CAS # 50-06-6	7.3	1.47			1,1*	Negative		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
Beta-blokers	Acebutolol CAS # 37517-30-9		1.71[i]				Positive		Chapter 2	Verlicchi and Zambello, 2015

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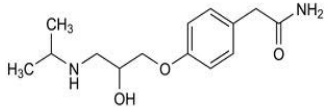
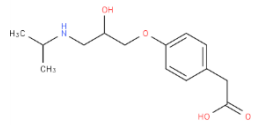
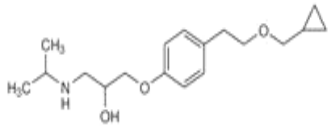
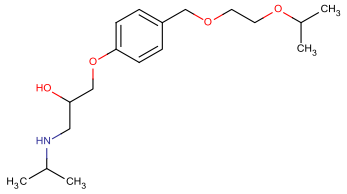
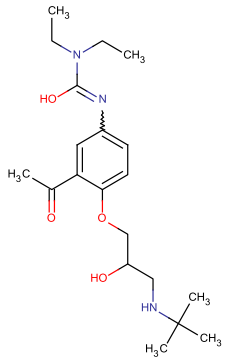
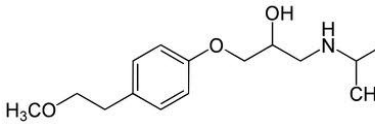
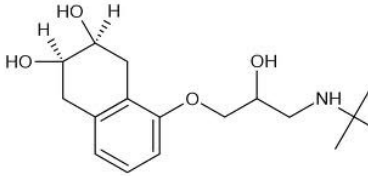
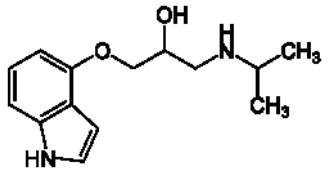
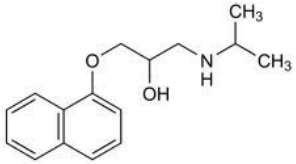
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Atenolol CAS # 29133-68-7	9.6	0.16	-0.68[i]	1.1-1.9 [j]	30	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Atenolol acid CAS #56392-14-4								Chapter 6	Verlicchi et al., 2015
	Betaxolol CAS # 63659-18-7	---	2.81		6.0 [j]	1,24*	Positive		Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Bisoprolol CAS # 66722-44-9		1.84		0.64-0.77 [j]		Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Carazolol CAS #57775-29-8	---	3.59			1,539*	Positive		Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Beta-blokers	Celiprolol CAS # 56980-93-9		1.93		0.18-0.24 [j]		Positive		Chapter 1 Chapter 2	Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Metoprolol CAS # 37350-58-6	9.6	1.88		0.35-0.40 [j]	8	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Nadolol CAS # 42200-33-9	9.67	0.81			110	Positive		Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Pindolol CAS #13523-86-9					3,175*	Positive		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Propranolol CAS # 525-66-6	9.42	3.48	2.6 [t]	0.36-0.46 [j]	0,244	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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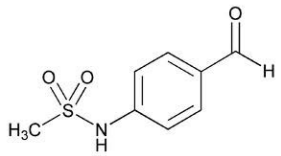
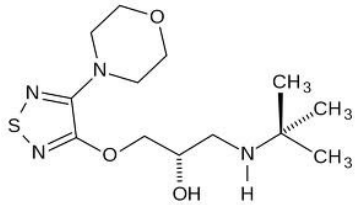
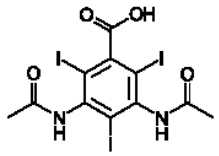
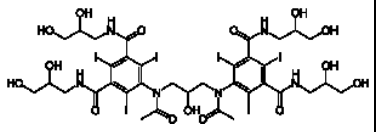
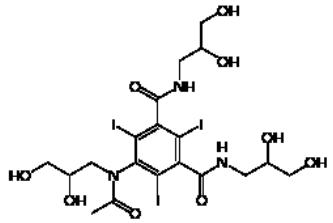
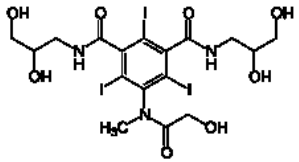
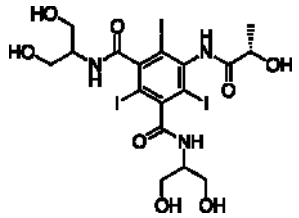
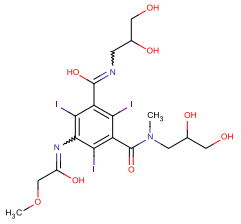
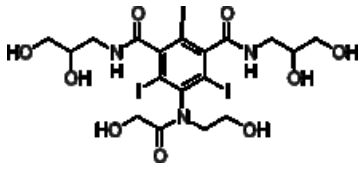
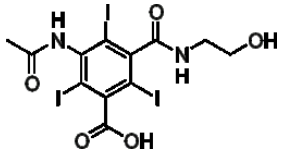
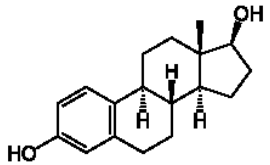
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Sotalol CAS # 3930-20-9	pK_1=8.2 pK_2=9.8	0.24		0.40-0.43 [j]	13*	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Timolol CAS # 26839-75-8	9.21	1.83			9	Positive		Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
Contrast mediaa	Diatrizoate CAS # 737-31-5						Negative		Chapter 6	Verlicchi et al., 2015
	Iodixanol CAS #92339-11-2						Negative		Chapter 6	Verlicchi et al., 2015
	Iohexol CAS # 66108-95-0					45705*	Negative		Chapter 6	Verlicchi et al., 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Iomeprol CAS # 78649-41-9						Negative		Chapter 6	Verlicchi et al., 2015
	Iopamidol CAS # 62883-00-5					5363*	Negative		Chapter 6	Verlicchi et al., 2015
	Iopromide CAS # 73334-07-3		-2.49	1[l]	1.6-2.5 1.0*-2.0* 0.12*- 0.026* [s]	370000	Pos./Neut.		Chapter 1 Chapter 2 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2015
	Ioversol CAS #87771-40-2						Positive		Chapter 6	Verlicchi et al., 2015
	Ioxitalamic acid CAS # 28179-44-4					2,857*			Chapter 6	Verlicchi et al., 2015
Hormones	Estradiol E2 CAS # 50-28-2	10.27[m]	3.94	2.4-2.8[l]	175-460 [r] 280*-950* [r]	0,000008*	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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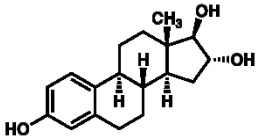
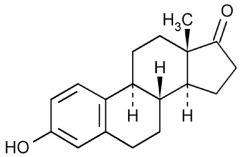
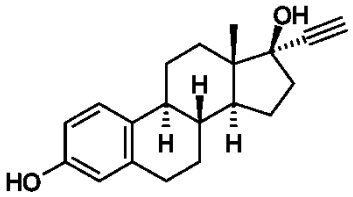
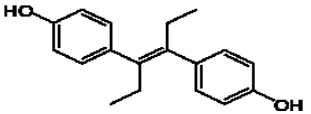
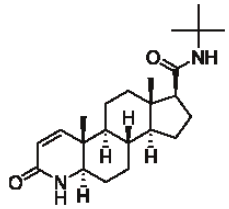
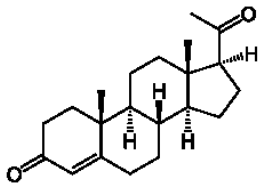
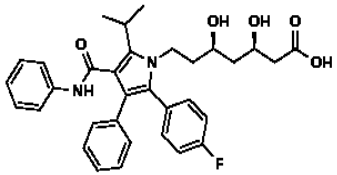
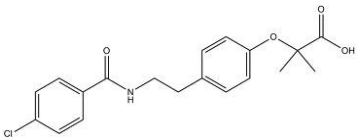
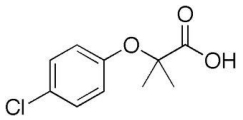
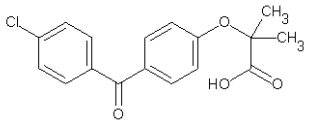
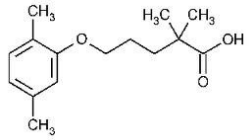
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Hormones	Estriol E3 CAS # 50-27-1		2.81			0,0075	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Estrone E1 CAS # 53-16-7	10.25[m]	3.43	2.4-2.9[l]	10-162 [r] 28*-430* [r] >20 [s]	0,00016	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Ethinylestradiol EE2 CAS # 57-63-6	10.24[m]	4.12	2.5-2.8[l]	0.4-20 [o] 1.2-8 [r] 1.5*-6* [r] >0.5->0.7 [s]	0,00004*	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Diethylstibestrol CAS # 56-53-1						Negative		Chapter 2	Verlicchi and Zambello, 2015
	Finasteride CAS # 98319-26-7					20	Neutral		Chapter 4	Verlicchi and Zambello, 2014

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Progesterone CAS # 57-83-0						Neutral		Chapter 2	Verlicchi and Zambello, 2015
Lipid regulators	Atorvastatin CAS # 134523-00-5	4.33	6.36			0,19*	Negative		Chapter 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015 Verlicchi et al., 2013a;
	Bezafibrate CAS # 41859-67-0	3.6[c]	4.25		2.1-3.0 3.4*-4.5* 0.77*->2.9*[s]	5,3	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Clofibric acid CAS # 882-09-7	-3.18[m]	2.57		0.3-0.8 0.1*-0.23* 0.09*-0.1*[s]	40,2	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015;
	Fenofibrate CAS # 49562-28-9	---	5.19			0,1	Neutral		Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2013a; Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015;
	Fenofibric acid CAS # 42017-89-0		2.9		7.2-10.8 0.4*-1.7*;	7,6	Negative		Chapter 1	Verlicchi et al., 2013b;
	Gemfibrozil CAS # 25812-30-0	4.8	4.77	1.28[t]	6.4-9.6 0.5*-1.8*	0,9	Negative		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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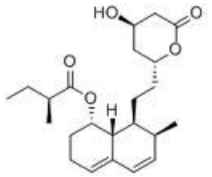
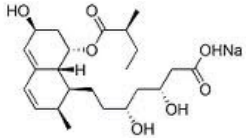
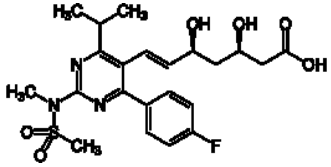
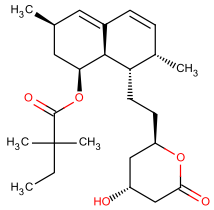
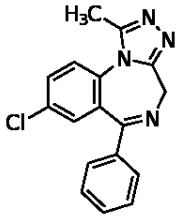
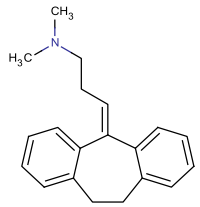
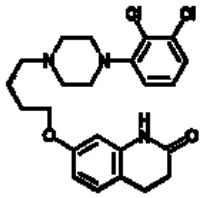
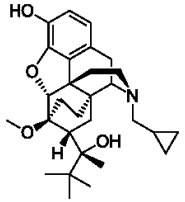
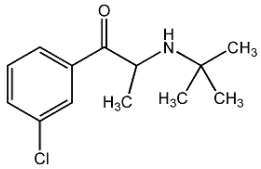
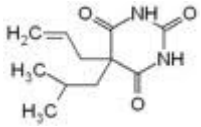
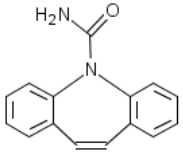
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Lipid regulators	Mevastatin CAS # 73573-88-3		3.95			0,239*	Negative		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Pravastatin CAS # 81093-37-0	---	-0.23			1,8	Negative		Chapter 1 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014
	Rosuvastatin CAS #287714-41-4						Negative		Chapter 4	Verlicchi and Zambello, 2014
	Simvastatin CAS # 79902-63-9		5.19			0,0002*	Neutral		Chapter 2	Verlicchi and Zambello, 2015;
Psychiatric drugs	Alprazolam CAS # 28981-97-7		3.87;2.12			0,000508	Positive		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015;

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Amitriptyline CAS # 50-48-6	9.76; 9.4	4.95				Positive		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015;
	Aripiprazole CAS # 129722-12-9		4.13				Positive		Chapter 2	Verlicchi and Zambello, 2015;
	Buprenorphine CAS #52485-79-7						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Bupropion CAS # 34841-39-9		3.85				Positive		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Butalbital CAS # 77-26-9		1.87				Negative		Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014
	Carbamazepine CAS # 298-46-4	13.9 [b]	2.45	0.1[l]	≤0.1 [j] <0.03-<0.06 [o] <0.005*- <0.008* [s]	13,8	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7 Chapter 8	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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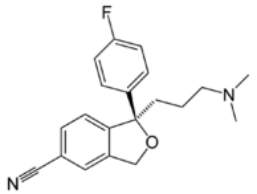
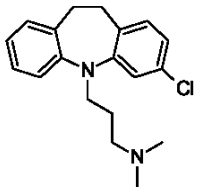
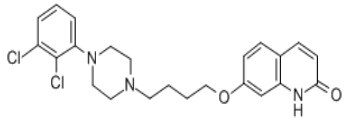
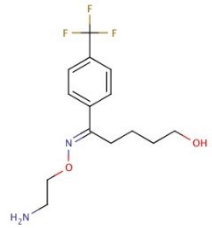
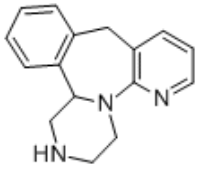
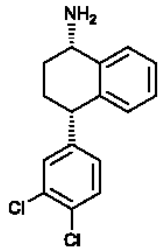
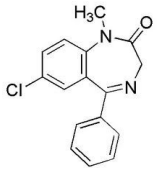
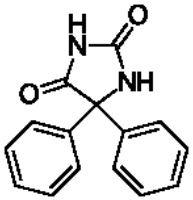
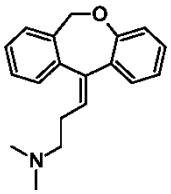
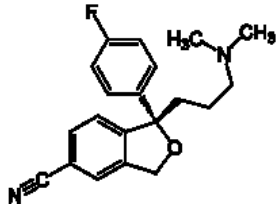
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Citalopram CAS # 59729-33-8		3.74			0,00635	Positive		Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Clomipramine CAS #303-49-1						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Dehydro-aripiprazole CAS # 129722-25-4		3.91				Positive		Chapter 2	Verlicchi and Zambello, 2015
	Desmethylfluvoxamine CAS # 192876-02-1						Positive		Chapter 2	Verlicchi and Zambello, 2015
	Desmethylmitrazepine CAS # 61337-68-6						Positive		Chapter 2	Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Desmethylsertraline CAS # 87857-41-8						Positive		Chapter 2	Verlicchi and Zambello, 2015
	Diazepam CAS # 439-14-5	3.4	2.82	1.3[l]	≤0.16 [j] <0.25-<0.4 [o]	2	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Phenytoin (Dilantin) CAS #57-41-0						Negative		Chapter 4	Verlicchi and Zambello, 2014
	Doxepine CAS # 1668-19-5		4.29				Positive		Chapter 2	Verlicchi and Zambello, 2015
	Escitalopram CAS # 128196-01-0						Positive		Chapter 2	Verlicchi and Zambello, 2015

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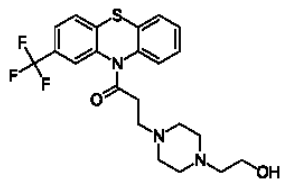
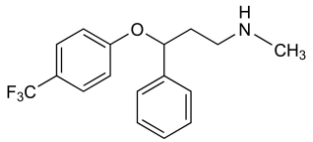
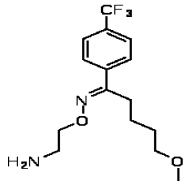
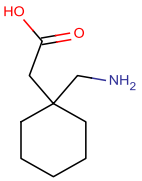
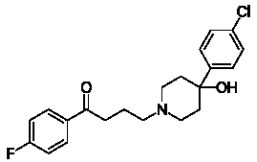
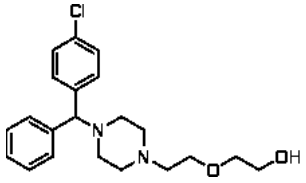
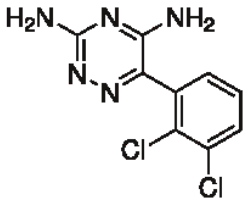
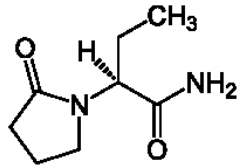
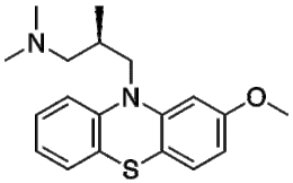
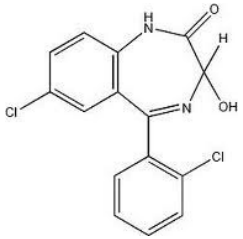
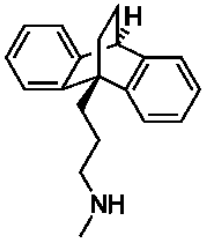
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Fluphenazine CAS #69-23-8						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Fluoxetine CAS # 54910-89-3	9.5	4.05	0.7[n]	5-9 [o]	0,05	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Fluvoxamine CAS # 54739-18-3						Positive		Chapter 2	Verlicchi and Zambello, 2015
	Gabapentin CAS # 60142-96-3					0,196*	Neutral		Chapter 1 Chapter 6	Verlicchi et al., 2015; Verlicchi et al., 2013b;
	Haloperidol CAS #52-86-8						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Hydroxyzine CAS #68-88-2						Positive		Chapter 4	Verlicchi and Zambello, 2014

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Psychiatric drugs	Lamotrigine CAS # 84057-84-1						Positive		Chapter 2	Verlicchi and Zambello, 2015
	Levetiracetam CAS # 102767-28-2						Neutral		Chapter 6	Verlicchi et al., 2015
	Levomepromazine CAS #60-99-1								Chapter 4	Verlicchi and Zambello, 2014
	Lorazepam CAS # 846-49-1	pK_1=1.3 pK_2=11.5	2.39			2*	Neutral		Chapter 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Maprotilin CAS #10262-69-8						Positive		Chapter 4	Verlicchi and Zambello, 2014

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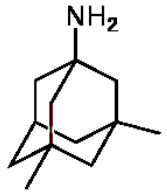
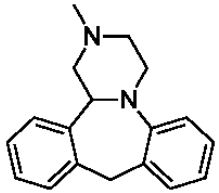
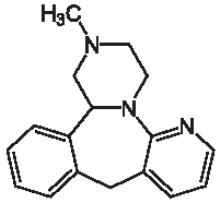
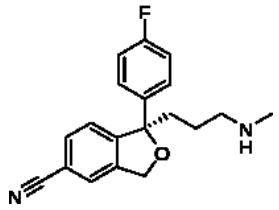
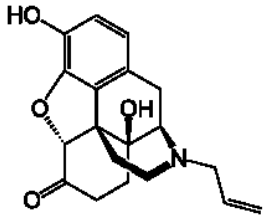
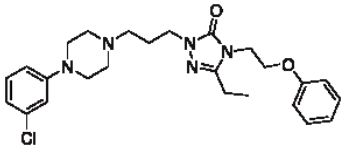
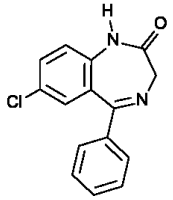
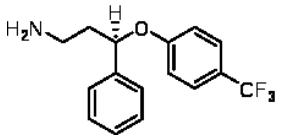
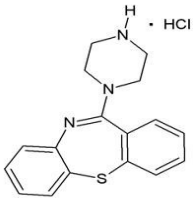
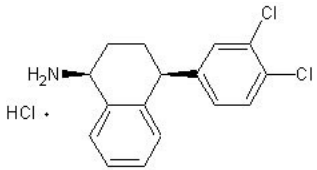
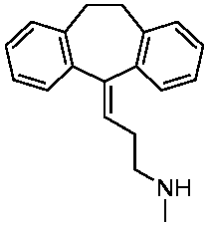
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Memantin CAS #19982-08-2						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Mianserin CAS #24219-97-4						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Mitrazapin CAS # 61337-67-5						Positive		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	N-desmethylcitalopram CAS # 62498-67-3						Positive		Chapter 2	Verlicchi and Zambello, 2015
	Naloxone CAS #465-65-6						Positive		Chapter 4	Verlicchi and Zambello, 2014

Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Nefazodone CAS #83366-66-9						Positive		Chapter 4	Verlicchi and Zambello, 2014
Nordiazepam CAS # 1088-11-5		3.89				Neutral		Chapter 2	Verlicchi and Zambello, 2015
Norfluoxetine CAS # 126924-38-7	9.05[d]	4.07[d]			0,242^	Positive		Chapter 2	Verlicchi and Zambello, 2015
Norquetiapine CAS # 753475-15-9						Pos./Neg.		Chapter 2	Verlicchi and Zambello, 2015
Norsertraline CAS # 91797-57-8		4.82				Pos./Neg.		Chapter 2	Verlicchi and Zambello, 2015
Nortriptyline CAS # 72-69-5	10.11	4.51				Positive		Chapter 2	Verlicchi and Zambello, 2015

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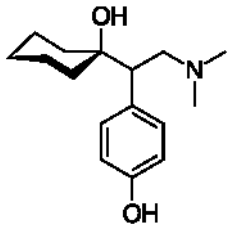
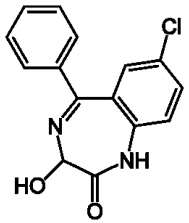
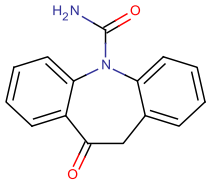
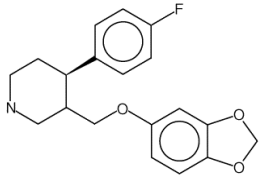
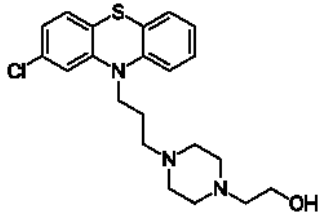
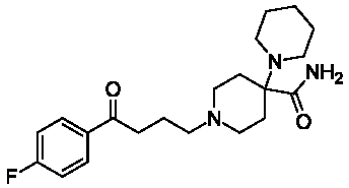
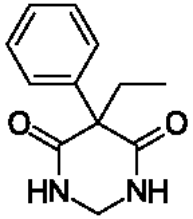
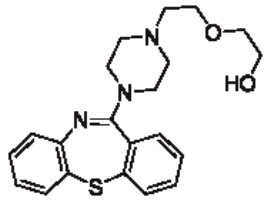
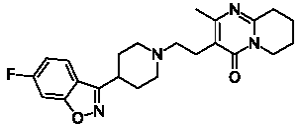
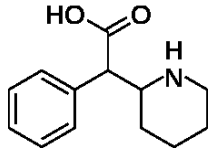
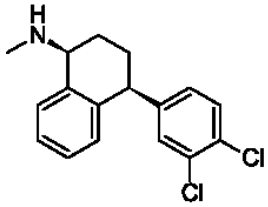
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	O-desmethylvenlafaxine CAS # 93413-62-8						Positive		Chapter 2	Verlicchi and Zambello, 2015
	Oxazepam CAS # 604-75-1		2.24; 3.37			0,0019*	Negative		Chapter 2 Chapter 4 Chapter 6	Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Oxcarbazepine CAS # 28721-07-5		1.11				Neutral		Chapter 6	Verlicchi et al., 2015
	Paroxetine CAS # 61869-08-7	9.0	3.95			8,8*	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 7	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Perphenazine CAS #58-39-9						Positive		Chapter 4	Verlicchi and Zambello, 2014

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Pipamperon CAS # 1893-33-0		2.02				Pos./Neg.		Chapter 2	Verlicchi and Zambello, 2015
	Primidone CAS # 125-33-7					0,069*	Negative		Chapter 6	Verlicchi et al., 2015
	Quetiapine CAS # 111974-69-7		1.94				Positive		Chapter 2	Verlicchi and Zambello, 2015
	Risperidone CAS # 106266-06-2					0,001*	Positive		Chapter 4	Verlicchi and Zambello, 2014
	Ritalinic acid CAS # 19395-41-6								Chapter 6	Verlicchi et al., 2015
	Sertraline CAS # 79617-96-2		5.29			0,242*	Positive		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

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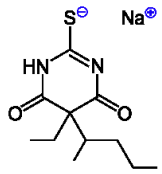
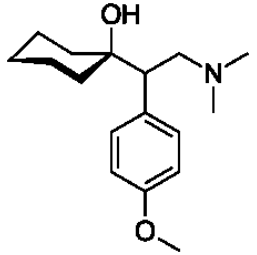
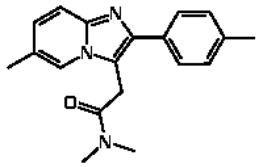
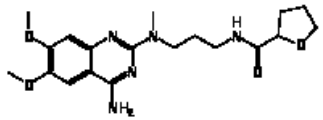
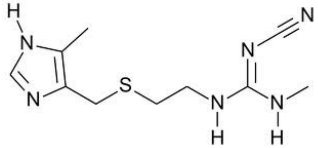
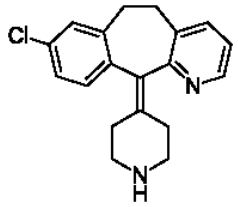
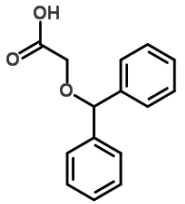
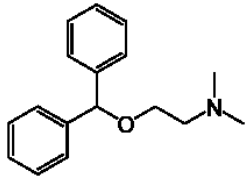
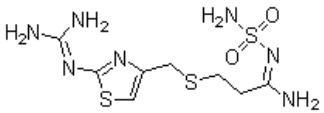
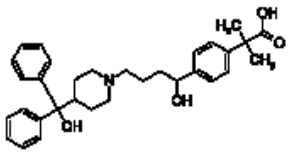
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Psychiatric drugs	Thiopental CAS # 76-75-5						Negative		Chapter 6	Verlicchi et al., 2015
	Venlafaxine CAS # 93413-69-5		3.28				Positive		Chapter 2 Chapter 6 Chapter 4	Verlicchi et al., 2015; Verlicchi and Zambello, 2014 Verlicchi and Zambello, 2015
	Zolpidem CAS # 82626-48-0		3.85				Positive		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
Receptor antagonists	Alfuzosin CAS #81403-80-7						Positive		Chapter 4	Verlicchi and Zambello, 2014;
	Cimetidine CAS # 51481-61-9	6.8	0.40			35	Pos./Neut.		Chapter 1 Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
Receptor antagonists	Desloratidin CAS # 100643-71-8								Chapter 4	Verlicchi and Zambello, 2014
	2-Diphenhylmethoxy acetic acid CAS # 21409-25-6						Negative		Chapetr 2	Verlicchi and Zambello, 2015
	Diphenhydramine CAS # 58-73-1	8.98	3.27; 3.11				Positive		Chapetr 2 Chapter 4	Verlicchi and Zambello, 2015 Verlicchi and Zambello, 2014
	Famotidine CAS # 76824-35-6	---	-0.64			22*	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Fexofenadine CAS #83799-24-0						Negative		Chapter 4	Verlicchi and Zambello, 2014

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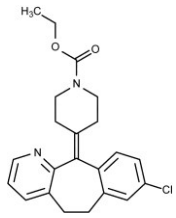
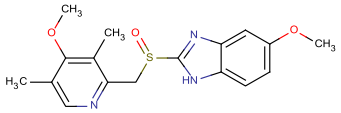
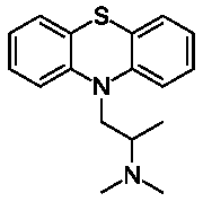
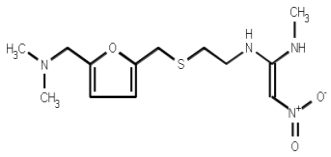
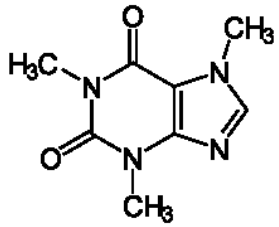
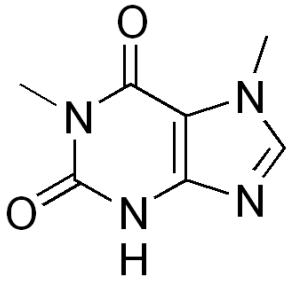
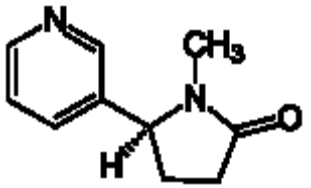
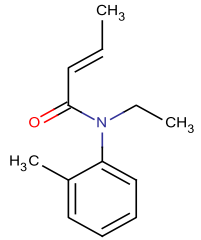
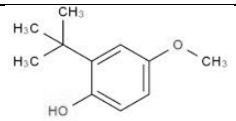
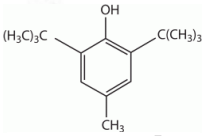
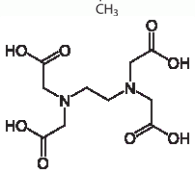
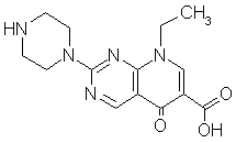
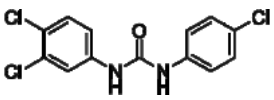
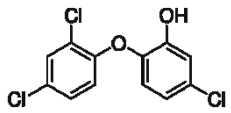
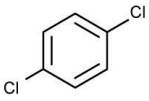
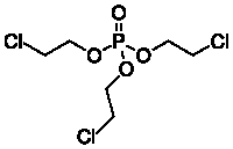
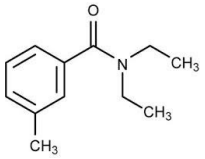
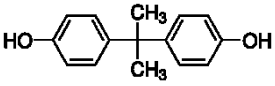
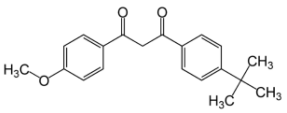
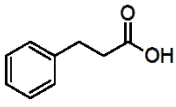
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Loratadine CAS # 79794-75-5	---	5.20	3.5[l]		0,021*	Neutral		Chapter 1 Chapter 2 Chapter 4 Chapter 5	Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Omeprazole CAS # 73590-58-6		3.4				Neutral		Chapter 2 Chapter 4	Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
	Promethazine CAS #67-87-7						Positive		Chapter 4	Verlicchi and Zambello, 2014
	Ranitidine CAS # 66357-35-5	2.4	0.27			63	Positive		Chapter 1 Chapter 2 Chapter 4 Chapter 5 Chapter 6 Chapter 7	Verlicchi et al., 2015; Verlicchi et al., 2013a; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
Stimulants	Caffeine CAS # 58-08-2	-0.92	0.16; -0.07			0,00005*	Neutral		Chapter 2 Chapter 4 Chapter 6 Chapter 8	Verlicchi et al., 2015; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015

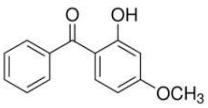
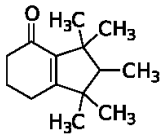
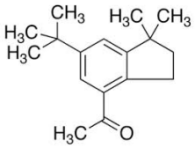
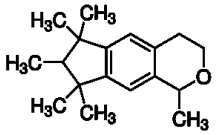
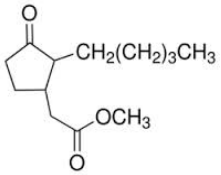
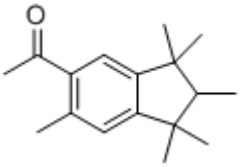
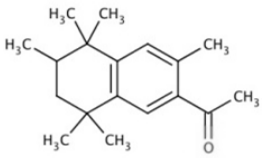
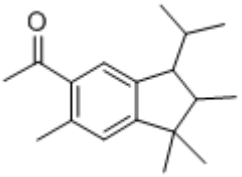
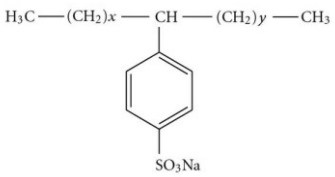
	Pharmaceutical	pK_a	Log K_{ow}	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	PNEC$_{water}$ (µg/L)	Charge at pH 7	Molecular structure	Chapter number	References
	Paraxanthine (1,7-Dimethylxanthine) CAS # 611-59-6		-0.39;-0.22				Negative		Chapter 2	Verlicchi and Zambello, 2015
	Cotinine CAS # 486-56-6		0.07				Positive		Chapter 2	Verlicchi and Zambello, 2015
Vasodilator	Dipyradamol CAS #								Chapter 4	Verlicchi and Zambello, 2014;
Topical Products	Crotamiton CAS # 483-63-6		2.73				Neutral		Chapter 1	Verlicchi et al., 2013b;

Appendix A

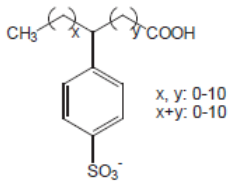
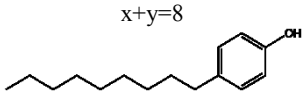
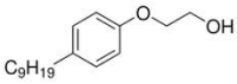
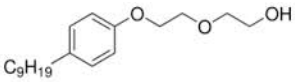
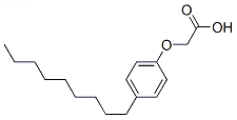
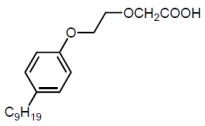
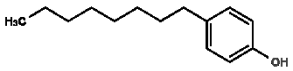
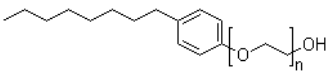
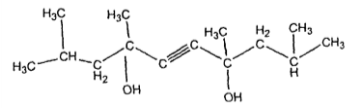
Table A 2 List of PCPs included in this thesis, grouped according to their class with the corresponding references

	Class	Compound	Molecular structure	Chapter number	References
1	Anti oxidant	Butylated hydroxyanisole (BHA) $C_{11}H_{16}O_2$ CAS # 25013-16-5		Chapter 3	Verlicchi et al., 2014
2	Anti oxidant	Butylated hydroxytoluene (BHT) $C_{15}H_{24}O$ CAS # 128-37-0		Chapter 3	Verlicchi et al., 2014
3	Anti oxidant	Ethylenediamine-tetraacetic acid (EDTA) $C_{10}H_{16}N_2O_8$ CAS # 60-00-4		Chapter 3	Verlicchi et al., 2014
4	Antiseptic	Pipemic acid CAS # 51940-44-4		Chapter 2	Verlicchi and Zambello, 2015
5	Antiseptic	Triclocarban $C_{13}H_9C_{13}N_2O$ CAS # 101-20-2		Chapter 2 Chapter 3 Chapter 4	Verlicchi et al., 2014; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
6	Antiseptic	Triclosan $C_{12}H_7Cl_3O_2$ CAS # 3380-34-5		Chapter 1 Chapter 2 Chapter 3 Chapter 4 Chapter 6	Verlicchi et al., 2012 Verlicchi et al., 2014; Verlicchi et al., 2015; Verlicchi et al., 2013b; Verlicchi and Zambello, 2014; Verlicchi and Zambello, 2015
7	Deodorant	1,4-Dichlorobenzene (*p*-DCB) $C_6H_4Cl_2$ CAS # 106-46-7		Chapter 3	Verlicchi et al., 2014
8	Flame retardant	tris (2-chloroethyl) phosphate (TCEP) $C_6H_{12}Cl_3O_4P$ CAS # 115-96-8		Chapter 3	Verlicchi et al., 2014
9	Insect repellent	Diethyl-3-methylbenzoyl-amide (DEET) $C_{12}H_{17}NO$ CAS # 134-62-3		Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
10	Plasticizer	4,4'-(propane-2,2-diyl) diphenol (Bisphenol A) $C_{15}H_{16}O_2$ CAS # 80-05-7		Chapter 3	Verlicchi et al., 2014
11	Sun screen product	Avobenzone (Parsol) $C_{20}H_{22}O_3$ CAS # 70356-09-1		Chapter 3	Verlicchi et al., 2014
12	Sun screen product	Hydrocinnamic acid $C_9H_{10}O_2$ CAS # 501-52-0		Chapter 3	Verlicchi et al., 2014

Appendix A

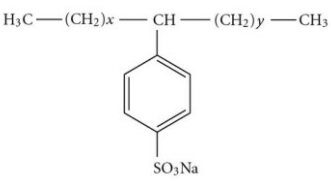
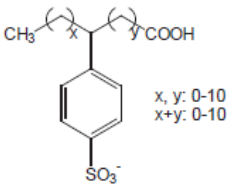
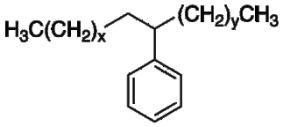
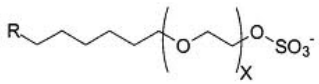
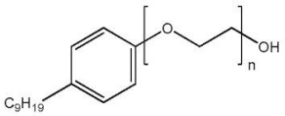
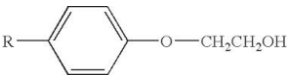
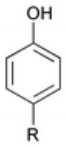
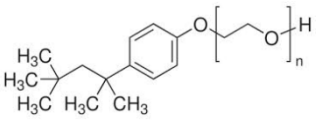
	Class	Compound	Molecular structure	Chapter number	References
13	Sun screen product	Oxybenzone $C_{14}H_{12}O_3$ CAS # 131-57-7		Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
14	Synthetic musk	Cashmeran $C_{14}H_{22}O$ CAS # 33704-61-9		Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
15	Synthetic musk	Celestolide $C_{17}H_{24}O$ CAS # 13171-00-1		Chapter 2 Chapter 3 Chapter 6	Verlicchi et al., 2014; Verlicchi et al., 2015; Verlicchi and Zambello, 2015
16	Synthetic musk	Galaxolide (HHCB) $C_{18}H_{26}O$ CAS # 1222-05-5		Chapter 2 Chapter 3 Chapter 6	Verlicchi et al., 2014; Verlicchi et al., 2015; Verlicchi and Zambello, 2015
17	Synthetic musk	Methyl dihydrojasmonate (MDHJ) $C_{13}H_{22}O_3$ CAS # 24851-98-7		Chapter 3	Verlicchi et al., 2014
18	Synthetic musk	Phantolide (AHDI) CAS # 15323-35-0		Chapter 2	Verlicchi and Zambello, 2015
19	Synthetic musk	Tonalide (AHTN) $C_{18}H_{26}O$ CAS # 1506-02-1		Chapter 2 Chapter 6	Verlicchi et al., 2014; Verlicchi et al., 2015; Verlicchi and Zambello, 2015
20	Synthetic musk	Traseolide (ATII) CAS # 68140-48-7		Chapter 2	Verlicchi and Zambello, 2015
21-24	Anionic surfacants	Linear alkylbenzene sulfonate (LAS) $NaSO_3C_{10}H_{13}(CH_2)_{x+y}$		Chapter 3	Verlicchi et al., 2014
		LAS C10 1322-98-1	x+y=7	Chapter 3	Verlicchi et al., 2014
		LAS C11 27636-75-5	x+y=8		
		LAS C12 25155-30-0	x+y=9		
		LAS C13 26248-24-8	x+y=10		

Appendix A

	Class	Compound	Molecular structure	Chapter number	References
25-27	Anionic surfactants	Sulfophenyl carboxylate SPC $C_9H_9SO_5Na(CH_2)_{x+y}$ SPC-C9, SPC-C10, SPC-C11	 x+y=6 x+y=7 x+y=8	Chapter 3	Verlicchi et al., 2014
28	Nonionic surfacant	Nonylphenol (NP) 25154-52-3 $C_{15}H_{24}O$		Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
29-30	Nonionic surfacants	Nonylphenol mono ethoxylate (NP1EO), Nonylphenol diethoxylate (NP2EO)	 	Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
31-32	Nonionic surfacants	Nonyl phenol mono ethoxycarboxylic acid (NP1EC) $C_{17}H_{26}O_3$ 3115-49-9 Nonyl phenol di ethoxycarboxylic acid (NP2EC) $C_{19}H_{30}O_4$ 106807-78-7	 	Chapter 3	Verlicchi et al., 2014
33	Nonionic surfacant	4-tert-octylphenol (OP) $C_{14}H_{22}O$ 140-66-9		Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
34-35	Nonionic surfacants	4-tert-octylphenolmono ethoxylate (OP1EO) $C_{16}H_{26}O_2$ 4-tert-octylphenoldi ethoxilate (OP2EO) $C_{18}H_{30}O_3$	 n=1 n=2	Chapter 2 Chapter 3	Verlicchi et al., 2014; Verlicchi and Zambello, 2015
36	Nonionic surfacant	Surfynol 104 $C_{14}H_{26}O_2$ 8043-35-4		Chapter 3	Verlicchi et al., 2014

Appendix A

Table A3 Classes of Surfactants included in this thesis

	Type	Class	Molecular structure	n. papers	References
A	Anionic surfacants	Methylen Blue Active Subtances MBAS		Chapter 3	Verlicchi et al., 2014
B	Anionic surfacants	Linear alkylbenzene sulfonate LAS $NaSO_3C_9H_{11}(CH_2)_{x+y}$		Chapter 3	Verlicchi et al., 2014
C	Anionic surfacants	Sulfophenyl carboxylate SPC		Chapter 3	Verlicchi et al., 2014
D	Anionic surfacants	Linear Alkyl benzene LAB $C_6H_5CHR_1R_2$ where $R_1 = C_nH_{2n+1}$ $R_2 = C_mH_{2m+1}$ m≥0, n≥1 (typically 10-16)		Chapter 3	Verlicchi et al., 2014
E	Anionic surfacants	Alkyl ethoxy sulfates AES $CH_3(CH_2)_y(O\ CH_2CH_2)_xOSO_3X$	 x=0-12 y=12-13 X most often being Na	Chapter 3	Verlicchi et al., 2014
F	Nonionic surfacants	NP(1-3)EO, NP(4-9)EO	 Mixture of NPnEO with n=1-3 with n=4-9	Chapter 3	Verlicchi et al., 2014
G	Nonionic surfacants	4 alkylphenol monoetoxylated APE $C_9H_{16}(CH_2)_nO_2$		Chapter 3	Verlicchi et al., 2014
H	Nonionic surfacants	Alkylphenols AP $C_7H_7O(CH_2)_n$		Chapter 3	Verlicchi et al., 2014
I	Nonionic surfacants	Triton X 100 (4-octylphenol polyethoxylate $C_{14}H_{22}O(CH_2\ CH_2O)_n$		Chapter 3	Verlicchi et al., 2014

APPENDIX B

Supplementary Data Lists

List of Supplementary data for Chapter 2

Published in

Verlicchi P, Zambello E. Pharmaceuticals and personal care products in untreated and treated sewage sludge: occurrence and environmental risk in the case of application on soil – A critical review. Sci Tot Environ 2015;538:750-767.

List of Supplementary data for Chapter 4

Published in

Verlicchi P, Zambello E. How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review. Sci Tot Enviro 2014;470-471;1281-1306.

Table SD-1 List of compounds included in the review grouped according to their therapeutic class, together with influent/effluent average concentrations (µg/L) and average removal values (%, in brackets) with respect to the treatment step (I, II, III, hybrid, no conventional CW). The last column reports the corresponding reference. The number in brackets after the name of each PhC represents the number of previous works dealing with it, included in the current survey.

Table SD-2 Number of provided data of influent /effluent concentrations and of removal (in brackets)for each compounds with respect to the treatment step and constructed wetland type.

Table SD-3. Main issues investigated and discussed in all the treatment lines included in the review (**in a separate file**).

Table SD-4 Rules of thumb for predicting potential behavior of pharmaceuticals during treatments

Table SD-5 PNEC values used in the environmental risk assessment

List of Supplementary data for Chapter 5

Published in

Verlicchi P, Galletti A, PetrovicM, Barceló D, Al Aukidy M, Zambello E. Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed—analysis of their respective contributions. Sci Total Environ 2013b;454–455:411–25.

Table SD1. Investigated pharmaceutical compounds: CAS number, formula, main physical and chemical properties, molecular structure and observed variability range in secondary biological effluent (literature data).

Fig. SD1. Occurrence in the H-SSF bed influent and effluent for the selected analgesics and anti-inflammatories and observed average removal efficiency.

Fig. SD2. Occurrence in the H-SSF bed influent and effluent for the selected antibiotics and observed average removal efficiency

Fig. SD3. Occurrence in the H-SSF bed influent and effluent for the selected PhCs belonging to therapeutic classes C-H and observed average removal efficiency.

Fig. SD4. Occurrence in the H-SSF bed influent and effluent for the selected PhCs belonging to therapeutic classe I-L and observed average removal efficiency.

List of Supplementary data for Chapter 6

Published in

Verlicchi P, Al Aukidy M, Zambello E. What have we learned from worldwide experiences on the management and treatment of hospital effluent? — An overview and a discussion on perspectives. Sci Total Environ. 2015;514:467-491.

Appendix C

International published works

Appendix C

International Article (2013)

Journal	Science of the Total Environment
Impact Factor	4.099
Citations (till March 2016)	10

Science of the Total Environment 454-455 (2013) 411–425

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Removal of selected pharmaceuticals from domestic wastewater in an activated sludge system followed by a horizontal subsurface flow bed — Analysis of their respective contributions

P. Verlicchi [a,b,*], A. Galletti [c], M. Petrovic [d,e], D. Barceló [e,f], M. Al Aukidy [a,b], E. Zambello [a,b]

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HIGHLIGHTS

- An activated sludge process followed by a horizontal subsurface flow bed were investigated.
- 73 selected pharmaceuticals of twelve therapeutic classes were analysed.
- The feeding was real domestic wastewater.
- Occurrence, mass load and removal efficiency of selected compounds were investigated.
- Contributions of the two steps in the removal of selected compounds were assessed.

International Article (2014)

Journal	Science of the Total Environment
Impact Factor	4.099
Citations (till March 2016)	18

Science of the Total Environment 470–471 (2014) 1281–1306

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review

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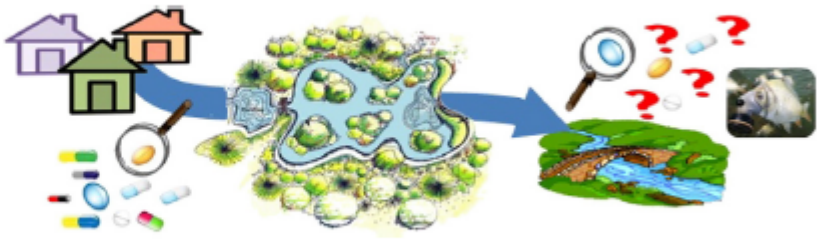
HIGHLIGHTS

- The review deals with the removal of 137 pharmaceuticals in different types of constructed wetlands.
- The plants under review are mainly surface flow and horizontal subsurface flow systems.
- Constructed wetlands under review acted as primary, secondary or tertiary step.
- Constructed wetlands do not completely reduce to low level the environmental risk due to pharmaceuticals in their effluent.
- The most critical compounds are five antibiotics and two analgesics anti-inflammatories.

GRAPHICAL ABSTRACT



Appendix C

International Article (2015)

Journal	Science of the Total Environment
Impact Factor	4.099
Citations (till March 2016)	2

Science of the Total Environment 538 (2015) 750–767

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Pharmaceuticals and personal care products in untreated and treated sewage sludge: Occurrence and environmental risk in the case of application on soil — A critical review

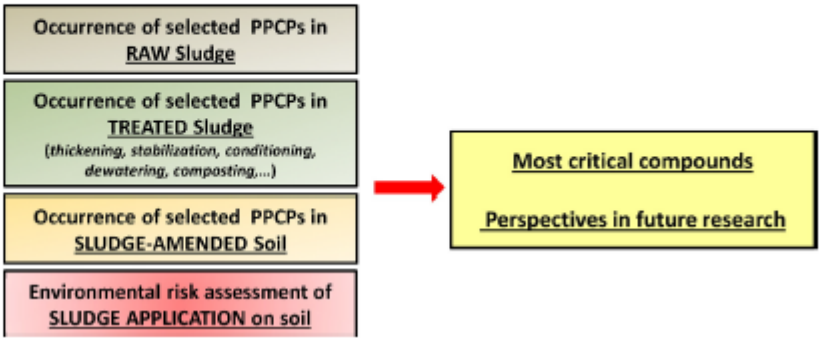
P. Verlicchi [a,b], E. Zambello [a]

[a] Department of Engineering, University of Ferrara, Via Saragat 1 44122 Ferrara, Italy
[b] Technopole Terra and Acqua Tech, Via Borsari 46, 44123 Ferrara, Italy

HIGHLIGHTS

- The review refers to concentrations of 169 PPCPs in different kinds of sludge.
- After digestion or composting, concentrations of most compounds reduced.
- K_d are reported for the selected compounds in different kinds of sludge and soils.
- Environmental risk due to PPCPs in case of sludge application on soil was assessed.
- The most critical compounds are triclosan, triclocarban, hormones and antibiotics.

GRAPHICAL ABSTRACT



International Article (2015)

Journal	Science of the Total Environment
Impact Factor	4.099
Citations (till March 2016)	9

Science of the Total Environment 514 (2015) 467–491

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Review

What have we learned from worldwide experiences on the management and treatment of hospital effluent? — An overview and a discussion on perspectives

P. Verlicchi [a,b,*], M. Al Aukidy [a], E. Zambello [a]

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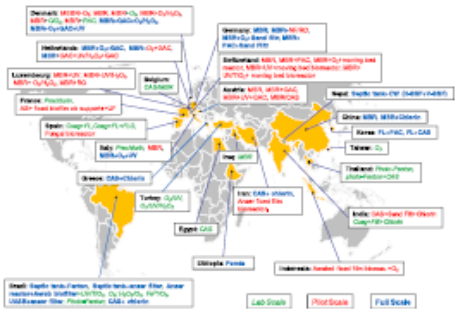
[b] TerraAcqua Tech Technopole of the University of Ferrara, Via Borsari 46, 44123 Ferrara, Italy

HIGHLIGHTS

- Different technologies for a dedicated treatment of hospital effluent are discussed.
- Photo-Fenton process seems to be a promising preliminary treatment. Membrane bioreactor is a proper secondary treatment for hospital effluent.
- AOPs showed a good removal efficiency for most classes of pharmaceuticals.
- UV irradiation is a promising technology in the removal of X-ray contrast media.

GRAPHICAL ABSTRACT



International Book Chapter (2013)

Editor	Elsevier
Book Title	Comprehensive Analytical Chemistry
Citations (till March 2016)	5

Chapter 8

Removal of Pharmaceuticals by Conventional Wastewater Treatment Plants

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Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62 http://dx.doi.org/10.1016/B978-0-444-62657-8.00008-2

International Book Chapter (2014)

Editors	Dr. Silvia Díaz Cruz and Prof. Dr. Damià Barceló
Book Title	Personal Care Products in the Aquatic Environment

Chapter 7
Removal of Personal Care Products in Constructed Wetlands

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Abstract This chapter is an overview of the occurrence of common personal care products in the influent and effluent of different types of constructed wetlands fed with domestic wastewaters, acting as primary, secondary or tertiary steps and the corresponding removal efficiency achieved by these treatments. The reviewed personal care products belong to 9 different classes: 3 antioxidants, 2 antiseptics, 1 deodorant, 1 flame retardant, 1 insect repellant, 1 plasticizer, 3 sun screen products, 5 synthetic musks and 16 surfactants (7 anionic and 9 nonionic).
Data are collated from 36 peer review papers, referring to investigations carried out in Europe (64 %), America (28 %) and Asia (8 %). Of the 88 treatment lines reviewed, the most common constructed wetland type was the horizontal subsurface flow (49 %) followed by the surface flow (39 %) and, in a few cases, the vertical subsurface flow. Removal was mainly influenced by redox potential, temperature, hydraulic retention time and influent concentration of the compound.
The highest values of removal were found for fragrances in secondary systems and fragrances and triclosan in polishing systems.
Due to the different and simultaneous removal mechanisms occurring within these systems and their buffer capacity, they might represent a reliable and feasible treatment which is able to control and reduce the spread of personal care products in the aquatic environment.

Keywords
Constructed wetlands, occurrence, personal care products, removal efficiencies, removal mechanisms

Appendix D

Excretion Rate

Table D1

Class	PhC compounds	Excretion Rates (%)	References
A	Acetaminophen	2/<5/10/80	Monteiro and Boxall, 2010/Jjemba, 2006, Lienert et al., 2007/ Perazzolo et al., 2010/Verlicchi et al., 2010
A	Codeine	3-16/40	Monteiro and Boxall, 2010/Lienert et al., 2007
A	Diclofenac	5-10/15/6-39/41	Verlicchi et al., 2010/Jjemba, 2006/Monteiro and Boxall, 2010/Perazzolo et al., 2010
A	Ibuprofen	10/1-8/5/25	Verlicchi et al., 2010/Jjemba, 2006/Monteiro and Boxall, 2010/Coetsier et al., 2009
A	Indomethacin	**10-20**/15	www.torrinomedica.it/ Monteiro and Boxall, 2010
A	Ketoprofen	10/**75-90**	Verlicchi et al., 2010*/ www.torrinomedica.it
B	Azithromycin	6/8	Monteiro and Boxall, 2010/Kummerer and Henniger, 2003
B	Chloramphenicol	5-10	Jjemba, 2006
B	Chlortetracycline	20/70	www.bioagrimix.com/Monteiro and Boxall, 2010
B	Ciprofloxacin	20/40/83,7/95	Verlicchi et al., 2010/Kummerer and Henniger, 2003/Jjemba, 2006/ Perazzolo et al., 2010
B	Clarithromycin	20/25	Kummerer and Henniger, 2003/Verlicchi et al., 2010
B	Doxycycline	70/41	Jjemba, 2006/Monteiro and Boxall, 2010
B	Erythromycin	5/8/12-15	Verlicchi et al., 2010/Kummerer and Henniger, 2003/Monteiro and Boxall, 2010
B	Metronidazole	40	Jjemba, 2006
B	Norfloxacin	30/40-69/74	Jjemba, 2006/Monteiro and Boxall, 2010/ Perazzolo et al., 2010
B	Ofloxacin	70/**46.5,95.2**	Kummerer and Henniger, 2003/ www.ncbi.nlm.nih.gov/pmc/articles
B	Sulfadiazine	**44**/57	www.ncbi.nlm.nih.gov/pubmed/3557734/ Kummerer and Henniger, 2003
B	Sulfamethoxazole	30/15/10-30/6-39/90/20	Verlicchi et al., 2010/Jjemba, 2006/ Monteiro and Boxall, 2010/ Monteiro and Boxall, 2010/Kummerer and Henniger, 2003/ Perazzolo et al., 2010
B	Tetracycline	80-90/70	Jjemba, 2006(Kühne et al., 2000)*/Monteiro and Boxall, 2010
B	Trimethoprim +	80/60/56/50-60;30-69	Verlicchi et al., 2010/ Jjemba, 2006/ www.ncbi.nlm.nih.gov/pubmed/3557734)/Monteiro and Boxall, 2010
G	Glibenclamide	16	www.medsafe.govt.nz/profs/datasheet/d/Daoniltab.pdf
E	Enalapril	36	Jjemba, 2006
E	Hydrochlorothiazide	24/100	Jjemba, 2006/Oosterhuis et al., 2013
E	Lisinopril	100	www.torrinomedica.it
N	Tamoxifen	30	Coetsier et al., 2009
M	Salbutamol	28	www.medsafe.govt.nz/profs/datasheet/b/Buventolinhalpwd.htm
G	Atenolol	50-90/96	Verlicchi et al., 2010/ Perazzolo et al., 2010
G	Metoprolol	10-30/15/39	Verlicchi et al., 2010/Lienert et al., 2007, Perazzolo et al., 2010/Monteiro and Boxall, 2010
G	Propranolol	0.5/24	Monteiro and Boxall, 2010/ Coetsier et al., 2009
G	Sotalol	>80/98	http://eurheartj.oxfordjournals.org/content/14/suppl_H/30.abstract/ Perazzolo et al., 2010
G	Timolol	20	Tocco et al., 1975
H	Furosemide	40	Jjemba, 2006
I	Atorvastatin	5	Jjemba, 2006
J	Carbamazepine	3/1-2/3/15	Verlicchi et al., 2010/Jjemba, 2006/ Ketter, 1999/Coetsier et al., 2009
J	Diazepam	1	Jjemba, 2006
J	Fluoxetine	2.5-11/60/5	Jjemba, 2006/Lienert et al, 2007/Monteiro and Boxall, 2010
J	Lorazepam	0.3/85	Verbeeck et al., 1976/Coetsier et al., 2009
J	Paroxetine	3	www.torrinomedica.it
K	Ranitidine	30-40/30-70/6-39;68-79	Verlicchi et al., 2010/Jjemba, 2006/Monteiro and Boxall, 2010

References of Table D1

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