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Title: Contributions of combined sewer overflows and treated effluents to the bacterial load released into a coastal area

Article Type: Research Paper

Keywords: Coastal area; combined sewer overflow; E. coli; Enterococci; wastewater management and treatment; wastewater treatment plant effluent.

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Corresponding Author's Institution: University of Ferrara

First Author: Mustafa Al Aukidy, PhD

Order of Authors: Mustafa Al Aukidy, PhD; Paola Verlicchi, Ph.D.

Abstract: The impact of combined sewer overflow (CSO) on the receiving water body is an issue of increasing concern, as it may lead to restrictions in the use and destination of the receiving body, such as bathing or recreational area closures, fish and shellfish consumption restrictions, and contamination of drinking water resources. Recent investigations have mainly referred to the occurrence and loads of suspended solids, organic compounds and, in some cases, micropollutants. Attempts have been made to find correlations between the discharged load and the size and characteristics of the catchment area, climate conditions, rainfall duration and intensity.

This study refers to a touristic coastal area in the north-east of Italy, which is characterized by a combined sewer network including 5 CSO outfalls which, in the case of heavy rain events, directly discharge the exceeding water flow rate into channels which, after a short distance, reach the Adriatic Sea. The study analyzed: i) rainfall events during the summer period in 2014 which led to overflow in the different outfalls, ii) the inter- and intra-event variability with regard to E. coli, Enterococci and conductivity, and iii) the hydraulic and pollutant (E. coli and Enterococci) loads discharged by the local wastewater treatment plant and by all the CSO outfalls. Finally, it estimated the contribution of each source to the released hydraulic and pollutant loads into the receiving water body. Moreover, it was also found that the modest water volume discharged by all CSO outfalls (only 8 % of the total volume discharged by the area) contains more than 90 % of the microbial load.

Response to Reviewers: Dear Reviewers,
I again thank you for your useful comments and suggestions that greatly improved the quality of our revised manuscript.

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Moreover, you add methods in Italian language and it is difficult to check these methods with currently valid standards. This information is crucial in confirmation of your results correctness!!!
As I have already remarked these methods are based on International recognized standards.

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R: I do agree partially with the authors. Some CSO regulations limit both concentrations - duration - frequency of the spills (OD, ammonia in river environments), concentrations and duration (microbiological pollutants) and loads (mainly for nitrogren and phosphorus in eutrophic waters). But the point is that the microbiological load is not considered as a reference parameter because it doesn't make sense. Again, you can capture a small volume of water with a large amount of microorganism but this fact won't solve the beach closing problem.

6. We think that L-V curves referred to E. coli and Enterococci provide useful information about the amount of microorganisms released load and to evaluate how to reduce the first part of this load by a dedicated treatment. In fact the results we presented show that 90 % of the load is associated in the first part of the flush. Our focus is before the discharge in the sea and not once the immission arrives in the sea.
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But, again, the main problem is that it does not make sense characterize the numbers of E. Coli. For instance at section 3.3 the authors state that MD outfall spills loads of 250 billions of E.Coli in June, but sincerely I cannot image if this number is a huge number or is the same number of bacteria which actually live in my body (not E.Coli, for sure). If authors talk about of tons of Suspended solids or Nitrogen, I could imagine the magnitude of the spill.

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12. We think that from an environmental and sanitary engineering point of view, these graphs and calculations provide useful information on the evaluation of possible treatment trains. Moreover other studies used the same graph types in presenting their results, see for instance Galfi et al.,2016a
R: I do not agree (see previous comments). Furthermore, although Galfi et al. use the same L-V curves these authors tried to correlate the bacterial load with TSS (and there are more differences in the work). I think that for the problem addressed in this paper the L-V are not a good indicator of bacterial pollution because the main factor affecting to bathing waters (such as the presented in the paper) are related with the concentrations and the duration of the spills.

DETAILED REVISION

1. INTRODUCTION
No comments here

2. MATERIAL & METHODS
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We added some explanations in the manuscript.

Also the information about the operation time of the pumps (in percentage) is relevant, because this is clearly related with the microbiological pollution released to the aquatic media. I think that this information is useful to compare the system presented in the manuscript with other sewer systems.

Added Table 2 reporting, for each CSO outfall, the percentages of time in which pumps/valve worked with respect to each month and to the whole observation period.

I also suggest changing the name of section "2.4 Calculations" to "2.4. Data analysis" or something similar.
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At this section authors present their findings related with the volume discharged by CSO and the WWTP, pollutant concentrations and loads. Regarding the first section (3.1) I think that authors can shorten a little bit the information presented as much of them can easily visualized at Figures 2 and 3.
Done.

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We added the requested information, on the basis of the data reported in table 1, see Table 2.

At section 3.2 authors should avoid some texts repetitions. For instance, the information provided at page 13, ln 422-424 and page 15, ln 505-509 was presented in the text before.
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Added in the supplementary material (Table S5 and Table S6)

From my point of view, the information of Figures 11 and 12 is not really well interpreted. My conclusion is that there is not a clear correlation between E.Coli concentrations and Flowrate. R2 values for MD are not significant, and the data provided is in log scales, so the scatter is artificially reduced. From my point of view, what we can see here is an almost constant concentration of E.Coli and Enterococci, with some scatter probably related with the disinfection events and some variability produced by the uncertainty in the determination of the microbiological pollutant.
We have greatly appreciated your comments and we modified the text accordingly.

Sections 3.3 and 3.4 doesn't make sense from my point of view, as stated previously. Especially the section related with the L-V curves and first flush.
We accepted this suggestion and we deleted this section, even if the other reviewers did not asked to erase them. We think that the concept of microbial load could be useful to understand the problems related to CSOs and could provide further information. In any case the manuscript without these sections provide sufficient elements and data for the reader.

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Ferrara, May 12th 2017

Dear Prof. Damia Barceló
Editor in Chief
Science of the Total Environment,

referring to the paper:

Contributions of combined sewage overflows and treated effluents to the bacterial load released into a coastal area

by

Mustafa Al Aukidy and Paola Verlicchi

in submitting it to Your international Journal, I would like to make the following remarks:

- the work described in this paper has not been previously published and it is not under consideration for publication elsewhere,
- the *Corresponding Author* is PAOLA VERLICCHI
- Her address is:

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I-44122 Ferrara
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Tel +39.(0)532.974938
Fax +39.(0)532.974870
mail paola.verlicchi@unife.it

Unique features of the study - This study refers to a touristic coastal area in the north-east of Italy, which is characterized by a combined sewage network including CSO outfalls which, in the case of heavy rain events, directly discharge the exceeding water flow rate into a channel which, after a short distance, reaches the Adriatic Sea. The study analyzed i) rainfall events during the summer period in 2014 which led to overflow in the different outfalls, ii) the inter- and intra-event variability with regard to *E. coli*, *Enterococci* and conductivity, and iii) the hydraulic and pollutant (*E. coli* and *Enterococci*) loads discharged by the local wastewater treatment plant and by all the CSO outfalls. It also estimated the contribution of the two sources to the released hydraulic and pollutant loads into the receiving water body. It emerged that the modest water volume discharged by all CSO outfalls (only 8 % of the total volume discharged by the area) contains more than 90 % of the microbial load. This could lead to restriction of recreational activities including prohibition of bathing in the touristic season with an unavoidable negative impact on the local economy.

Sincerely Yours

Paola Verlicchi

Replies to reviewers’ comemnts and suggestions

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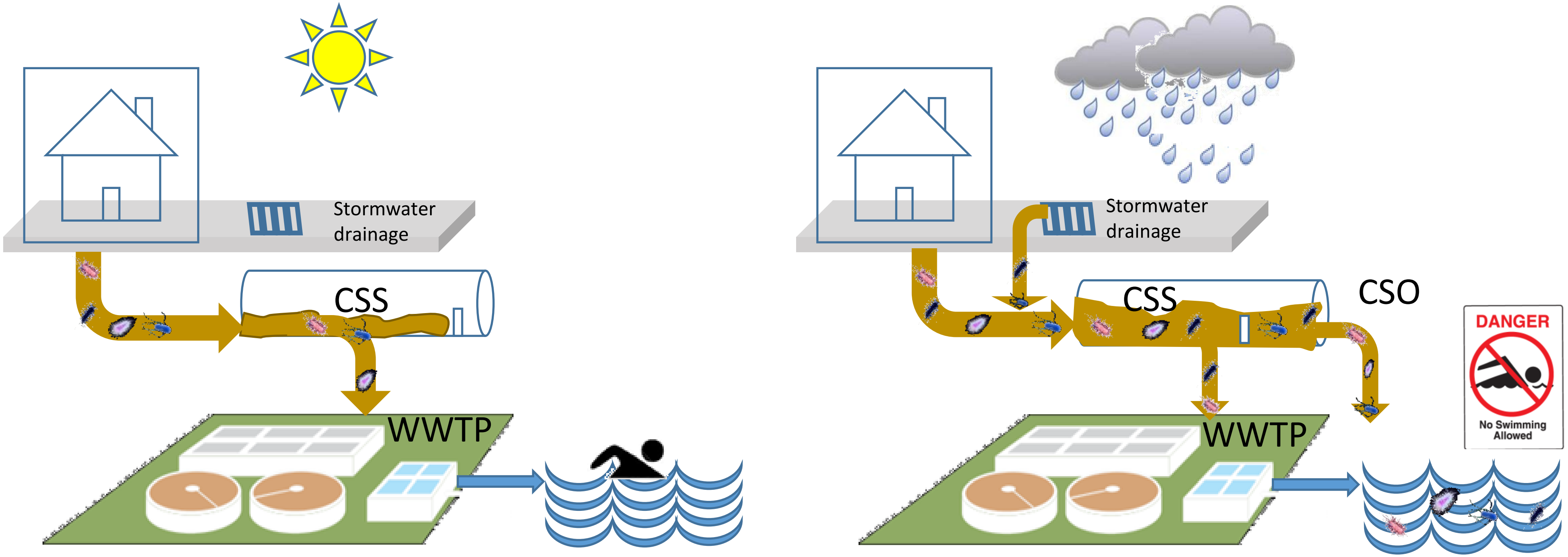
***Graphical Abstract**



Highlights

The impact of combined sewer overflows (CSOs) in a coastal area was assessed.

Microbiological load of CSOs and WWTP effluent was investigated in the study area

The study refers to a summer period

The contribution of CSOs is 8 % in terms of discharged water volume

CSOs are responsible for more than 90 % of microbial discharged load.

Contributions of combined sewer overflows and treated effluents to the bacterial load released into a coastal area

Al Aukidy M.[a], Verlicchi P.[a,b,]*

[a] Department of Engineering, University of Ferrara, Via Saragat 1, 441122 Ferrara, Italy
[b]Terra & Acqua Tech, Technopole of the University of Ferrara, Via Borsari, 46, 44121 Ferrara Italy
mustafakether.alaukidi@unife.it, paola.verlicchi@unife.it

* Corresponding Author

Graphical abstract

Abstract

The impact of combined sewer overflow (CSO) on the receiving water body is an issue of increasing concern, as it may lead to restrictions in the use and destination of the receiving body, such as bathing or recreational area closures, fish and shellfish consumption restrictions, and contamination of drinking water resources. Recent investigations have mainly referred to the occurrence and loads of suspended solids, organic compounds and, in some cases, micropollutants. Attempts have been made to find correlations between the discharged load and the size and characteristics of the catchment area, climate conditions, rainfall duration and intensity.

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Keywords: Coastal area, combined sewer overflow, *E. coli*, Enterococci, ~~first flush effect~~, wastewater management and treatment, wastewater treatment plant effluent.

List of abbreviations and acronyms used in the manuscript: ADP= antecedent dry periods; BIO_D: secondary effluent within the WWTP; BY= bypass; CSO = combined sewer overflow; CSS= combined sewer system; EMC = event mean concentration; EMF= event mean flow; MD = combined sewer overflow outfall upstream the wastewater treatment plant; WWTP = wastewater treatment plant

1 Introduction

In many urbanized areas, domestic wastewater and rainwater (a mixture that, according to the Council Directive 91/271/EEC, is called *urban wastewater*) are collected and conveyed to the wastewater treatment plant (WWTP) by the same network, known as a combined sewer system (CSS).

Combined sewer overflows (CSOs) may occur in the case of intense rainfall (Barco et al., 2008) and/or periods of melting snow (Madoux et al., 2013), resulting in a higher water flow rate within the sewer network due to the occasional, but sometimes consistent, contribution of surface runoff, as well as rainfall. Surface runoff conveyed to the public sewer system may contain suspended solids, organic matter, microorganisms, heavy metals, or pesticides depending on the type, destination and use, width and imperviousness of washing surfaces, rain event frequency and duration, and number of antecedent dry days (Diaz-Fierros et al., 2002; Barco et al., 2008; Galfi et al., 2016b). CSO pollutant concentrations are the result of mixing domestic wastewater and drained stormwater as well as the internal re-suspension of sewer deposits due to flow-induced turbulence. Wastewater and stormwater concentrations as well as their flow rates define the content of the different pollutants (Passerat et al., 2011; Rechenburg et al., 2006).

Receiving water body contaminations by CSOs are intermittent and strictly correlated to the catchment area sewer network ~~size~~ (namely pipe diameters and network size), and climate conditions. Their frequency is site-specific and may also vary from one year to another. These overflows are quite often directly released into a surface water body without any kind of treatment (Ouattara et al., 2014).

Due to their pollutant load, this practice can seriously degrade the receptor water quality, causing depletion of oxygen~~,~~ and an increment in suspended solids, nutrients, organic matter, and heavy metals (Barco et al., 2008; Diaz-Fierros et al., 2002; Hanner et al., 2004; Kafi et al., 2008). Moreover, soon after intense rain events, surface water was found to be affected by an increment in the concentrations of *Giardia* and *Cryptosporidium* (Mac Kenzie et al., 1994; Gibson et al., 1998), Norovirus (Campos et al., 206), and micropollutants (Launay et al., 2016).

This issue is of great concern for water quality control authorities as it could lead to a restriction in the use and destination of the receiving surface body, and consequently, to negative economic impacts. In fact, it could lead to the closure of bathing areas (Burton and Pitt, 2002; Jalliffier-Verne et al., 2016; NYC Global Partners, 2011), restrictions to the consumption of fish and shellfish (Line et al., 2008), and contamination of drinking water resources (McLellan et al., 2007; Galfi et al., 2016b).

It is well known that expensive implementations at large urban WWTPs manage to reduce the residual pollutant load of the treated effluent and thus greatly contribute to improvements in the quality of the receiving surface water body. But these actions cannot attenuate the effects of the short-term disturbances induced by the release of untreated CSOs. This is the case of the catchment area of Brussels, crossed by the Zenne River (Ouattara et al., 2014). The river quality has greatly benefited from the recent upgrade of two large urban WWTPs placed along the river course. However, during intense rain periods, which are quite frequent in the area, a rapid worsening of the microbiological river quality occurs due to untreated CSO releases, resulting in an increment of more than a 2 log factor in the concentrations of *E. coli* and Enterococci in the surface water. Similar negative impacts periodically affect other rivers: the Seine (Servais et al., 2007), the Thames (Tryland et al., 2002) and St. Clair River (Ontario, Marsalek et al., 1994). This decrease in quality is much more evident in cases where the receiving receptor is an effluent-dominant river (Buerge et al., 2006).

It was found that *E. coli* concentrations in stormwater runoff may vary from 2 orders of magnitude lower than in raw wastewater (Passerat et al., 2011; Madoux-Humery et al., 2013) to similar wastewater concentrations in the case of septic cross-connections (Sauvé et al., 2012). Moreover, sediment deposits contribute to the occurrence of bacteria in the first phase of intense rainfall (Madoux-Humery et al., 2015) due to their re-suspension induced by the flow turbulence.

Increasing attention has recently been paid to CSO composition and pollutant load. Most studies have investigated overflow occurrence and the temporal-spatial variability of macropollutants (among them Barco et al., 2008; Kafi et al., 2008) as well as micropollutants (mainly organic compounds and pharmaceuticals: Madoux-Humery et al., 2013, 2015; Phillips et al., 2012; Chèvre et al., 2013); the apportionment of the different sources (wastewater, sewer deposit re-suspension and stormwater) in terms of conductivity, total suspended solids (TSS), *E. coli* during a rain event leading to CSO (Madoux et al., 2015; Passerat et al., 2011), in terms of heavy metals (Diaz-Fierros et al. 2002), and their spatial and temporal variability during different seasons (Madoux et al., 2015, 2013; Galfi et al., 2016a).

Attempts to quantify and simulate the load of CSOs on surface water have also been recently carried out. Among these, Chèvre et al. (2013) applied the substance flow analysis approach to the town of Lausanne, Switzerland, in order to evaluate how to attenuate the load of pharmaceuticals on the aquatic systems due to CSOs and WWTP effluents, while Pongmala et al. (2015) estimated the dynamics of suspended solids, *E. coli* and the micropollutant carbamazepine in the combined sewer network in a sub-catchment of the large area of Montréal (Canada).

From a regulation point of view, the situation varies from country to country. For instance, U.S.EPA (1993) provided a guidance document regarding the disinfection of CSOs. In particular, it highlights that an acceptable treatment should guarantee a removal of at least 4 log units in bacteria, in detention times of less than the conventional 15-30 minutes. Canadian Provincial Regulations restrict the frequency of CSO discharges at each outfall location depending on the time of the year, the type of precipitation (rainfall or snowmelt) and the assimilative capacity of the receiving water (Madoux-Humery et al., 2013, 2015). In the United Kingdom, the Urban Pollution Management (UPM) Manual set wet weather standards for protecting river aquatic life, bathing water, shellfish water, amenity use and location of CSO outfalls (Foundation for Water Research, 2012). In Italy, only a few Regions set out guidelines regarding the management of rainwater. For instance, those set out by the Region of Emilia Romagna *suggest* collecting and treating the first 2.5-5 mm of rain which has fallen on an impervious surface (DGR, 2005) while the remainder may be directly discharged. There are no specific prescriptions in cases where the CSO is directly released into the sea.

This study aims to provide new insights in this context, through an assessment of *E. coli* and Enterococci loads due to CSOs in a typical Italian coastal area during summertime (the observation period is June-September 2014), and comparing them to those released by the effluent of the local municipal WWTP during the whole observation period (dry+wet days). The aim is to identify which are the most important sources in terms of microbiological pollution in the receiving water body ~~(the sea)~~ and also to suggest attenuation measures in order to avoid the bathing area closures which have unfortunately occurred on a regular basis over the last few summers.

2 Materials and Methods

2.1 The site under study

The study site refers to the area of the municipality of Comacchio (coordinates: 44°42’N 12°11’E), situated in the eastern side of the Po Valley, north-east Italy. The area is adjacent to a lagoon (Comacchio Lagoon) and is characterized by an altitude of 1 m over the sea level. The study catchment basin has an extension of 850 ha; the land use is 72 % residential, 12 % institutional and commercial, 15 % open lands and 1 % industrial. The area can be classified as a residential centre; its impervious surface varies between 31 % and 60 % in the different sub-catchment basins and, with respect to the whole catchment, it is equal to 44 %.

This is a typical coastal town characterized by a high density of tourists in summer (up to 180,000 persons) and a resident population of about 25,000 inhabitants during the remaining months. As in all the Mediterranean touristic coastal towns, the population presents consistent fluctuations between May and September: an increment in population is generally registered in weekends in May, June and September and the highest peaks of presences occur during July and end of August.

During the observation period (June-September 2014), the minimum temperature varied between 10 °C and 25 °C and the maximum one between 19 °C and 39 °C. In June, the maximum solar radiation was in the range 870-1317 W/m^2 and the average solar radiation was equal to 258 W/m^2; in July the maximum values were in the range 923-1114 W/m^2 and the average value was 287 W/m^2; in August the maximum value was in the range 895-1190 W/m^2 and the average value equal to 254 W/m and in September the maximum was between 490 and 1000 W/m^2 and the average value was 180 W/m^2. The number of sunshine hours was 15 h and 30 min in June and decreased to 11 h and 45 min in September.

Domestic wastewater and rainwater are collected and conveyed to the local WWTP by a combined sewer system consisting of numerous pipelines discharging the sewer by gravity into a main collector, whose diameter varies between 1000 and 1600 mm, along which a series of lifting pump stations are present in order to convey the sewer towards the central WWTP (Figure 1). The WWTP consists of two treatment lines (one permanently in operation, the second one only between May and September), each of them including preliminary treatments, primary sedimentation, secondary treatments by conventional activated sludge process, and disinfection tanks. The treated effluent is released into a channel which after a distance of 3.5 km reaches the Adriatic Sea.

During heavy rain events, when the influent wastewater flow rate exceeds the capacity of the WWTP and/or the overflow threshold inside the CSS, the exceeding volume is directly discharged in the surface water network through submerged pumps installed for this purpose. This aliquot is the combined sewer overflow (CSO). The Comacchio sewer network under study has five CSO outfalls. Figure 1 reports the sewer network (purple lines), the CSO outfall positions (red squares), the WWTP (rectangle) and the rain gauges (black triangles) placed in the study area.

In particular, the CSO outfalls are located within the lifting pump stations (S6, S8, S13 and S14) receiving urban wastewater from different sub-catchments, and immediately upstream the WWTP (called MD) receiving urban wastewaters from the whole catchment area, as shown in Fig. 1. Overflows are released in five different points of the surface water network at a distance varying between 1.26 km and 6.1 km from the final receptor (Adriatic Sea). On the basis of the characteristics of the channels receiving these overflows, it was estimated an average water speed equal to 0.4 m/s. This means that the time to reach the sea is in the range 0.88 – 4.2 h. The water flowing in these channels are quite turbid and thus the expected decay of microorganisms during their transport to the sea due to sunlight (UV irradiation) is quite modest with respect to the case of clear water.

Figure 1.

Within the WWTP, when the received wastewater flowrate exceeds the nominal capacity of the treatment train, a bypass (called BY) between the primary and secondary steps directly conveys part of the primary effluent to the disinfection tank (avoiding the biological treatment), together with the secondary effluent (called BIO_D in Figure 1). Once disinfected, the total effluent is discharged into the receiving water body.

Each outfall contains submerged pumps with different nominal capacities that can work concurrently, depending on the intensity of the rain event. The characteristics of S6, S8, S13 and S14 outfalls are reported in Table S1. Each outfall is responsible for a determined part of the sewer network and it is designed in a way that pumps start to operate when the water flow rate is 4 times the average dry weather flow rate of that sewer network part.

MD outfall is different, as it consists of a particular valve, characterized by 24 steps, which are 24 degrees of valve opening, adjustable in accordance with the volume of water to be moved. Table S2 reports its working details.

All pumps and MD valve are connected to a data logger that records the date, starting time and duration every time the device (pump or valve) starts working. On the basis of these recorded data and the nominal flow rate for each device ~~pump~~, the total discharged water volume were calculated for each CSO event.

2.2 Characteristics of the recorded rain events

The study refers to the period of June - September 2014. Precipitation data such as event time, total duration and intensity were obtained using three rain gauges installed in the study area (Figure 1). These gauges registered the total depth of rainfall every 9 minutes. Then, 3 hours after the rain event, the cumulative height measurement of each gauge was reset to consider the occurrence of a new event. This separation of one event from another takes into account the speed with which the summer storms evolve. Therefore, in order to define the rainfall events that cause CSO, events separated by at least three hours are considered as individual events, even when they occurred in the same day.

With regard to the studied area, the annual precipitation patterns for 2013, 2014 and 2015 are reported in Figure S1 in terms of monthly precipitation depth. A comparison of the three years shows that there could be some differences from one year to another - recorded annual precipitations were 870 mm in 2013, 740 mm in 2014 and 612 mm in 2015 and summertime (June-September) contribution to the total annual rain water was equal to 28 % (2013), 35 % (2014) and 24 % (2015). An analysis of the precipitation pattern in a wider temporal period highlights that rainy summers alternate with dry ones, or even periods of drought and in any case, the pollutograph referring to 2014 represents a worse scenario in terms of frequency of summer CSO with 93 mm and 99.8 mm falling respectively in June and July.

During the studied period a total of 20 rain events were recorded with an event precipitation depth ranging from 3.01 to 41.4 mm and an average of 17.6 mm. An overview is provided in Table S3 in Supplementary Material: six events occurred both in June (14th, 17th, 19th, 25th,26th, 30th) and July (10th, 12th, 24th, 25th, 26th, 30th), and four in both August (3rd, 15th, 20th, 24th) and September (1st, 9th, 10th, 20th).

The main characteristics of the rainfall events leading to CSO are given in Table 1. For each event, the antecedent dry periods (ADPs) are also reported. The characteristics of the rain events relative to MD outfall are derived from almost the same rain events involving CSO in other outfalls, so data are omitted in the aforementioned Table 1.

The highest cumulative precipitations were observed on June 14th, July 10th, July 26th, July 30thand September 20th, and were always anticipated by a low intensity rainfall event a few hours previously.

Table 1

The recorded rain events leading to a CSO occurred between early morning (about 5 AM) and late evening (11.30 PM) with only a few exceptions when the events occurred before 5 AM (June 30th, July 10th and 26th, September 10thsee Figures 6-10). This implies that during the recorded rain events, in the sewer system there was generally a consistent contribution of domestic wastewater flowing to the WWTP.

2.3 Sampling and analysis

The field investigation was conducted between June and September 2014 for 20 rainfall events leading to CSO in at least one monitored point. Grab water samples were collected every 30 minutes at the five CSO outfalls and processed for *E. coli*, Enterococci and conductivity. Altogether, 154 samples were withdrawn and processed.

The influent and effluent of the municipal WWTP were regularly monitored by the local Water Managing Body staff members for the whole period of investigation in terms of flow rate and concentrations of the two selected bacteria.

All samples were collected manually using 500 mL plastic bottles which had been rinsed with clean water before being used. Samples were refrigerated and analyzed within 3 h of collection.

All analyses were carried out in accordance with the official analytical methods of the Italian legislation, issued by the IRSA-CNR Institute for Water Research of the Italian National Research Council and APAT (Agency for the Protection of the Environment and Technical Services) (IRSA - APAT 2003). In particular analyses of *E. coli* have been performed according to Method B 7030, corresponding to the Standard Methods for the Examination of Water and Wastewater based on the Enzyme standard test (APHA, 1998). Analyses of Enterococci were done according to Method B 7040, corresponding to the standard method ISO 7899-1: 1998 (ISO, 1998), also included in the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Conductivity was analyzed according to the Italian official standard Method 2030, based on electrodes with a surface of 1 cm^2 at 25 °C in a 200 ml water sample.

~~The analysis of *E. coli,* Enterococci and conductivity has been performed according to the standard methods provided by IRSA – CNR (2003) (Method B 7030, Method B 7040 and Method 2030, respectively).~~

Uncertainties in flow rate measures can be assumed to be less than 10 % according to the considerations made by Madoux-Humery et al. (2013) and uncertainties in *E. coli* and Enterococci concentrations less than 25 %, according to Madoux-Humery et al. (2015).

Unfortunately, there were some events in which it was not possible to collect overflow samples and process them for the analytes of interest. These occurred in MD for the events of June 19th, June 26th, August 15th, and August 20th; and for S6 referring to the events of June 25th, July 24th, August 24th, and September 1st.

With regard to WWTP effluent quality, we prudently assumed that the treated effluent (chemically disinfected effluent) always had a content of *E. coli* equal to the maximum value allowed by the local control body authorization (5000 MPN/100 mL, according to the current law: D. Lgs 152/2006, reported in Table S4 in the Supplementary materials). This value corresponds to the 85° percentile of the measured values. Accordingly, for Enterococci, the assumed average concentration in the WWTP effluent was equal to the 85° percentile of the collected data and corresponds to 2,500 MPN/100 mL.

2.4 Data analysis ~~Calculations~~

Collected data of *E. coli* and Enterococci concentrations in each CSO outfall were reported in terms of:

- box-plots;
- concentration profiles *vs*. event time for all the events in order to evaluate the intra-event variability at each CSO outfall and to compare the profiles of different CSO outfalls;
- event mean concentration *EMT vs*. event mean flow rate *EMF*,
- loads discharged by the different CSO outfalls in the studied period.

Moreover, the study evaluated and compared the percentage contribution of each CSO outfall and the WWTP with respect to the total discharged volume in the observation period on a monthly and seasonal basis.

~~Finally, the impact of each CSO on the receiving water body was evaluated by means of:~~

- ~~the L-V curves reporting the normalized cumulative mass *vs.* the normalized cumulative flow rate,~~
- ~~an analysis of the occurrence and magnitude of the mass first flush in all the recorded events.~~

2.4.1 Load of fecal indicator bacteria, event mean concentration and event mean flow rate

The bacterial loads for each event (*EL*) were calculated by eq. 1.

$$EL = \int_0^T C(t)Q(t)dt = \sum_{i=1}^{m} C_i Q_i \Delta t_i \qquad \text{(eq. 1)}$$

where *T* is the duration of each CSO event (s), *m* is the number of samples collected for each CSO event, *C(t)* and *Q(t)* are the pollutant concentration (MPN/100 mL) and outfall flow rate (L/s) as functions of time, and C_i and Q_i are the monitored pollutant concentration (MPN/100 mL) and outfall flow rate (L/s) at each time interval Δt_i (s).

The last sample concentration was also assigned to the total volume discharged until the end of the event, as proposed by other studies (Madoux 2015; Bach et al. 2010). In the case of events with only one value of concentration available, this concentration was assumed to be constant for the whole event. This was the case of the following events: July 13th in MD; June 14th, June 17th, June 30th in S6; June 14th and June 17th in S13; and June 14th and June 30th in S14.

If no concentration value was available for a CSO due to the brevity of the overflow, its modest entity or other technical reasons, we assumed the concentration value measured in another outfall referring to the same event, or occurring at the same outfall for an event with similar characteristics in terms of rainfall duration and intensity and antecedent dry days. This occurred for the following events: June 19th; June 26th, August 15th and August 20th in MD; June 25th, July 24th, August 24th, and September 1st in S6.

Event mean concentration (*EMC*) was calculated using equation 2 and event mean flow rate (*EMF*) using equation 3.

$$EMC = \frac{\int_0^T C(t)Q(t)dt}{\int_0^T Q(t)dt} = \frac{\sum_{i=1}^{m} C_i Q_i \Delta t_i}{\sum_{i=1}^{m} Q_i \Delta t_i} \qquad \text{(eq. 2)}$$

$$EMF = \frac{\int_0^T Q(t)dt}{T} \qquad \text{(eq. 3)}$$

~~2.5 L-V curves~~

~~For each CSO, collected data are reported in terms of cumulative bacteria load divided by the total pollutant load *vs.* cumulative flowing water volume divided by the total water volume per event (the so-called L-V curve). In this pollutograph, the 45° line (the diagonal) represents a storm event in which the concentration of the pollutant is constant for the whole event (reference event).~~

~~If the resulting L-V curve is placed above the 45° line, it means that at the beginning of the rain event, the discharged flow rate was higher than that of the reference event. If instead, the curve is below the diagonal, the mass load of the selected pollutant was lower with respect to the reference case.~~

~~On the basis of these pollutographs, it is possible to evaluate the potential impact of the rainfall on the receiving surface water body and also to compare the impacts of different events.~~

~~2.6 The mass first flush MFF~~

~~Attempts have been made in the past to evaluate the distribution of the pollutant load during a rain event. Different authors have tried to measure and compare the normalized cumulative pollutant load discharged at the *beginning* of the rainfall event. In the early 70s, the concept of "*first flush*" was introduced and discussed by many researchers. In the following years, different definitions were provided for it. Geiger (1987) hypothesized that the phenomenon occurs when the L-V curve always has a slope of more than 45°. The definition was changed by others: according to Saget et al. (1996), the *first flush* is defined when at least 80 % of the pollution load is discharged in the first 30 % of the discharged water volume. The percentage of the discharged load in the first 30 % of water volume was set greater than 50 % by Flint and Davis (2007), whereas McCharty (2009) posed a threshold for the water volume and defined the *first flush* as the normalized mass load of pollutant discharged in the first 30 % of stormwater runoff volume.~~

~~In this study we assumed that a first flush occurs when the curve L-V is above the diagonal and, in order to quantify its magnitude, we evaluated the so-called mass first flush ratio *MFF* (Han et al., 2006), as defined by eq. 4:~~

~~$MFF_n = \frac{\frac{\int_0^T c(t)Q(t)dt}{M}}{\frac{\int_0^T Q(t)dt}{V}}$ (eq. 4)~~

~~where *n* represents a point in the CSO event, and corresponds to the percentage of the runoff, ranging from 0% to 100%. *M* is the total mass of emitted pollutant (MPN), *V* is the total CSO volume (L or m^3). By definition, *MFF* is 0 at the beginning of the rain event and is 1 at the end of the storm. Values greater than 1 mean that a first flush occurs. We assumed *n*= 20 and we analyzed MFF_{20} for the recorded rain events.~~

~~If we found MFF_{20} = 3, it means that the first 20 % of the discharged volume contains 60 % of the mass load of contaminant, if MFF_{20} = 2.5, it means that the first 20 % of the discharged volume contains 50 % of the mass load of contaminant.~~

3 Results and discussion

3.1 Water volume discharged by CSO outfalls and WWTPs

CSO outfalls were analyzed in terms of working frequency and discharged flow rates during the June-September 2014 period. Table S3 shows the operation days and the corresponding discharged flow (m^3/d) for each CSO outfall, as well as the WWTP daily volume (in terms of the completely treated effluent BIO_D and also the partially treated effluent BY) discharged into the receiving water body.

Data regarding CSO event duration and the average and maximum flow rates are compiled in Table 1. The CSO duration ranged between 0.4 min to 930 min (=15.5 h), with an average of 214 min and a 95° percentile of 611 min. ~~The highest frequency of working occurred for MD and S6 (15 and 14 events, respectively) followed by S14 (5 events), S8 (4 events) and S13 (3 events).~~

~~Table 1 shows that during each rain event, the number of CSO outfalls in operation and the discharged flow varied, depending on rain intensity and duration as well as the surface extent affected by the intense rainfall.~~

An analysis of the device (pumps and MD valve) operation time recorded during the observation period is reported in Table 2, on the basis of data reported in the third column of Table 1, in percentage with respect to each month and the whole observation period. As it was expected, the highest values were found for MD (ranging from 2.26 and 6.28 % on a monthly basis). If we consider each CSO outfall, the highest values were always in the month of July in the order: MD > S6 > S13 > S8 > S14.

Table 2

During the same rainfall event, not all CSO outfalls were in operation, and this is attributed to the spatial variability and the rain intensity associated with that event, and also according to the extension of the urban basin surface for which the outfall is responsible ~~and the nominal capacity of the pumps of each outfall~~.

An analysis of data reported in Table S3 shows that the working frequency of the different outfalls varied from event to event. In particular: overflow occurred 18 times in MD outfall, 13 times in S6, 5 times in S14 and 4 times in S8 and S13. ~~With respect to the number of outfalls which generated overflow, it emerged that overflow interested 5 outfalls in 5 % of the rain events, 4 outfalls in 10 % of the recorded events, 3 outfalls in 24 % of events, 2 outfalls in 14 % of events and 1 outfall (generally MD) in 48 % of cases.~~

The MD outfall exhibited a greater number of CSO events with respect to the other outfalls, and this is due to its position and function. It receives urban wastewater from the whole catchment area through different collectors and is the last “hydraulic protection” for the WWTP. In particular, it receives all the wastewater coming from the north part of the study area, whose sewer network does not have any CSO outfall (Figure 1). Outfalls situated downstream the sewer network (S14 and S6) were in operation a greater number of times than S13 and S8 due to the larger drained surface area.

With regard to Table S3, overflow events are reported using a color code, each of which is also attributed to the rain event which causes the corresponding CSOs.

The analysis of the overflow events in terms of the percentage contributions of discharged water volume by each CSO outfall on a monthly basis with respect to the total flow in the sewer system is given in Fig. 2.

The highest contribution of CSO outfalls for the total discharged overflow was observed in July (17%) and to a lesser extent in June (9 %), whereas the lowest one occurred in August (2%).

The overall volumes discharged from the outfall points during the study period were in the following order: MD (70,362 m^3) > S6 (60,538 m^3) > S13 (40,527 m^3) > S14 (33,502 m^3) > S8 (15,233 m^3), whereas the total volume discharged from the WWTP (that is BIO_D + BY) was 2.23×10^6 m^3. An analysis of the discharged water volume by each point is reported in Figure 3. For each specific outfall we evaluated the percentage of water volume discharged in each month (see Table S3) with regard to the total volume discharged by the point under evaluation in the four months (corresponding to the sum of the four discharged values of the point reported in Table S3). It emerges that the monthly percentage contribution to its total discharged water volume varies depending on the point and the month of July mostly contributed for all the points, with the exception of BIO_D, to the discharged water volume (Fig. 3). In particular: BIO_D equally contributed to the discharged volume over the observation period (as expected) and BY mostly contributed during the first two months. In S8 and S13, overflow events occurred only in two months, whereas in S14 occurred in three, and in MD and S6 in four months.

Figure 2.

Figure 3

~~As can be seen from the analysis of Table 1 and Table S3, rainfall events with a high cumulative precipitation depth with respect to their duration were always responsible for overflow events with high discharge volumes (for instance, the events occurring on June 14th in S14 and July 30th in S6). With respect to each single rain event, the water volume discharged by CSO outfalls varies between 0.07 % to 75 % of the total volume collected in the sewer system (domestic wastewater + rainwater).~~

Some overflows occurred during rainfall events on summer days with a lower tourist presence in the study area. This mainly happened for CSO outfalls draining large basin areas even when modest rain intensity occurred (e.g. June 17th, 25th and 30th, August 24th, and September 1st and 10th). On these days, however, the CSO flow rate was modest, with only one exception (June 17th) being the last day of a long and intense rain event which had started on June 14th.

Generally, overflow events of long duration occurred after prolonged rainfall events in terms of total cumulative precipitation depth (Table 1). Finally, the rainfall event of July 26thcaused flooding around the urban basin, in particular on the southern beaches, due to the intensity and duration of the event and the consequent fall of water on impervious surfaces.

3.2 Concentrations of investigated pollutants

Figure 4 represents the range of variability of the concentrations of both indicator bacteria observed during the monitoring campaign in all CSO outfalls, together with the Italian limits of *E. coli* and/or Enterococci for the direct discharge of WWTP effluents into surface water bodies and into inland (internal water) bodies, as well as marine bathing water. Table S4 ~~in the Supplementary data section~~ provides details about these legal values as well as the definition of inland and marine water according to the current regulations.

The widest variability ranges for the two indicator bacteria were always observed for MD and the lowest for S13.

The corresponding median concentrations, reported in descending order were:

- *E coli* (MPN/100 mL): 2.40 x 10^6 (MD), 1.64 x 10^6 (S14), 1.49 x 10^6 (S8), 1.05 x 10^6 (S13) and 4.89 x 10^5 (S6).
- Enterococci (MPN/100 mL): 2.66 x 10^5 (S14), 2.06 x 10^5 (MD), 1.99 x 10^5 (S13), 1.48 x 10^5 (S6) and 1.18 x 10^5 (S8).

It is important to observe that the first quartile, median and third quartile of the measured concentrations were *always* above the reported legal limits. The only exception was observed for S6, where the minimum and first quartile concentrations were below the limit for the direct discharge of a WWTP effluent (limit A = 5 x 10^3 MPN/100 mL).

The interval of variability found in the studied area ranged between 10 and 1.3 x 10^7 MPN/100 mL for *E. coli* and 10 and 7.27 x 10^5 MPN/100 mL for Enterococci. Table S5 and S6 summarize minimum, maximum and median concentrations for each outfall.

Figure 4.

These ranges are in fairly good agreement with those reported in literature and, in particular, with Arnone and Walling (2006) (900-7 x 10^4 MPN/100 mL for *E. coli* and 1.1 x 10^4 –3 x 10^5 MP/100 mL for Enterococci), Marsalek et al.(1994) (*E coli* in the range 2.8 x 10^4-1.1 x 10^6 MPN/100 mL for) and Passerat et al. (2011) (3.8 x 10^5-6.4 x 10^6 MPN/100 mL for *E. coli* and 1.2 x 10^5-1.2 x 10^6 MPN/100 mL for Enterococci)

In a separate sewer system, a concentration of *E. coli* and Enterococci was found in the range of 10-4 x 10^4 CFU/100 mL and 10 -9 x 10^4 CFU/100 mL, respectively during rainfall events and between 10 and 5.7 x 10^4 CFU/100 mL and 10 and 8 x 10^3 CFU/100 mL, respectively in snowmelt periods (Galfi et al., 2016b).

Figure 5 reports all the measured concentrations for *E. coli* in CSOs *vs*. the corresponding sampling time. It confirms that *E. coli* concentrations depend on many factors (rain intensity and duration, moisture, temperature, nutrient availability, adsorption/desorption processes, hydrologic processes and predation). It also highlights the fact that although one could expect that during the night values should be lower due to the modest contribution of domestic wastewater, they are generally in the range of 10^5-10^7 MPN/100 mL.

Fig. 5.

Dry weather concentrations of *E. coli* and Enterococci in the raw WWTP influent measured by Local Water Managing Body CADF were on average 3.6 x 10^6 and 1.7 x 10^5, respectively. It emerges that for both *E. coli* and Enterococci, the median values found at the different outfalls are in the same order of magnitude of the average value measured in the raw influent WWTP in dry weather.

During the whole observation period, the average concentration of *E. coli* and Enterococci in the treated effluent (data not reported) were 2.5 x 10^3 MPN/100 mL and 1.12 x 10^3/100 mL, with only a few exceptions related to the occasional escapes of suspended solids from the secondary clarifier.

3.2.1 Intra-event variability of monitored parameters

Figures 6-10 present the profiles of *E. coli* and Enterococci concentrations as well as the conductivity for all CSO outfalls during the different rainfall events. The X-axis reports the sampling time for each outfall. Note that the Y-axis on the left is in a log scale and it refers to bacteria concentration, whereas for conductivity the scale is on the right side and is a normal scale.

Measured concentrations of *E. coli* and Enterococci showed similar variations in all CSO outfalls and they generally vary within one order of magnitude during each event, with only a few exceptions. Some events were characterized by lower concentrations, between 10 and 1000 MPN/100 mL for both indicators: this occurred in MD on June 17th and September 1st and in S6 on July 30th and August 3rd and S8 on July 30th. ~~Bacteria concentrations were found between 10 and 100 MPN/100 mL in MD (June 17th and September 1st) and in S6 and S8 (July 30th). The rainfall event of July 30th was quite long (more than 510 min), with 3 antecedent dry days and, regarding S6, the event with the largest discharged overflow volume (11,078 m^3). The July 30th event in S6 and S8 shows lower concentrations than all the other events.~~ These S6 and S8 lower ~~unusual~~ values were due to the disinfection treatment by means of peracetic acid applied at these two outfall points by the local water management body in order to protect the receiving water body during the summer season and to guarantee adherence to marine bathing limits. This represents a strategy suggested and adopted in different countries, as it will be discussed in section 3.6. The concentration profiles found in the two events in MD seem to exhibit the occurrence of a first flush phenomenon in the investigated outfall that is a more polluted overflow discharged at the beginning of the CSO.

~~With regard to the whole set of collected data, peak maximum concentrations for *E. coli* varied between 2.5 x 10^6 and 1.1 x 10^7 MPN/100 mL (Fig. 4), with the highest value occurring in MD and the lowest in S14. For Enterococci the maximum values varied between 4 x 10^5 and 8 x 10^5 MPN/100 mL, with the highest value in MD and the lowest in S13.~~

The maximum values of bacterial concentrations in CSOs were found at the beginning of the rain event in 60 % of cases for *E. coli* and 55 % for Enterococci. The concentration profiles (see Figures 6-10) are strictly related to rainfall duration and intensity and antecedent precipitations, as discussed by Pongmala et al. (2015).

It is important to know the maximum concentrations occurring for microbiological contaminants, as they represent the most critical situations for the receiving water body and could seriously affect and threaten its expected use and purpose (drinking needs, bathing, and recreational activities in general).

E. coli concentration profiles are in good agreement (variability ranges and trends) with the curves found by Madoux-Humery et al (2015) in summertime in a residential area with only 11 % of open lands.

With regard to conductivity, its variation over time during CSO generally shows a peak at the beginning of the event, then rapidly decreases and sometimes reaches a minimum before progressively increasing until the end of the overflow. This profile is in good agreement with that found by Passerat et al. (2011) for the CSO monitored in a French urban area.

As shown in Figures 6-10, the conductivity varied in the following ranges: 1782 - 5460 μS/cm in MD, 320 - 3010 μS/cm in S6, 1090-17,650 μS/cm in S8, 210 - 1727 μS/cm in S13 and 210-14200 μS/cm in S14.

Based on the collected data, the corresponding median conductivity values for the different CSO discharges were 3550 μS/cm (MD), 1080 μS/cm (S6), 2655 μS/cm (S8), 650 μS/cm (S13) and 2430 μS/cm (S14). CSO conductivity is lower than the typical values found for raw wastewater. It was found that conductivity of the WWTP influent in the dry period was about 6210 μs/cm (CADF, Report 2013), highlighting the dilution effect of wastewater due to urban stormwater runoff (with a much lower conductivity). The values reported by Passerat et al. (2011) for the CSO are instead considerably lower (the maximum is equal to 500 μS/cm, the minimum to 150 μS/cm and the median to 200 μS/cm), and the same is true of the average conductivity in raw wastewater (1175 μS/cm). The differences are mainly due to the consistent apportionment of saline intrusion in the sewer network under study.

Figure 6.

Figure 7.

As reported in literature, there is a great inter-event and intra-event variation in the concentration of microorganisms in CSO depending on catchment characteristics, rainfall/runoff duration and intensity, stormwater quality, climate characteristics (namely air and water temperature), and the number of dry days before the event. The sources of bacteria in stormwater runoff are attributed to the presence of debris, human activities and animal feces in urbanized areas, and to wildlife feces, recreational activities and soil and vegetation in low-imperviousness surfaces (Galfi et al., 2016a).
~~The interval of variability found in the studied area ranges between 10 and 1.3 x 10^7 MPN/100 mL for *E. coli* and 10 and 7.83 x 10^5 MPN/100 mL for Enterococci.~~

~~Bacteria concentrations were found between 10 and 100 MPN/100 mL in MD (June 17th and September 1st) and in S6 and S8 (July 30th). The rainfall event of July 30th was quite long (more than 510 min), with 3 antecedent dry days and, regarding S6, the event with the largest discharged overflow volume (11,078 m^3).~~

Figure 8.

Figure 9.

Figure 10.

~~These ranges are in fairly good agreement with those reported in literature and, in particular, with Arnone and Walling (2006) (900-7 x 10^4 MPN/100 mL for *E. coli* and 1.1 x 10^4 –3 x 10^5 MP/100 mL for Enterococci), Marsalek et al.(1994) (*E coli* in the range 2.8 x 10^4-1.1 x 10^6 MPN/100 mL for) and Passerat et al. (2011) (3.8 x 10^5-6.4 x 10^6 MPN/100 mL for *E. coli* and 1.2 x 10^5-1.2 x 10^6 MPN/100 mL for Enterococci)~~

~~In a separate sewer system, a concentration of *E. coli* and Enterococci was found in the range of 10-4 x 10^4 CFU/100 mL and 10 -9 x 10^4 CFU/100 mL, respectively during rainfall events and between 10 and 5.7 x 10^4 CFU/100 mL and 10 and 8 x 10^3 CFU/100 mL, respectively in snowmelt periods (Galfi et al., 2016b).~~

3.2.2 Event mean concentration

Most studies have presented and compared results on the basis of the *Event Mean Concentration* (*EMC*) (Madoux-Humery et al., 2015; Hathaway and Hunt, 2010). This parameter provides a macro-snapshot of the event under study, but does not consider the dynamics of microbial concentrations during the event. Tables S5 and S6 report the estimated *EMC* for each event and each outfall for *E. coli* and Enterococci respectively, whereas Figures 11 and 12 show the curves of *EMC*, for all the observed CSO events, *versus* the corresponding event mean flowrate *EMF*, for each CSO outfall.

It emerges that *EMCs* ranged from 5.45 x 10^2 to 9.69 x 10^6 MPN/100 mL for *E. coli* and from 7.56 x 10^2 to 6.58 x 10^5 MPN/100 mL for Enterococci.

EMCs of *E. coli* in all CSO outfalls were mostly in the range of 10^6 MPN/100 mL, with the exception of two events (July 30th at S6 and S8 and August 3rd at S6), where considerably lower *EMC* values were observed because the water managing body decided to disinfect this stream as it will be discussed later. ~~by means of peracetic acid before its release into the surface water channel~~. A similar pattern was also observed for the Enterococci but with one order of magnitude less.

With regard to literature data, we found that *EMCs* of *E. coli* were one order of magnitude lower than *EMCs* found by Madoux-Houmery et al.(2015).

In Figures 11 and 12, the interpolating line of the data regarding MD outfall has a slightly positive slope for both *E. coli* and Enterococci. The low value of R^2 means that, on the basis of the collected data, the correlation is not clear. On the contrary, Hathaway and Hunt (2011) and Dickenson and Sansalone (2012) found a good correlation (slope < 1) between bacteria concentration and flow rate, indicating that there is a contribution of a *less concentrated* water stream (i.e. stormwater) to the total load of both bacteria (dilution effect). They found that the content of *E. coli* in stormwater was 2 orders of magnitude lower than in raw wastewater.

With regard to the other monitored CSO outfalls, due to the low quantity of data, concentration-discharge slopes were not considered.

Figure 11.

Figure 12.

3.3 Discharged bacterial load – Contribution of occasional and continuous points

The total discharged load of *E. coli* and Enterococci from each CSO outfall and the WWTP has been calculated and depicted in Figure 13 on a monthly basis in absolute terms (as the amount discharged from each point, see rectangles), and as a percentage of the discharged load with respect to the total load discharged into the receiving water body (see bold lines). The contribution of each point and the main sources in each month are immediately evident.

With regard to *E. coli*, the highest discharged amount in all months was due to MD outfall, with loads of 2.5 x 10^{14}, 8.6 x 10^{14}, 4.2 x 10^{14}, 9.4 x 10^{13} MPN/month from June to September. The second source varied: S6 in June and July, WWTP effluent in August and S14 in September.

With regard to Enterococci, the main contribution was due to S6 in June and September and to MD in July and August, followed by WWTP effluent in June and August, S8 in July, and MD in September.

The differences between the monthly load emitted by the main two sources were extremely high in August for *E. coli* and high in June for *E. coli* and Enterococci. In the other cases, the differences were quite modest.

Although the water flow discharged from CSO outfalls (Fig. 2) is much lower than that discharged from the WWTP into surface water (9% in June, 17% in July, 2% in August and 5% in September), the discharged load of bacteria from these points is consistently higher throughout the studied period (Fig. 13): on a monthly basis, they contribute more than 90 % for *E. coli* and more than 77 % on average for Enterocci.

Figure 13.

~~3.4 Intra-event bacteria variation and first flush effect~~

~~Curves of normalized cumulative pollutant mass load *versus* cumulative normalized flow (L-V curves) are reported in Figure 14 for the different CSO outfalls.~~

~~For both indicator bacteria, L-V curves showed similar patterns in MD, S6 and S13, where a consistent mass load was emitted during the initial stage of the event. In fact, in most cases at these outfalls, the slope of the mass emission line exceeded the bisector, resulting in a first flush phenomenon according to the approach developed by Geiger (1987).~~

~~S8 and S14 instead showed no clear trend with different patterns among events: a first flush was observed for some events, whereas for others, high discrepancies in bacterial concentrations occurred. For instance, in S8, an event presents an *end-flush* (McCarthy, 2009), that is it was the final step which mostly contributed to the bacterial discharged load. Generally, these situations are due to a consistent contribution of wastewater intrusion (McCarthy, 2009) or a highly-polluted surface runoff in the last rainfall phase (Hathaway and Hunt 2011).~~

~~The L-V curves referring to MD, S13 and S14 are quite similar to those found by Galfi et al. (2016b) for a separate storm sewage draining in a large (40 ha) catchment area, with 60 % imperviousness in Ostersund (Sweden); plots referring to S6 are similar to those found for a separate storm sewage draining in a small (5 ha) catchment area, with 80 % imperviousness in Sweden, and plots referring to S8 present similarities with those found for a separate stormwater network in Raleigh, NC, by Hathaway and Hunt (2011).~~

~~Figure 14~~.

~~Existence of the first flush was investigated by many authors with regard to different pollutants, namely SS, COD, BOD, TN, P, Pb and Zn (Barco et al., 2008), *E. coli*, Enterococci (McCarthy et al., 2012) and in combined sewer networks (Barco et al., 2008;) as well as separate sewer systems (McCarthy et al., 2009); in different catchment sizes and types, including industrial poles, residential areas with different populations (McCarthy et al., 2012) and green recreational areas (Galfi et al., 2016a).~~

~~All studies concluded that its occurrence is strictly related to the type of pollutant, size and imperviousness of the catchment area, surface characteristics, type of sewer network, duration of antecedent dry periods and climate conditions. In order to investigate its magnitude, some authors evaluated the mass first flush ratio at a specific point (n) MFF_n in the storm event with respect to the pollutant of interest.~~

~~In this study, we compared events in terms of MFF_{20} with regard to *E. coli* and Enterococci. Table 4 reports the values for all the rain events occurring in the different CSO outfalls.~~

~~It clearly emerges that MFF_{20} varied between 0.09 and 5.0. MFF_{20} lowest values were observed during the rain event of September 20th in S6 (Enterococci), S8 (*E. coli* and Enterococci) and S14 (Enterococci), even though the precipitation was intense (25.8 mm) and long (306 min). This fact could be due to the short antecedent dry period, which was only 0.6 day (McCarthy et al., 2012) and the modest contribution of sediment resuspension.~~

~~The highest values of MFF_{20} observed in S6 of both indicator bacteria during the event of July 30th were due to their relative high measured concentrations at the initial stage of the event (in the order of 10^3 MPN/100 mL) and their low concentrations or absence (disinfected point) during the rest of the event.~~

~~Table 2~~

3.5 Fate of the released fecal indicator bacteria in the water environment

Once *E. coli* and Enterococci are released in the water environment (channels and then the Adriatic Sea) their elimination/survival is strictly correlated to the receiving water characteristics (mainly temperature, turbidity, salinity, residence time in the channel) and the environmental conditions (namely sunlight hours, UV irradiation, sunny/cloudy weather conditions). In addition the tide may also affect bacteria elimination processes. Enterococci can generally survive longer than *E. coli* in water (Byappanahalli et al., 2012). A brief presentation of the influence af the cited parameters is reported in Table 3 and an interesting discussion on their influence on the microbiological quality of the sea in all the Spanish beaches and in a Lake Michigan swimming beach are reported in Aragonés et al. (2016) and in Whitman et al. (2004) respectively.

Table 3

With regard to the study area, the channels receiving overflows are characterized by a turbidity equal to 40-90 mg/L SiO_2 in ~~laminar~~ dry weather conditions and much higher after rain events, due to the induced turbulence leading to re-supspension of settled material. The water depth in these channels is between 2.5 and 4 m and water temperature after rainfall events in summer is between 17°C and 20 °C. The distance between the overflow release points and the Adriatic Sea varies between 1.26 km and 6.1 km. Assuming a water speed in the channels of 0.4 m/s, the residence time varies between 0.88 h and 4.2 h.

In this short period of time, in case of overflow, fecal bacteria elimination from the outfall release points to the final receiver (the Sea) is modest. In fact, after rainfall events, the sky is generally cloudy and thus the solar radiation is not able to efficienctly remove these microorganisms, even if there are many sunlight hours (from 15 h in June to 12 h in September). Moreover, water turbidity hinders light penetration. The really modest natural attenuation of the exceptionally high load/concentration of microorganisms released after an intense rainfall event in the channels is demonstrated by the fact that during each summer, soon after intense rain events, in the beaches near the immission of these channels in the Adriatic Sea, bathing is prohibited as bacteriological standards in sea water are exceeded. Unfortunately this is happening in many other coastal towns in Italy.

3.6 CSO management and treatment

In order to reduce and attenuate the pollutant load of intermittent CSOs in the receiving water body, correct management and treatment should be adopted.

Enlargement of the existing sewer network is possible but extremely expensive due to the wide extension of the sewer network and the necessary upgrading of the receiving WWTP in terms of an increment of the nominal hydraulic capacity and upgrading of the existing treatment capacity.

Lessons learned from recent experiences show that in combined sewer networks, adequate measures refer to a dedicated treatment of the occasional overflow rate. They must guarantee a high level of removal of suspended solids and bacteria and that in the vicinity of swimming beaches disinfection becomes a necessity.

Recently, technologies and/or treatment trains were tested in pilot and full scale plants. Of these, the most promising seem to be:

- chemical pre-treatments prior to UV disinfection. Investigations were carried out for alum ($Al_2(S0_4)_3 \bullet 12H_2O$), ferric chloride ($FeCl_3$) and cationic polymers. Higher UV light transmission (UVT) and suspended solid removal were observed with alum (20 mg/L increased the UVT of the raw CSO from 30 to 60% after settling; a dose of 100 mg/L of alum maximized UVT that reached approximately 85%). Flocculation, although not increasing UVT did improve the removal of total suspended solids. Cationic polymers worked quickly, compared to metal coagulants, but reached a maximum UVT of 60 % (Gibson et al., 2016).

Interesting results have been achieved by treating CSO in a ballasted flocculation unit (BFU), that is a compartment employing microsand in order to favor bloc formation acting as a ballast agent, thus reducing hydraulic retention time and increasing the nominal overflow rate (Gasperi et al., 2012). The full scale BFU unit, equipped at the Seine Aval WWTP near Paris, showed that the treatment seems to be less sensitive to the influent concentration fluctuations and hydraulic peak load than to the control and adjustments of chemical doses and sand injection;

- vegetated and unvegetated horizontal subsurface flow beds as discussed by Pisoeiro et al. (2016). In a bed (size: 55.5 cm long, 36.1 cm wide and 40 cm high; filling material (35 cm height): 4-8 mm, 30 % porosity) fed with CSO (Enterococci concentration was on average 1,15 x 10^6 MPN/100 mL (standard deviation 8.21 10^5), TSS 120 mg/L (standard deviation 48) and COD 233 mg/L (standard deviation 53)), with a hydraulic retention time of 1 d and 7 days, an average removal rate was found of 90-100 % for TSS, 60-90 % for COD and 2-6 log units for Enterococci; most of TSS and bacteria were removed in the first 24 hours. Moreover, plant species (*Phragmites australis*) did not influence the removal of TSS and bacteria;
- peracetic acid (PAA) disinfection: it was found that PAA concentration in the range of 5 - 15 mg/L and contact times from 2 to 10 mins are able to reduce the *E. coli* concentration from 10^5-10^6MPN/100 mL to below the limits posed by the Kentucky Administrative Regulation (KAR 2012) of 240 MPN/100 mL for the instantaneous samples and 130 MPN/100 mL for the geometric mean of samples taken over a 30-day period (see Table S2 in the Supplementary Data section) (Coyle et al., 2014);
- performic acid (PFA) disinfection: investigations on the disinfection of CSO using PFA in a sea-outfall pipe of a large WWTP in Copenaghen showed a removal of 1-3.5 log units for *E. coli* and 1.0-2.44 log removal for Enterococci at doses ranging in the interval 1-8 mg/L (Chhetry et al. 2015). These results, although interesting, are still at an early stage of development. (Chhetry et al., 2014).

On the basis of these findings and the characteristics of the area under study, attenuation measures have recently been discussed - in order to reduce the impact of the intermittent CSOs of the Comacchio area in the Adriatic Sea, the Local Water Management Body has planned to build a specific treatment plant for the MD CSO, consisting of a sedimentation tank (for the removal of suspended solids) and of a PAA disinfection step for a maximum flow rate of 350 L/s. This should guarantee respect of the Italian limits for bathing on beaches.

4 Conclusions

The analysis of the pollutant loads discharged by intermittent CSO outfalls compared to those released by the local WWTP highlights that although the CSO water volume is much lower than that released by the WWTP, the CSO microbiological load is much higher than that of the WWTP, particularly during periods of heavy rain in the summer. Once the overflow is released into the surface water network, auto-purification processes take place in the receiving system. Among these, UV irradiation is very effective in removing microorganisms in water environment. But after an intense rain event, this effect is modest due to different reasons: UV irradiation cannot well penetrate in water due to water turbidity and UV intensity is reduced by cloudy weather conditions. Moreover, released microorganisms stay 1-4.9 h in the channels before reaching the Sea and this period is not sufficient to guarantee a good removal under sunlight conditions. This fact could have an immediate acute negative impact on the quality of the receiving water body, and in the worst case scenario could lead to the prohibition of bathing as bacteriological limits in sea water are exceeded. Unfortunately, these events have frequently occurred during previous summer seasons in most of the Italian coastal area. The case study highlights that a correct measure could be disinfection for the effluent for the most critical CSO outfall in terms of discharged microbial load. This would greatly reduce the risk of compromising quality in recreational areas, mainly with regard to bathing.

5 Additional materials

Supplementary data to this article can be found at: ….

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References

American Public Health Association (APHA), American Water Work Association (AWWA) and Water Environment Federation (WEF). Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998. Washington DC, USA.

Aragonés L, López I, Palazón A, López-Ubeda R, García C. Evaluation of the quality of coastal bathing waters in Spain through fecal bacteria *Escherichia coli* and Enterococcus. Sci Total Environ 2016;566-567:288-97.

Arnone RD, Walling JP, Evaluating *Cryptosporidium* and *Giardia* concentrations in combined sewer overflow, J Water Health. 2006;4(2):157-65

Bach PM, McCarthy DT, Deletic A. Redefining the stormwater first flush phenomenon. Water Res 2010;44(8):2487-98.

Barco J, Papiri S, Stenstrom MK. First flush in a combined sewer system. Chemosphere 2008;71(5):827-33.

Bathing Water Committee, 2009. Bathing Water Profiles: Best Practice and Guidance. European Environment Agency (EC DG ENV 2009 URL: http://ec.europa.eu/environment/water/water-bathing/pdf/profiles_dec_2009.pdf).

Buerge IJ, Poiger T, Muller MD, Buser HR. Combined Sewer Overflows to Surface Waters Detected by the Anthropogenic Marker Caffeine. Environ Sci Technol 2006;40(13):4096-4102, doi:10.1021/es052553l

Burton GA, Pitt RE. Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists and Engineers. Lewis Publishers, Boca Raton. 2002

Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ. Enterococci in the environment. Microbiol. Mol. Biol. Rev. 2012;76 (4), 685–706.

CADF, Piano di campionamento delle acque interne in prossimità del litorale Comacchiese, Report 2013, available at the web site: http://www.cadf.it/pdf/balneaz_settott2013.pdf (*in Italian*, last access May 2017).

Campos CJA, Avant J, Lowther J, Till D, Lees DN. Human Norovirus in untreated sewage and effluents from primary, secondary and tertiary treatment processes. Water Res 2016; 103:224-32.

Chèvre N, Coutu S, Margot J, Wynn HK, Bader H, Scheidegger R, Rossi L. Substance flow analysis as a tool for mitigating the impact of pharmaceuticals on the aquatic system. Water Res 2013;47(9):2995-3005.

Chhetri RK, Flagstad R, Munch ES, Hørning C, Berner J, Kolte-Olsen A, Thornberg D, Andersen HR. Full scale evaluation of combined sewer overflows disinfection using performic acid in a sea-outfall pipe, Chemical Engineering Journal 2015;270:133–139

Chhetri RK, Thornberg D, Berner J, Gramstad R, Ojstedt U, Sharma AK, Andersen HR, Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids, Sci. Total Environ. 2014;490:1065–1072.

Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment, Official Journal L 135, 30/05/1991.

Coyle EE, Ormsbee LE, Brion GM. Peracetic acid as an alternative disinfection technology for wet weather flows. Water Environ Res 2014;86(8), pp. 687-697

DGR-Deliberazione della Giunta Regionale 14 febbraio 2005, n. 286. Direttiva concernente indirizzi per la gestione delle acque di prima pioggia e di lavaggio da aree esterne (art. 39, DLgs 11 maggio 1999, n. 152)

Diaz-Fierros TF, Puerta J, Suarez J, Diaz-Fierros VF. Contaminant loads of CSOs at the wastewater treatment plant of a city in NW Spain. Urban Water 2002;4(3):291-9.

Dickenson JA, Sansalone JJ. Distribution and disinfection of bacterial loadings associated with particulate matter fractions transported in urban wet weather flows. Water Res 2012;46(20):6704-14.

~~Flint KR, Davis AP. Pollutant mass flushing characterization of highway stormwater runoff from an ultra-urban area. J Environ Eng, 2007;133(6):616–626.~~

Foundation for Water Rresearch, Urban Pollution Management Manual, 3rd edition, Marlow, 2012, document available at the web site: http://www.fwr.org/UPM3, last access June 12th 2017).

Galfi H, Haapala J, Nordqvist K, Westerlund C, Blecken G, Marsalek J, Viklander M. Inter-event and intra-event variations of indicator bacteria concentrations in the storm sewer system of the city of Östersund, Sweden. J Environ Eng 2016a;142(7).

Galfi H. Österlund H., Marsalek J, ViklanderM Indicator bacteria and associated water quality constituents in stormwater and snowmelt from four urban catchments, J Hydrol 2016b;539:125-140

Gasperi J, Laborie B, Rocher V. Treatment of combined sewer overflows by ballasted flocculation: Removal study of a large broad spectrum of pollutants. Chem Eng J 2012;211-212:293-301.

~~Geiger W (1987) Flushing effects in combined sewer systems in: Proceedings of the 4th International Conference Urban Drainage, Lausanne, Switzerland, 40-46.~~

Gibson III CJ, Stadterman KL, States S, Sykora J. Combined sewer overflows: A source of *Cryptosporidium* and *Giardia*? Water Sci Technol 1998;38(12):67-72.

Gibson J, Farnood R., Seto P. Chemical pretreatment of combined sewer overflows for improved UV disinfection Water Sci Tecnol 2016;73(2): 375-381.

~~Han YH., Lau SL., Kayhanian M, Stenstrom MK. Correlation analysis among highway stormwater pollutants and characteristics. Water Sci. Technol. 2006;53 (2), 235–243.~~

Hanner N, Mattsson A, Gruvberger C, Nyberg U, Aspegren H, Fredriksson O, Nordqvist A, Andersson B. Reducing the total discharge from a large WWTP by separate treatment of primary effluent overflow. Water Sci Technol 2004;50(7):157-62.

Hathaway JM, Hunt WF. Evaluation of first flush for indicator bacteria and total suspended solids in urban stormwater runoff. Water Air Soil Pollut 2011;217(1-4):135-47.

IRSA-APAT Metodi analitici per le acque, Istituto Poligrafico e Zecca dello Stato, 2003 ~~1994~~.

ISO International Standard Organization ISO 7899-1:199, Water quality -- Detection and enumeration of intestinal enterococci -- Part 1: Miniaturized method (Most Probable Number) for surface and waste water, 1998-11 (www.iso.org/standard/14852.html)

Jalliffier-Verne I, Heniche M, Madoux-Humery A, Galarneau M, Servais P, Prévost M, Dorner S. Cumulative effects of fecal contamination from combined sewer overflows: Management for source water protection. J Environ Manage 2016;174:62-70.

Kafi M, Gasperi J, Moilleron R, Gromaire MC, Chebbo G. Spatial variability of the characteristics of combined wet weather pollutant loads in Paris. Water Res 2008;42(3):539-49.

Kentucky Administrative Regulations KAR 2012. Surface Water Standards; 401 K.A.R. 10.031. http://lrc.ky.gov/kar/401/010/031.htm (last accessed April 2017).

Launay MA, Dittmer U, Steinmetz H Organic micropollutants discharged by combined sewer overflows – Characterisation of pollutant sources and stormwater-related processes. Water Res 2016;104:82-92

Line DE, White NM, Kirby-Smith WW, Potts JD. Fecal coliform export from four coastal north carolina areas. J Am Water Resour Assoc 2008;44(3):606-17.

Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. New Engl J Med 1994;331(3):161-7.

Madoux-Humery AS, Dorner S, Sauvé S, Aboulfadl K, Galarneau M, Servais P, Prévost M. Temporal variability of combined sewer overflow contaminants: Evaluation of wastewater micropollutants as tracers of fecal contamination. Water Res 2013;47(13):4370-82

Madoux-Humery AS, Dorner S, Sauvé S, Aboulfadl K, Galarneau M, Servais P, Prevost M. Temporal analysis of microbiological and wastewater micropollutant loads from combined sewer overflows: implications for management. Environ Sci Process Impacts 2015;17(5):965-974.

Marsalek J, Dutka BJ, Tsanis IK. Urban impacts on microbiological pollution of the St. Clair river in Sarnia, Ontario. Water Sci Technol 1994;30:177-84.

~~McCarthy DT, Hathaway JM, Hunt WF, Deletic A. Intra-event variability of *Escherichia coli* and total suspended solids in urban stormwater runoff. Wat Res, 2012;46:6661-6670~~

~~McCarthy DT. A traditional first flush assessment of *E. coli* in urban stormwater runoff. Water Sci Technol 2009;60(11):2749-57.~~

McLellan SL, Hollis EJ, Depas MM, Van Dyke M, Harris J, Scopel CO. Distribution and fate of escherichia coli in lake michigan following contamination with urban stormwater and combined sewer overflows. J Great Lakes Res 2007;33(3):566-80.

McLellan SL, Hollis EJ, Depas MM, Van Dyke M, Harris J, Scopel CO. Distribution and fate of *Escherichia coli* in Lake Michigan following contamination with urban stormwater and combined sewer overflows. J Great Lakes Res 2007;33(3):566-80.

MetCalfe & Eddy, Wastewater Engineering : Treatment and Reuse, McGraw-Hill Education – Europe, 2003

NYC Global Partners. Best practice: redeveloping industrial harbor for public swimming [WWW Document]. URL http://www.nyc.gov/html/ia/gprb/downloads/pdf/Copenhagen_HarbourBath.pdf, 2011. [accessed April 2017]

Ouattara NK, Garcia-Armisen T, Anzil A, Brion N, Servais P. Impact of wastewater release on the faecal contamination of a small urban river: The Zenne river in Brussels (Belgium). Water Air Soil Pollut 2014;225(8):1-12.

Passerat, J., Ouattara, N.K., Mouchel, J.-M., Rocher, V., Servais, P., 2011. Impact of an intense combined sewer overflow event on the microbiological water quality ofthe Seine River. Water Res. 45 (2), 893-903.

Phillips PJ, Chalmers AT, Gray JL, Kolpin DW, Foreman WT, Wall GR. Combined sewer overflows: An environmental source of hormones and wastewater micropollutants. Environ Sci Technol 2012;46(10):5336-43.

Pisoeiro, J., Galvão, A., Ferreira, F., Matos, J. Potential for CSO treatment with horizontal flow constructed wetlands: influence of hydraulic load, plant presence and loading frequency, Environ Sci Pollution Res 2016;23(20): 20591-99

Pongmala K, Autixier L, Madoux-Humery A-, Fuamba M, Galarneau M, Sauvé S, Prévost M, Dorner S. Modelling total suspended solids, *E. coli* and carbamazepine, a tracer of wastewater contamination from combined sewer overflows. J Hydrol 2015;531:830-9.

Rechenburg A, Koch C, Claßen T, Kistemann T. Impact of sewage treatment plants and combined sewer overflow basins on the microbiological quality of surface water. Water Sci Technol 2006;54(3):95-9.

~~Saget A, Chebbo G, Brtrand-Krajewski J. The first flush in sewer systems, Wat Sci Technol 1996;33(9):101-108~~

Sauvé S, Aboulfadl K, Dorner S, Payment P, Deschamps G, Prévost M. Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. Chemosphere 2012;86(2):118-123.

Servais P, Garcia-Armisen T, George I, Billen G. Fecal bacteria in the rivers of the seine drainage network (France): Sources, fate and modelling. Sci Total Environ 2007;375(1-3):152-67

Tryland I, Surman S, Berg JD. Monitoring faecal contamination of the Thames estuary using a semi-automated early warning system. Water Sci Technol 2002;46(3):25-31.

U.S. Environmental Protection Agency (1993) Combined Sewer Overflow Control; EPA 625-R-93-007; U.S. Environmental Protection Agency, Office of Research and Development: Washington, D.C.

Whitman RL, Nevers MB, Korinek GC, Byappanahalli MN. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. Appl. Environ. Microbiol. 2004;70 (7):4276–4285.

TABLES

Table 1: Characteristics of the studied rain events and CSO discharges. In the first column, in brackets, after the outfall name, the number of events occurring in summer 2014 at the specific site.

CSO point	Event	Overflow duration	Overflow discharged volume	Average Flowrate	Maximum Flowrate	Precipitation duration	Antecedent dry period (ADP)	Cumulative precipitations during the overflow event	Mean intensity of precipitation
		[min]	[m^3]	[L/s]	[L/s]	[min]	[d]	[mm]	[mm/h]
S6 (14)	14 June	167.4	5,550.3	553	754	202.7	0.7	16.2	4.9
	17 June	115.8	5,418.2	780	1300	117.6	2.9	14.8	7.5
	25 June	19.2	505.5	440	754	90.5	5.6	4.2	1.0
	30 June	75.3	1,871.4	414	754	232.4	3.4	10.6	3.6
	10 July	207.6	6760	543	754	733.0	0.4	25.8	2.1
	12 July	117.5	3,595	510	754	279.3	2.3	15.0	2.9
	24 July	34.8	845	405	405	126.6	2.9	3.01	1.4
	26 July	179.2	8967	834	1300	153.6	0.4	41.4	16.3
	30 July	253.0	11,078	730	1300	517.1	3.0	35.8	4.2
	3 August.	140.0	6943	826	1300	186.0	3.4	15.4	8.1
	24 August	47.8	1161	405	405	35.8	0.7	4.0	6.7
	1 September	24.4	593	405	405	108.3	7.0	6.4	3.5
	10 September	43.8	1063	405	405	207.0	6.7	9.0	2.6
	20 Sep.	202.2	5866	484	754	306.2	0.6	25.8	5.1
S8 (4)	12 July	134.1	3737	464	700	279.3	2.3	15.0	3.2
	26 July	226.0	6218	459	700	164.6	0.4	41.4	16.1
	30 July	96.4	2025	350	350	511.1	3.0	35.8	4.2
	20 September	154.9	3253	350	350	306.2	0.6	25.8	5.1
S13 (3)	14 June	367.9	16,299	738	1100	189.0	0.6	32.0	10.2
	17 June	0.4	24	1075	1100	153.0	2.9	6.6	2.6
	26-27 July	461.9	15,243	550	550	308.0	0.2	36.4	9.0

CSO point	Event	Overflow duration	Overflow discharged volume	Average Flowrate	Maximum Flowrate	Precipitation duration	Antecedent dry period (ADP)	Cumulative precipitations during the overflow event	Mean intensity of precipitation
S14 (5)		271.5	8961	550	550				
	14 June	240.0	12,748	885	1800	189.0	0.6	32.0	10.2
	30 June	1.9	73	650	650	269.0	3.7	14.6	3.1
	26 July	199.8	16,247	1355	1800	308.0	0.2	36.4	9.0
	10 September	94.4	3681	650	650	252.0	0.4	22.8	5.4
	20 September	19.3	753	650	650	279.0	0.6	16.8	3.6
MD (15)	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	11,618	430	651				
		135	966	119	321				
	30-31 July	735	30,383	689	2023				
		150	36	4	4				
	3-4 August	420	6710	266	775				
		30	29	16	16				
	15-16 August	450	2415	89	170				
		90	168	31	65				
	20 August	30	18	10	16				
	1 September	75	61	14	16				
	10 September	450	1669	62	240				
	20 September	450	4164	154	321				

~~**Table 2**: *MFF*$_{20}$ for CSO events in different outfalls~~

		~~**Jun 14th**~~	~~**Jun 17th**~~	~~**Jun 30th**~~	~~**Jul 10th**~~	~~**Jul 12th**~~	~~**Jul 26th**~~	~~**Jul 30th**~~	~~**Aug 3rd**~~	~~**Sep. 10th**~~	~~**Sep. 20th**~~
~~**MD**~~	~~*E. coli*~~	~~3.04~~	~~1.53~~	~~1.94~~	~~1.80~~	~~0.97~~	~~2.38~~	~~1.66~~	~~1.49~~	~~2.18~~	
	~~Enterococci~~	~~2.30~~	~~1.53~~	~~1.18~~	~~1.46~~	~~1.58~~	~~1.91~~	~~0.97~~	~~1.68~~	~~1.58~~	
~~**S6**~~	~~*E. coli*~~				~~1.22~~	~~1.18~~	~~0.72~~	~~4.98~~	~~2.68~~	~~0.83~~	~~1.56~~
	~~Enterococci~~				~~1.04~~	~~2.00~~	~~1.08~~	~~**5.00**~~	~~2.48~~	~~1.38~~	~~0.48~~
~~**S8**~~	~~*E. coli*~~					~~1.28~~	~~0.98~~	~~3.35~~			~~**0.09**~~
	~~Enterococci~~					~~2.01~~	~~1.21~~	~~0.31~~			~~0.16~~
~~**S13**~~	~~*E. coli*~~						~~1.60~~				
	~~Enterococci~~						~~1.59~~				
~~**S14**~~	~~*E. coli*~~						~~1.56~~			~~0.79~~	~~1.04~~
	~~Enterococci~~						~~1.57~~			~~1.24~~	~~0.39~~

Table 2. Percentage of time with CSO outfalls in operation with respect to each month and the whole period

	June	**July**	**August**	**September**	**Whole period**
MD	3.19	6.28	2.28	2.26	3.52
S6	0.87	1.77	0.42	0.63	0.93
S8	0	1.02	0	0.36	0.35
S13	0.85	1.64	0	0	0.63
S14	0.56	0.45	0.00	0.26	0.32

Table 3. Main parameters affecting the elimination/survival of fecal bacteria in water environment

Parameter	**Effect**
Water temperature	According to the Bathing Water Committee (2009), elimination of 90 % of *E. coli* and Entetococci requires respectively 35 h and 70 h in cloudy weather and 5 h and 15 h in sunny weather.
Turbidity	According to Whitman et al. (2004) water turbidity reduces the light penetration in the water column and thus it hinders the elimination of bacteria.
Salinity	High salinity waters are generally correlated to low microorganism concentration. Enterococci are more tolerant to higher values than *E. coli* (Aragonés et al., 2016).
Residence time in the water compartment	Bacteria elimination is proportional to their time spent in the channel before reaching the final receptor during which they may undergo to the different auto-purification processes.
Sunlight hours	Bacteria natural decay is associated to light exposure of microorganisms. During the night in fact, there is a replenishment (in terms of growth and or resuscitation) of bacteria (Withman et al., 2004)
UV irradiation	Light exposure and in particular the exposure to a light wavelength of 254 nm is responsible of a decay of the concentration of fecal bacteria. This is the working principle of UV reactors used for water and wastewater (=filtered biological effluent disinfection). The removal efficiency of bacteria by UV irradiation is higher in water with a high transmittance, that is with low turbidity: suspended particles shield microorganisms and radiation cannot reach them (Metcalf & Eddy, 2003).
Cloudy/sunny weather conditions	Clouds act as a shield for bacteria reducing the effect of the solar radiation that is responsible of their decay.
Tide	Tidal cycles may influence bacteria concentrations in water, depending on the tide height (Aragonés et al., 2016).

Captions

Figure 1. Schematics of the area under study with a focus on the combined sewer network, CSO outfalls, rain gauges and WWTP.

Figure 2. Volume of water discharged monthly into the receiving water body: percentage contribution of untreated CSOs and treated WWTP effluent (sum of BIO_D and BY).

Figure 3 Monthly discharged water volume (in percentage) by each point with respect to the corresponding total volume discharged in the four months. (BY is the effluent bypassing the secondary treatment and conveyed to the disinfection tank; BIO_D is the secondary effluent within the WWTP conveyed to the disinfection tank; MD is the combined sewage overflow outfall upstream the WWTP).

Figure 4. Box-plots of *E. coli* and *Enterococci* concentrations in the different CSOs. The dot lines refer to current Italian limits. In detail, A = suggested limit for release of a WWTP effluent (*E. coli)* into a surface water body; B = Inland bathing water limit (*E. coli*); C = Marine bathing water limit (*E. coli*) and Inland bathing water limit (*Enterococci*); D = Marine bathing water limit (*Enterococci*).

Figure 5. *E. coli* concentration in overflow *vs*. sampling time. The same symbol means a measurement referring to the same event in a specific CSO outfall.

Figure 6. Profiles of *E. coli*, Enterococci and conductivity during the overflows at MD outfall.

Figure 7. Profiles of *E. coli,* Enterococci and conductivity during the overflows at S6 outfall.

Figure 8: Profiles of *E. coli,* Enterococci and conductivity during the overflows at S8 outfall.

Figure 9. Profiles of *E. coli,* Enterococci and conductivity during the overflows at S13 outfall.

Figure 10: Profiles of *E. coli,* Enterococci and conductivity during the overflows at S14 outfall.

Figure 11. EMCs of *E. coli vs.* mean CSO event flowrate in log-log plots. Symbols with a star indicate disinfected events.

Figure 12. EMCs of *Enterococci vs.* mean CSO event flowrate in log-log plots. Symbols with a star indicate disinfected events.

Figure 13. Monthly discharged load of *E. coli* and *Enterococci* in the different CSO outfalls and released by the local WWTP effluent (dry and wet weather) as well as the cumulative percentage contribution to the total discharge (bold line).

~~**Figure 14**. Normalized cumulative mass load *vs*. normalized cumulative flow for the 5 CSO outfalls.~~

Figures

Click here to view linked References

Figures

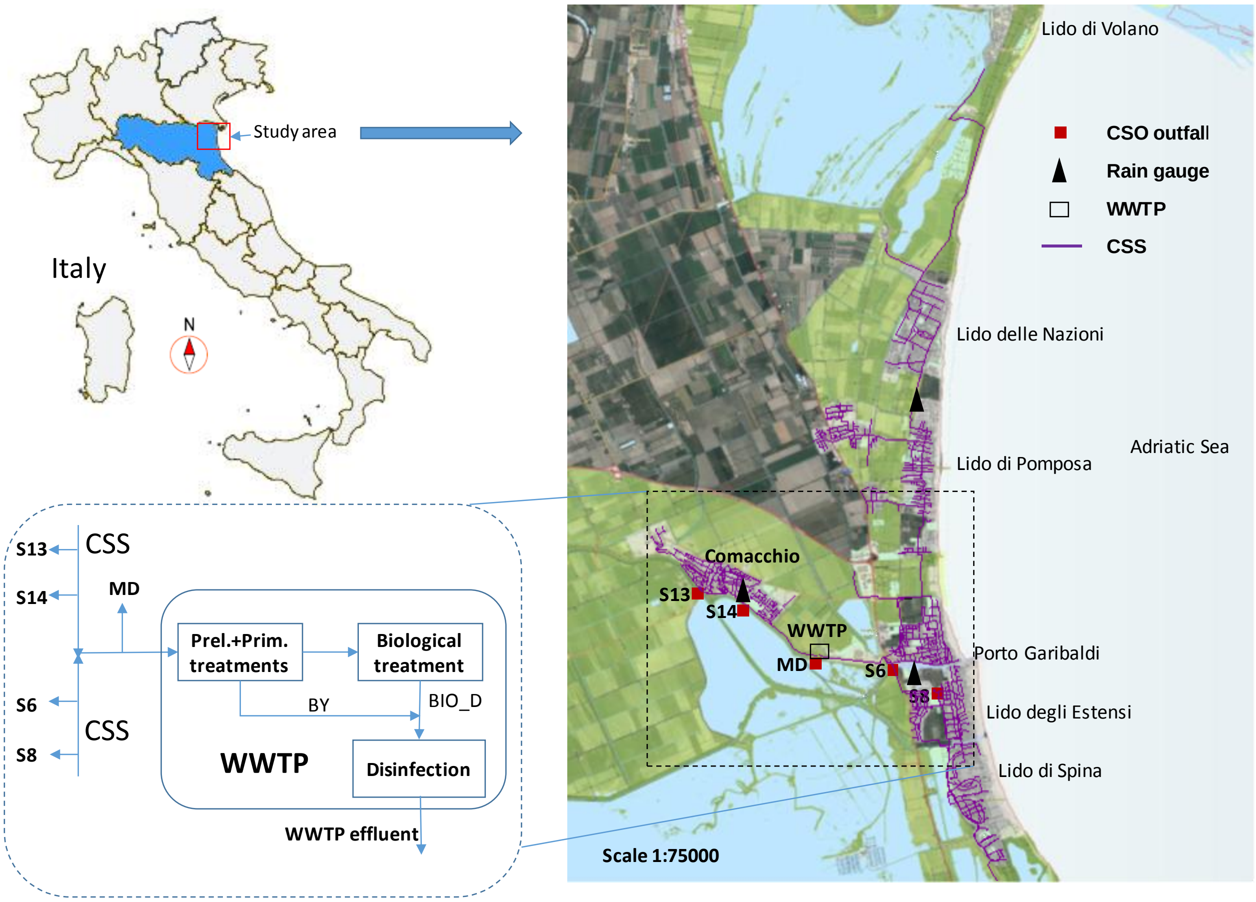


Figure 1.

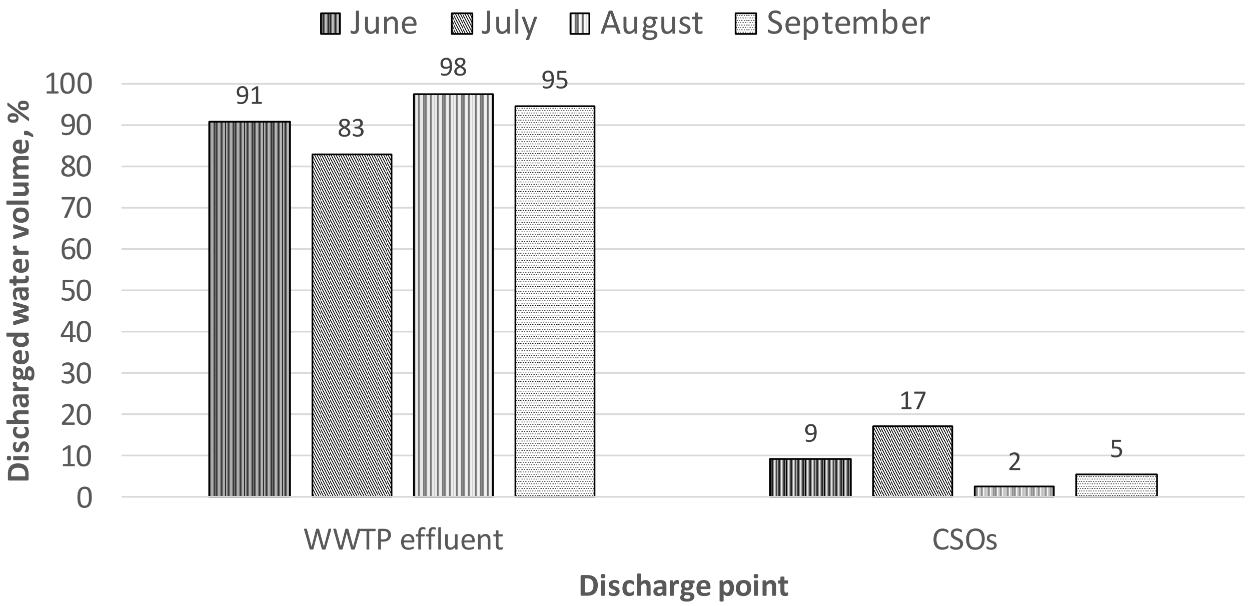

June
July
August
September
Discharged water volume, %
100
90
80
70
60
50
40
30
20
10
0
91
83
98
95
9
17
2
5
WWTP effluent
CSOs
Discharge point

Figure 2.

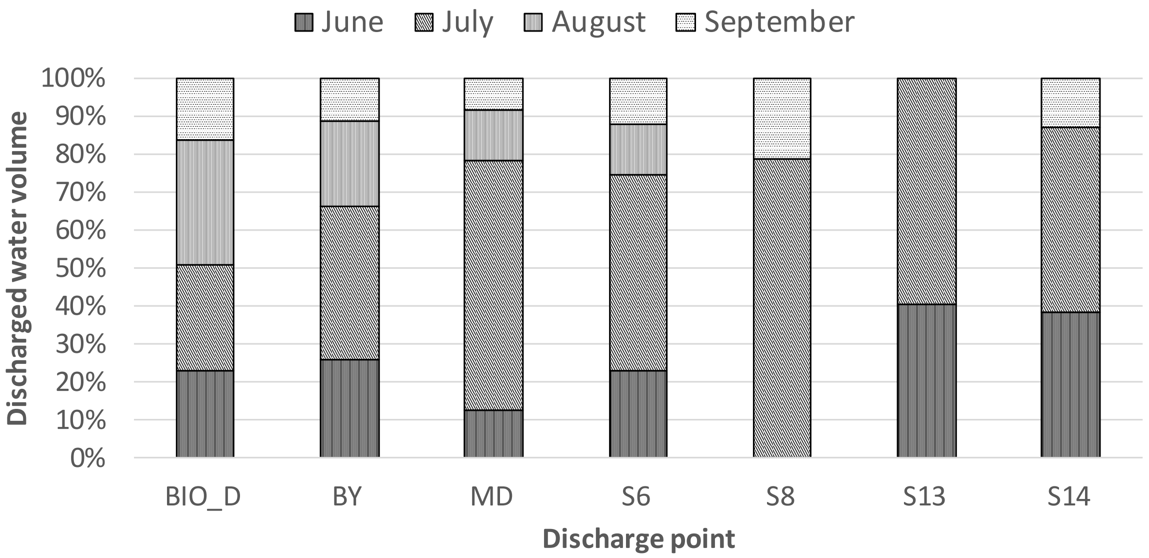

June
July
August
September
Discharged water volume
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
BIO_D
BY
MD
S6
S8
S13
S14
Discharge point

Figure 3.

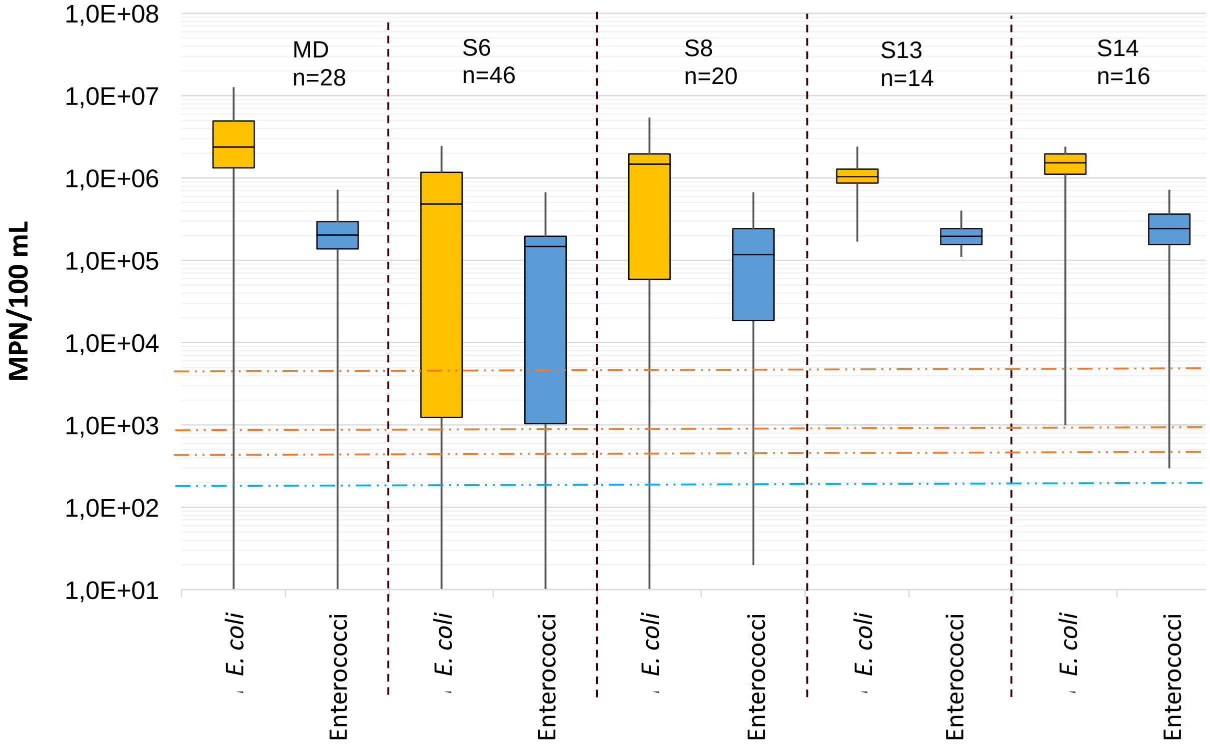

1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
MPN/100 mL
MD
n=28
S6
n=46
S8
n=20
S13
n=14
S14
n=16
E. coli
Enterococci
E. coli
Enterococci
E. coli
Enterococci
E. coli
Enterococci
E. coli
Enterococci

Figure 4.

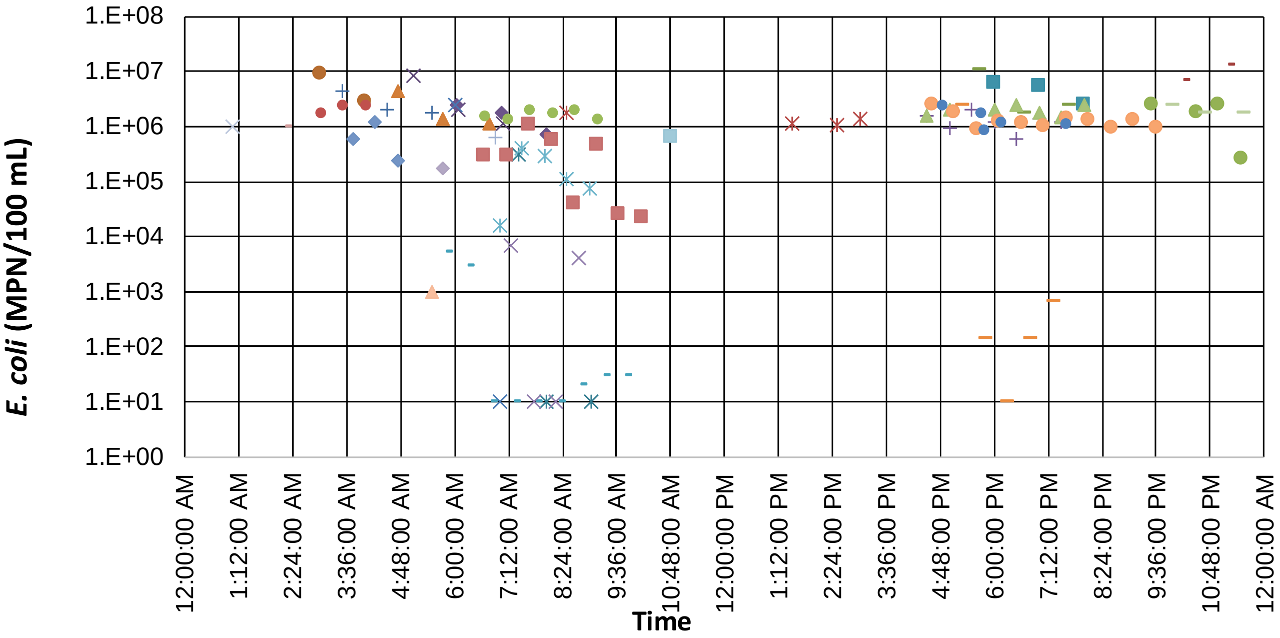

1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
E. coli (MPN/100 mL)
12:00:00 AM
1:12:00 AM
2:24:00 AM
3:36:00 AM
4:48:00 AM
6:00:00 AM
7:12:00 AM
8:24:00 AM
9:36:00 AM
10:48:00 AM
12:00:00 PM
1:12:00 PM
2:24:00 PM
3:36:00 PM
4:48:00 PM
6:00:00 PM
7:12:00 PM
8:24:00 PM
9:36:00 PM
10:48:00 PM
12:00:00 AM
Time

Figure 5.

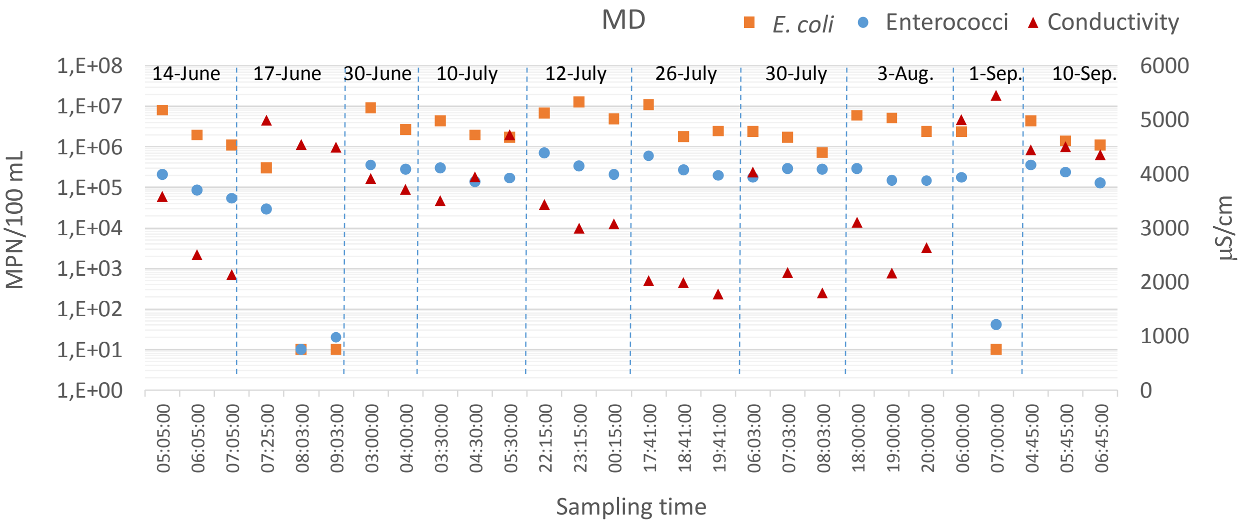


Figure 6

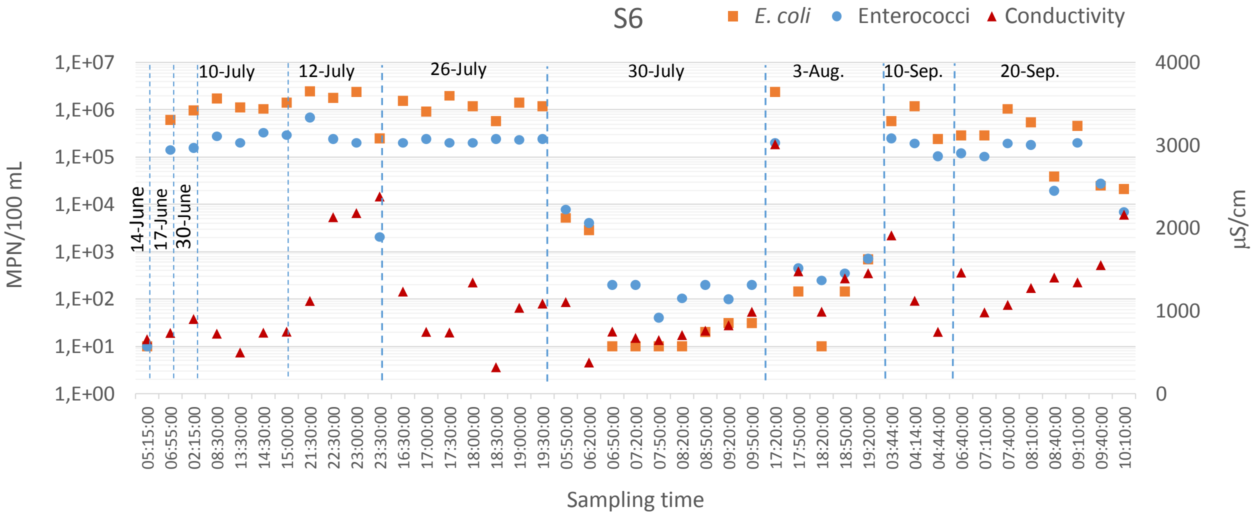


Figure 7

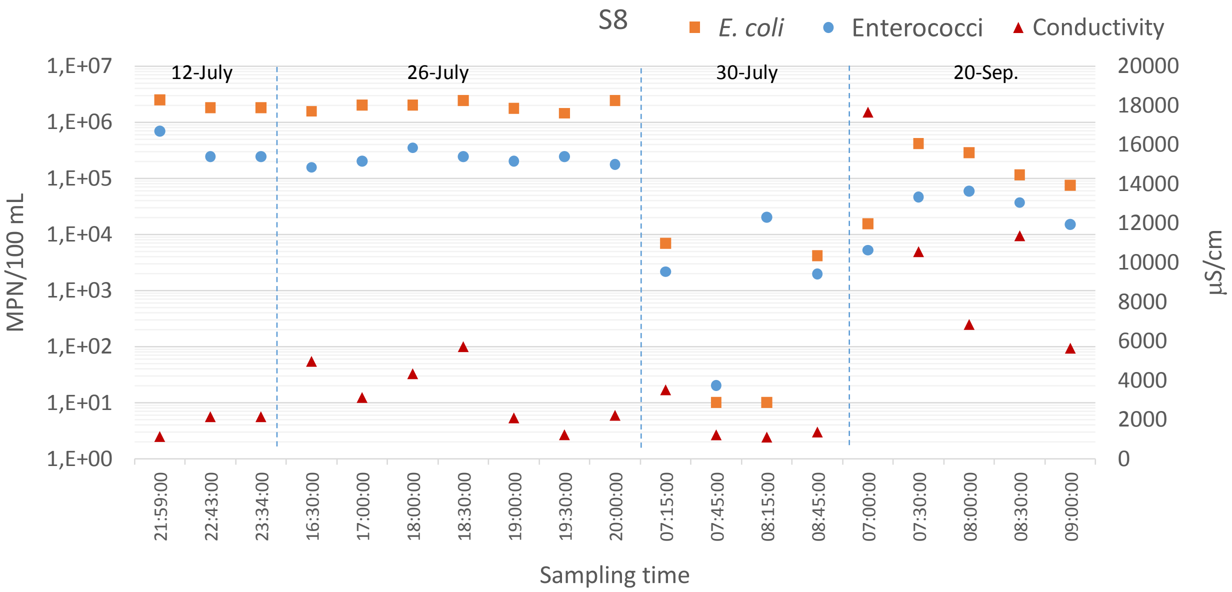


Figure 8

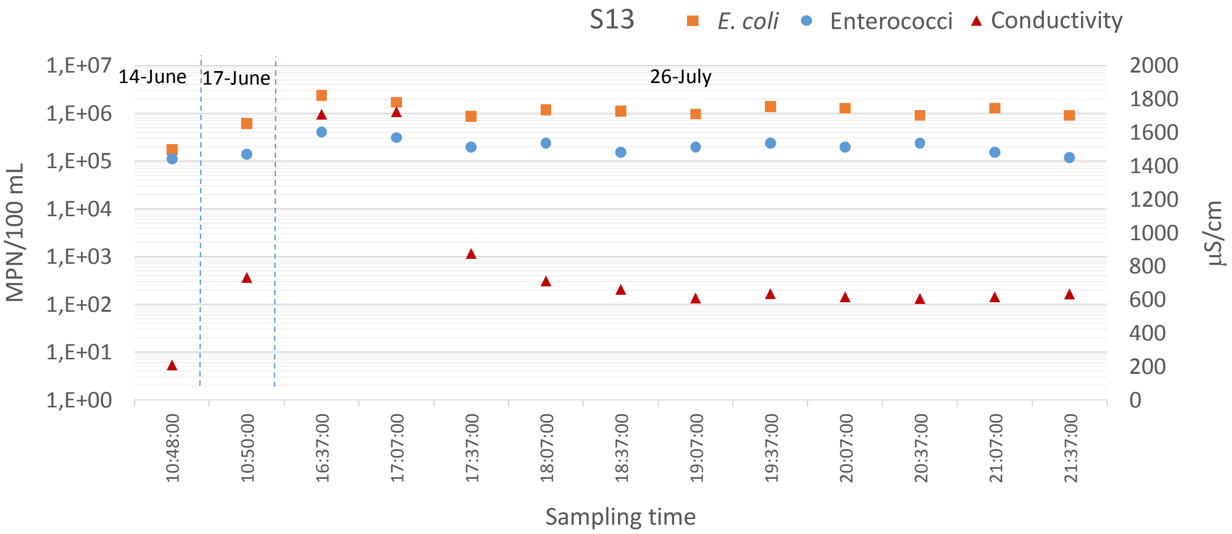


Figure 9

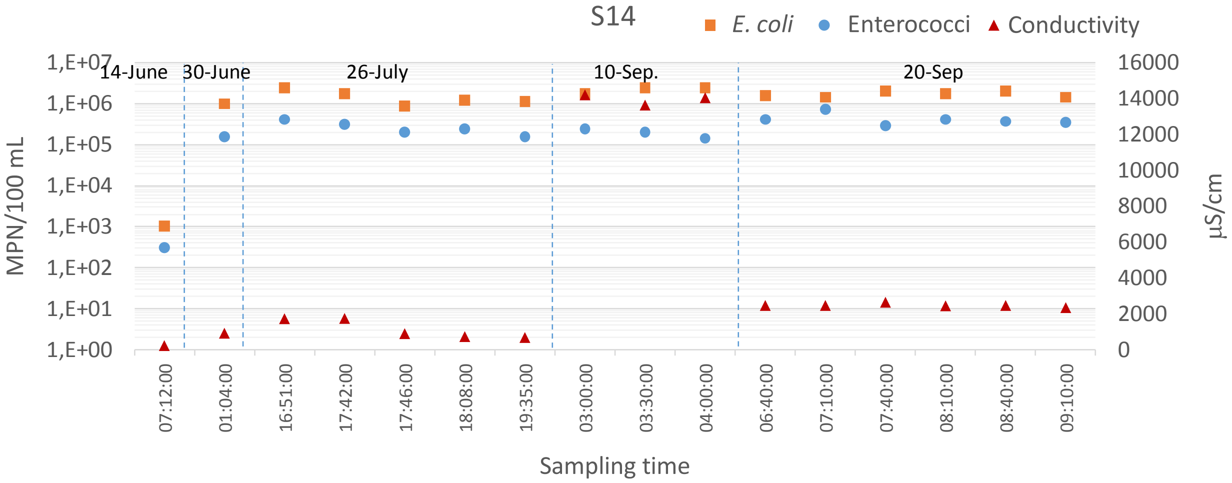
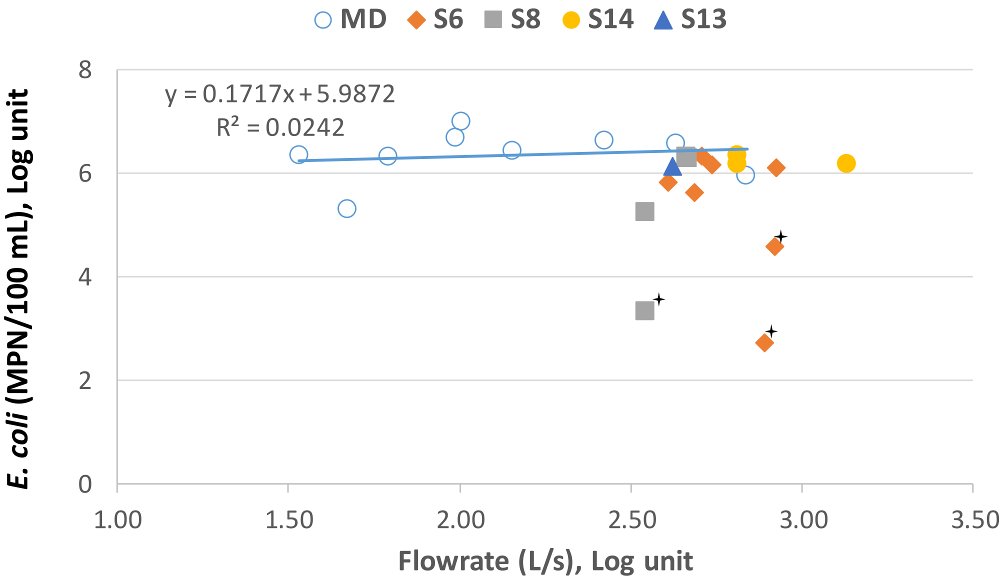

S14
■ E. coli
● Enterococci
▲ Conductivity
MPN/100 mL
µS/cm
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
16000
14000
12000
10000
8000
6000
4000
2000
0
14-June
30-June
26-July
10-Sep.
20-Sep
07:12:00
01:04:00
16:51:00
17:42:00
17:46:00
18:08:00
19:35:00
03:00:00
03:30:00
04:00:00
06:40:00
07:10:00
07:40:00
08:10:00
08:40:00
09:10:00
Sampling time

Figure 10



○ MD
◆ S6
■ S8
● S14
▲ S13
y = 0.1717x + 5.9872
R² = 0.0242
E. coli (MPN/100 mL), Log unit
8
6
4
2
0
1.00
1.50
2.00
2.50
3.00
3.50
Flowrate (L/s), Log unit

Figure 11.

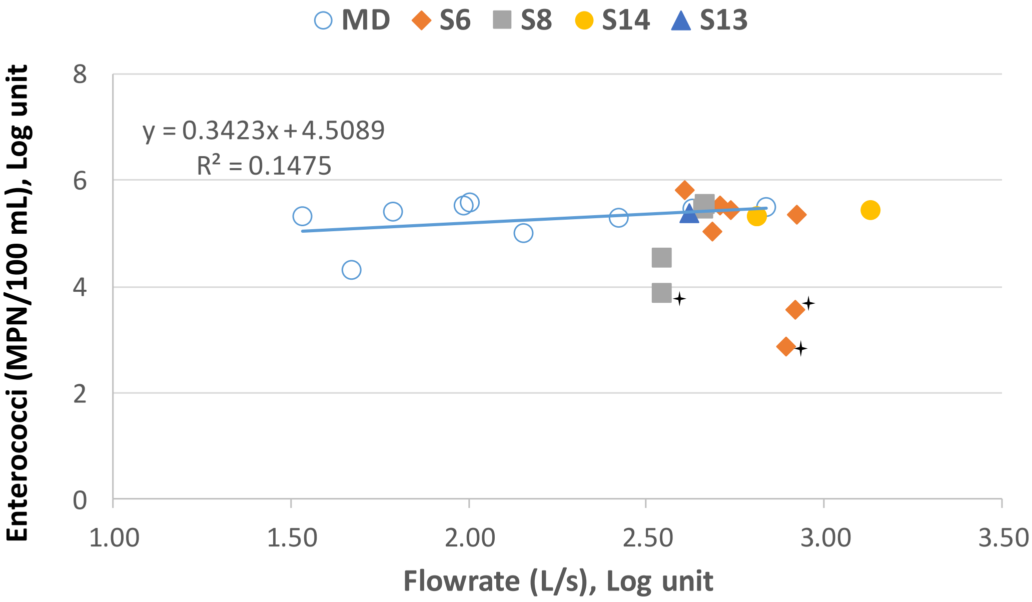

○ MD
◆ S6
■ S8
● S14
▲ S13
y = 0.3423x + 4.5089
R² = 0.1475
Enterococci (MPN/100 mL), Log unit
0
2
4
6
8
1.00
1.50
2.00
2.50
3.00
3.50
Flowrate (L/s), Log unit

Figure 12.

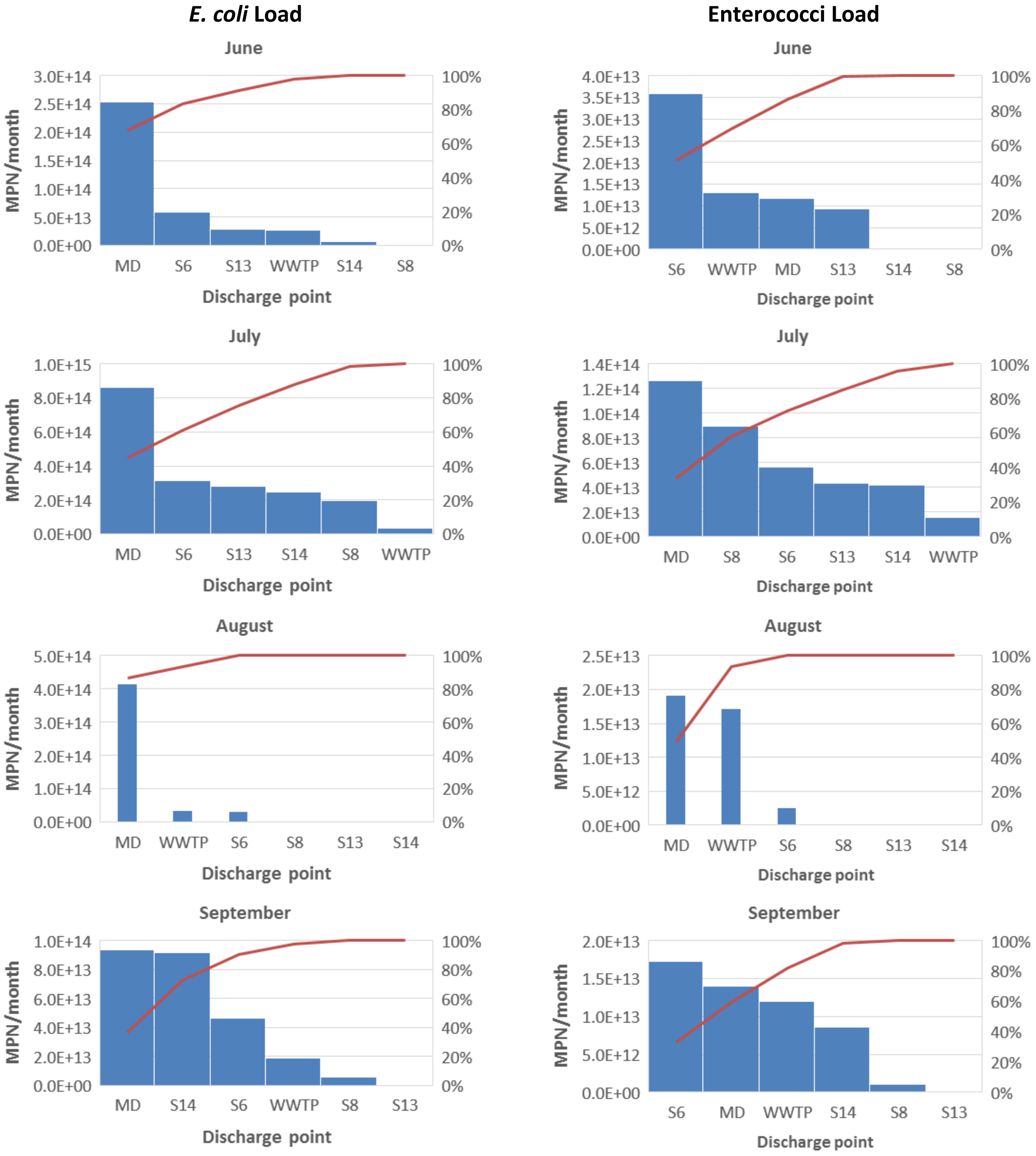
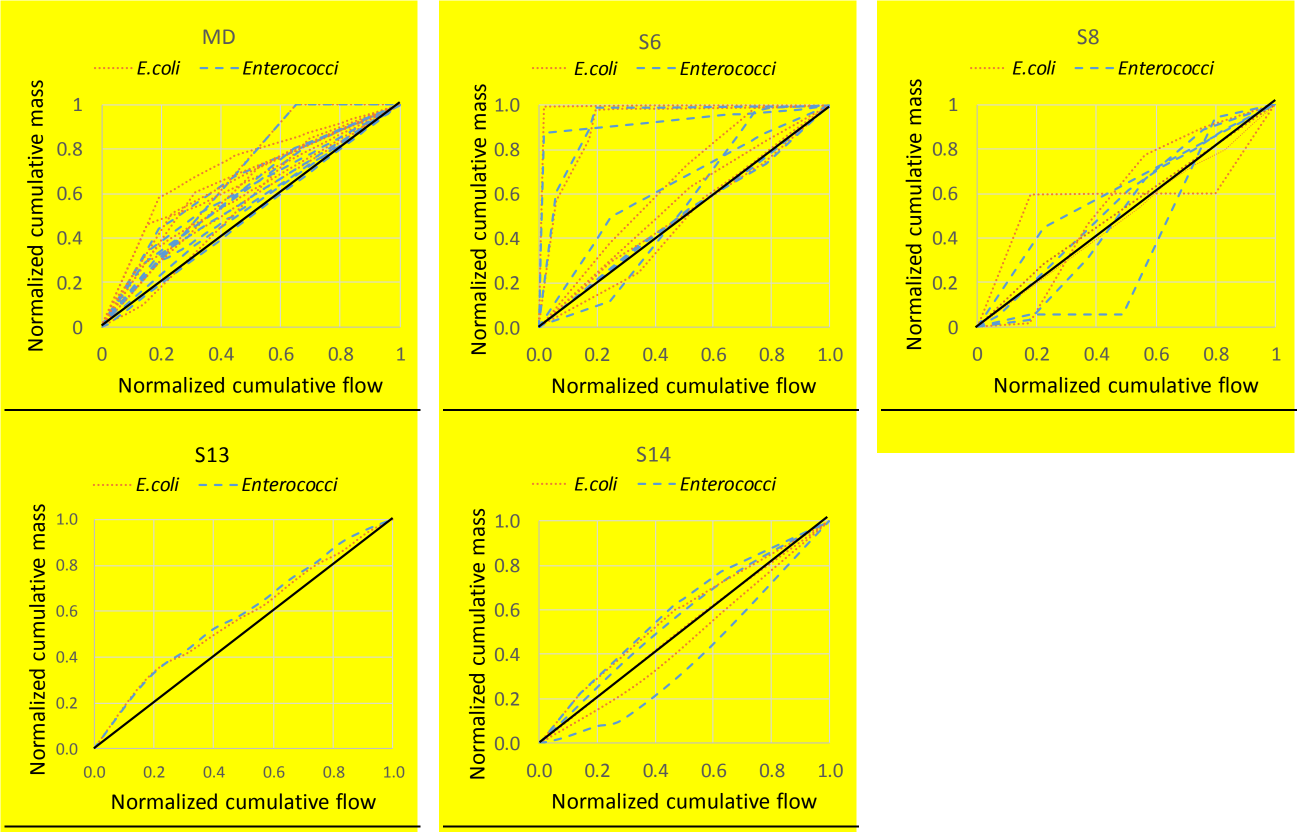

E. coli Load
Enterococci Load
June
3.0E+14
2.5E+14
2.0E+14
1.5E+14
1.0E+14
5.0E+13
0.0E+00
MPN/month
100%
80%
60%
40%
20%
0%
MD
S6
S13
WWTP
S14
S8
Discharge point
June
4.0E+13
3.5E+13
3.0E+13
2.5E+13
2.0E+13
1.5E+13
1.0E+13
5.0E+12
0.0E+00
MPN/month
S6
WWTP
MD
S13
S14
S8
Discharge point
July
1.0E+15
8.0E+14
6.0E+14
4.0E+14
2.0E+14
0.0E+00
MD
S6
S13
S14
S8
WWTP
Discharge point
July
1.4E+14
1.2E+14
1.0E+14
8.0E+13
6.0E+13
4.0E+13
2.0E+13
0.0E+00
MD
S8
S6
S13
S14
WWTP
Discharge point
August
5.0E+14
4.0E+14
3.0E+14
2.0E+14
1.0E+14
0.0E+00
MD
WWTP
S6
S8
S13
S14
Discharge point
August
2.5E+13
2.0E+13
1.5E+13
1.0E+13
5.0E+12
0.0E+00
MD
WWTP
S6
S8
S13
S14
Discharge point
September
1.0E+14
8.0E+13
6.0E+13
4.0E+13
2.0E+13
0.0E+00
MD
S14
S6
WWTP
S8
S13
Discharge point
September
2.0E+13
1.5E+13
1.0E+13
5.0E+12
0.0E+00
S6
MD
WWTP
S14
S8
S13
Discharge point

Figure 13.


MD
E.coli
Enterococci
Normalized cumulative mass
Normalized cumulative flow
0
0.2
0.4
0.6
0.8
1
S6
1.0
S8
S13
S14

~~Figure 14~~.

Contributions of combined sewer overflows and treated effluents to the bacterial load released into a coastal area

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Graphical abstract

Abstract

The impact of combined sewer overflow (CSO) on the receiving water body is an issue of increasing concern, as it may lead to restrictions in the use and destination of the receiving body, such as bathing or recreational area closures, fish and shellfish consumption restrictions, and contamination of drinking water resources. Recent investigations have mainly referred to the occurrence and loads of suspended solids, organic compounds and, in some cases, micropollutants. Attempts have been made to find correlations between the discharged load and the size and characteristics of the catchment area, climate conditions, rainfall duration and intensity.

This study refers to a touristic coastal area in the north-east of Italy, which is characterized by a combined sewer network including 5 CSO outfalls which, in the case of heavy rain events, directly discharge the exceeding water flow rate into channels which, after a short distance, reach the Adriatic Sea. The study analyzed: i) rainfall events during the summer period in 2014 which led to overflow in the different outfalls, ii) the inter- and intra-event variability with regard to *E. coli*, Enterococci and conductivity, and iii) the hydraulic and pollutant (*E. coli* and Enterococci) loads discharged by the local wastewater treatment plant and by all the CSO outfalls. Finally, it estimated the contribution of each source to the released hydraulic and pollutant loads into the receiving water body. Moreover, it was also found that the modest water volume discharged by all CSO outfalls (only 8 % of the total volume discharged by the area) contains more than 90 % of the microbial load.

Keywords: Coastal area, combined sewer overflow, *E. coli*, Enterococci, wastewater management and treatment, wastewater treatment plant effluent.

List of abbreviations and acronyms used in the manuscript: ADP= antecedent dry periods; BIO_D: secondary effluent within the WWTP; BY= bypass; CSO = combined sewer overflow; CSS= combined sewer system; EMC = event mean concentration; EMF= event mean flow; MD = combined sewer overflow outfall upstream the wastewater treatment plant; WWTP = wastewater treatment plant

1 Introduction

In many urbanized areas, domestic wastewater and rainwater (a mixture that, according to the Council Directive 91/271/EEC, is called *urban wastewater*) are collected and conveyed to the wastewater treatment plant (WWTP) by the same network, known as a combined sewer system (CSS).

Combined sewer overflows (CSOs) may occur in the case of intense rainfall (Barco et al., 2008) and/or periods of melting snow (Madoux et al., 2013), resulting in a higher water flow rate within the sewer network due to the occasional, but sometimes consistent, contribution of surface runoff, as well as rainfall. Surface runoff conveyed to the public sewer system may contain suspended solids, organic matter, microorganisms, heavy metals, or pesticides depending on the type, destination and use, width and imperviousness of washing surfaces, rain event frequency and duration, and number of antecedent dry days (Diaz-Fierros et al., 2002; Barco et al., 2008; Galfi et al., 2016b). CSO pollutant concentrations are the result of mixing domestic wastewater and drained stormwater as well as the internal re-suspension of sewer deposits due to flow-induced turbulence. Wastewater and stormwater concentrations as well as their flow rates define the content of the different pollutants (Passerat et al., 2011; Rechenburg et al., 2006).

Receiving water body contaminations by CSOs are intermittent and strictly correlated to the catchment area sewer network (namely pipe diameters and network size), and climate conditions. Their frequency is site-specific and may also vary from one year to another. These overflows are quite often directly released into a surface water body without any kind of treatment (Ouattara et al., 2014).

Due to their pollutant load, this practice can seriously degrade the receptor water quality, causing depletion of oxygen~~,~~ and an increment in suspended solids, nutrients, organic matter, and heavy metals (Barco et al., 2008; Diaz-Fierros et al., 2002; Hanner et al., 2004; Kafi et al., 2008). Moreover, soon after intense rain events, surface water was found to be affected by an increment in the concentrations of *Giardia* and *Cryptosporidium* (Mac Kenzie et al., 1994; Gibson et al., 1998), Norovirus (Campos et al., 206), and micropollutants (Launay et al., 2016).

This issue is of great concern for water quality control authorities as it could lead to a restriction in the use and destination of the receiving surface body, and consequently, to negative economic impacts. In fact, it could lead to the closure of bathing areas (Burton and Pitt, 2002; Jalliffier-Verne et al., 2016; NYC Global Partners, 2011), restrictions to the consumption of fish and shellfish (Line et al., 2008), and contamination of drinking water resources (McLellan et al., 2007; Galfi et al., 2016b).

It is well known that expensive implementations at large urban WWTPs manage to reduce the residual pollutant load of the treated effluent and thus greatly contribute to improvements in the quality of the receiving surface water body. But these actions cannot attenuate the effects of the short-term disturbances induced by the release of untreated CSOs. This is the case of the catchment area of Brussels, crossed by the Zenne River (Ouattara et al., 2014). The river quality has greatly benefited from the recent upgrade of two large urban WWTPs placed along the river course. However, during intense rain periods, which are quite frequent in the area, a rapid worsening of the microbiological river quality occurs due to untreated CSO releases, resulting in an increment of more than a 2 log factor in the concentrations of *E. coli* and Enterococci in the surface water. Similar negative impacts periodically affect other rivers: the Seine (Servais et al., 2007), the Thames (Tryland et al., 2002) and St. Clair River (Ontario, Marsalek et al., 1994). This decrease in quality is much more evident in cases where the receiving receptor is an effluent-dominant river (Buerge et al., 2006).

It was found that *E. coli* concentrations in stormwater runoff may vary from 2 orders of magnitude lower than in raw wastewater (Passerat et al., 2011; Madoux-Humery et al., 2013) to similar wastewater concentrations in the case of septic cross-connections (Sauvé et al., 2012). Moreover, sediment deposits contribute to the occurrence of bacteria in the first phase of intense rainfall (Madoux-Humery et al., 2015) due to their re-suspension induced by the flow turbulence.

Increasing attention has recently been paid to CSO composition and pollutant load. Most studies have investigated overflow occurrence and the temporal-spatial variability of macropollutants (among them Barco et al., 2008; Kafi et al., 2008) as well as micropollutants (mainly organic compounds and pharmaceuticals: Madoux-Humery et al., 2013, 2015; Phillips et al., 2012; Chèvre et al., 2013); the apportionment of the different sources (wastewater, sewer deposit re-suspension and stormwater) in terms of conductivity, total suspended solids (TSS), *E. coli* during a rain event leading to CSO (Madoux et al., 2015; Passerat et al., 2011), in terms of heavy metals (Diaz-Fierros et al. 2002), and their spatial and temporal variability during different seasons (Madoux et al., 2015, 2013; Galfi et al., 2016a).

Attempts to quantify and simulate the load of CSOs on surface water have also been recently carried out. Among these, Chèvre et al. (2013) applied the substance flow analysis approach to the town of Lausanne, Switzerland, in order to evaluate how to attenuate the load of pharmaceuticals on the aquatic systems due to CSOs and WWTP effluents, while Pongmala et al. (2015) estimated the dynamics of suspended solids, *E. coli* and the micropollutant carbamazepine in the combined sewer network in a sub-catchment of the large area of Montréal (Canada).

From a regulation point of view, the situation varies from country to country. For instance, U.S.EPA (1993) provided a guidance document regarding the disinfection of CSOs. In particular, it highlights that an acceptable treatment should guarantee a removal of at least 4 log units in bacteria, in detention times of less than the conventional 15-30 minutes. Canadian Provincial Regulations restrict the frequency of CSO discharges at each outfall location depending on the time of the year, the type of precipitation (rainfall or snowmelt) and the assimilative capacity of the receiving water (Madoux-Humery et al., 2013, 2015). In the United Kingdom, the Urban Pollution Management (UPM) Manual set wet weather standards for protecting river aquatic life, bathing water, shellfish water, amenity use and location of CSO outfalls (Foundation for Water Research, 2012). In Italy, only a few Regions set out guidelines regarding the management of rainwater. For instance, those set out by the Region of Emilia Romagna *suggest* collecting and treating the first 2.5-5 mm of rain which has fallen on an impervious surface (DGR, 2005) while the remainder may be directly discharged. There are no specific prescriptions in cases where the CSO is directly released into the sea.

This study aims to provide new insights in this context, through an assessment of *E. coli* and Enterococci loads due to CSOs in a typical Italian coastal area during summertime (the observation period is June-September 2014), and comparing them to those released by the effluent of the local municipal WWTP during the whole observation period (dry+wet days). The aim is to identify which are the most important sources in terms of microbiological pollution in the receiving water body and also to suggest attenuation measures in order to avoid the bathing area closures which have unfortunately occurred on a regular basis over the last few summers.

2 Materials and Methods

2.1 The site under study

The study site refers to the area of the municipality of Comacchio (coordinates: 44°42′N 12°11′E), situated in the eastern side of the Po Valley, north-east Italy. The area is adjacent to a lagoon (Comacchio Lagoon) and is characterized by an altitude of 1 m over the sea level. The study catchment basin has an extension of 850 ha; the land use is 72 % residential, 12 % institutional and commercial, 15 % open lands and 1 % industrial. The area can be classified as a residential centre; its impervious surface varies between 31 % and 60 % in the different sub-catchment basins and, with respect to the whole catchment, it is equal to 44 %. This is a typical coastal town characterized by a high density of tourists in summer (up to 180,000 persons) and a resident population of about 25,000 inhabitants during the remaining months. As in all the Mediterranean touristic coastal towns, the population presents consistent fluctuations between May and September: an increment in population is generally registered in weekends in May, June and September and the highest peaks of presences occur during July and end of August.

During the observation period (June-September 2014), the minimum temperature varied between 10 °C and 25 °C and the maximum one between 19 °C and 39 °C. In June, the maximum solar radiation was in the range 870-1317 W/m^2 and the average solar radiation was equal to 258 W/m^2; in July the maximum values were in the range 923-1114 W/m^2 and the average value was 287 W/m^2; in August the maximum value was in the range 895-1190 W/m^2 and the average value equal to 254 W/m and in September the maximum was between 490 and 1000 W/m^2 and the average value was 180 W/m^2. The number of sunshine hours was 15 h and 30 min in June and decreased to 11 h and 45 min in September.

Domestic wastewater and rainwater are collected and conveyed to the local WWTP by a combined sewer system consisting of numerous pipelines discharging the sewer by gravity into a main collector, whose diameter varies between 1000 and 1600 mm, along which a series of lifting pump stations are present in order to convey the sewer towards the central WWTP (Figure 1). The WWTP consists of two treatment lines (one permanently in operation, the second one only between May and September), each of them including preliminary treatments, primary sedimentation, secondary treatments by conventional activated sludge process, and disinfection tanks. The treated effluent is released into a channel which after a distance of 3.5 km reaches the Adriatic Sea.

During heavy rain events, when the influent wastewater flow rate exceeds the capacity of the WWTP and/or the overflow threshold inside the CSS, the exceeding volume is directly discharged in the surface water network through submerged pumps installed for this purpose. This aliquot is the combined sewer overflow (CSO). The Comacchio sewer network under study has five CSO outfalls. Figure 1 reports the sewer network (purple lines), the CSO outfall positions (red squares), the WWTP (rectangle) and the rain gauges (black triangles) placed in the study area.

In particular, the CSO outfalls are located within the lifting pump stations (S6, S8, S13 and S14) receiving urban wastewater from different sub-catchments, and immediately upstream the WWTP (called MD) receiving urban wastewaters from the whole catchment area, as shown in Fig. 1. Overflows are released in five different points of the surface water network at a distance varying between 1.26 km and 6.1 km from the final receptor (Adriatic Sea). On the basis of the characteristics of the channels receiving these overflows, it was estimated an average water speed equal to 0.4 m/s. This means that the time to reach the sea is in the range 0.88 – 4.2 h. The water flowing in these channels are quite turbid and thus the expected decay of microorganisms during their transport to the sea due to sunlight (UV irradiation) is quite modest with respect to the case of clear water.

Figure 1.

Within the WWTP, when the received wastewater flowrate exceeds the nominal capacity of the treatment train, a bypass (called BY) between the primary and secondary steps directly conveys part of the primary effluent to the disinfection tank (avoiding the biological treatment), together with the secondary effluent (called BIO_D in Figure 1). Once disinfected, the total effluent is discharged into the receiving water body.

Each outfall contains submerged pumps with different nominal capacities that can work concurrently, depending on the intensity of the rain event. The characteristics of S6, S8, S13 and S14 outfalls are reported in Table S1. Each outfall is responsible for a determined part of the sewer network and it is designed in a way that pumps start to operate when the water flow rate is 4 times the average dry weather flow rate of that sewer network part.

MD outfall is different, as it consists of a particular valve, characterized by 24 steps, which are 24 degrees of valve opening, adjustable in accordance with the volume of water to be moved. Table S2 reports its working details.

All pumps and MD valve are connected to a data logger that records the date, starting time and duration every time the device (pump or valve) starts working. On the basis of these recorded data and the nominal flow rate for each device, the total discharged water volume were calculated for each CSO event.

2.2 Characteristics of the recorded rain events

The study refers to the period of June - September 2014. Precipitation data such as event time, total duration and intensity were obtained using three rain gauges installed in the study area (Figure 1). These gauges registered the total depth of rainfall every 9 minutes. Then, 3 hours after the rain event, the cumulative height measurement of each gauge was reset to consider the occurrence of a new event. This separation of one event from another takes into account the speed with which the summer storms evolve. Therefore, in order to define the rainfall events that cause CSO, events separated by at least three hours are considered as individual events, even when they occurred in the same day.

With regard to the studied area, the annual precipitation patterns for 2013, 2014 and 2015 are reported in Figure S1 in terms of monthly precipitation depth. A comparison of the three years shows that there could be some differences from one year to another - recorded annual precipitations were 870 mm in 2013, 740 mm in 2014 and 612 mm in 2015 and summertime (June-September) contribution to the total annual rain water was equal to 28 % (2013), 35 % (2014) and 24 % (2015). An analysis of the precipitation pattern in a wider temporal period highlights that rainy summers alternate with dry ones, or even periods of drought and in any case, the pollutograph referring to 2014 represents a worse scenario in terms of frequency of summer CSO with 93 mm and 99.8 mm falling respectively in June and July.

During the studied period a total of 20 rain events were recorded with an event precipitation depth ranging from 3.01 to 41.4 mm and an average of 17.6 mm. An overview is provided in Table S3 in Supplementary Material: six events occurred both in June (14th, 17th, 19th, 25th,26th, 30th) and July (10th, 12th, 24th, 25th, 26th, 30th), and four in both August (3rd, 15th, 20th, 24th) and September (1st, 9th, 10th, 20th).

The main characteristics of the rainfall events leading to CSO are given in Table 1. For each event, the antecedent dry periods (ADPs) are also reported. The characteristics of the rain events relative to MD outfall are derived from almost the same rain events involving CSO in other outfalls, so data are omitted in the aforementioned Table 1.

The highest cumulative precipitations were observed on June 14th, July 10th, July 26th, July 30thand September 20th, and were always anticipated by a low intensity rainfall event a few hours previously.

Table 1

The recorded rain events leading to a CSO occurred between early morning (about 5 AM) and late evening (11.30 PM) with only a few exceptions when the events occurred before 5 AM (June 30th, July 10th and 26th, September 10th see Figures 6-10). This implies that during the recorded rain events, in the sewer system there was generally a consistent contribution of domestic wastewater flowing to the WWTP.

2.3 Sampling and analysis

The field investigation was conducted between June and September 2014 for 20 rainfall events leading to CSO in at least one monitored point. Grab water samples were collected every 30 minutes at the five CSO outfalls and processed for *E. coli*, Enterococci and conductivity. Altogether, 154 samples were withdrawn and processed.

The influent and effluent of the municipal WWTP were regularly monitored by the local Water Managing Body staff members for the whole period of investigation in terms of flow rate and concentrations of the two selected bacteria.

All samples were collected manually using 500 mL plastic bottles which had been rinsed with clean water before being used. Samples were refrigerated and analyzed within 3 h of collection.

All analyses were carried out in accordance with the official analytical methods of the Italian legislation, issued by the IRSA-CNR Institute for Water Research of the Italian National Research Council and APAT (Agency for the Protection of the Environment and Technical Services) (IRSA - APAT 2003). In particular analyses of *E. coli* have been performed according to Method B 7030, corresponding to the Standard Methods for the Examination of Water and Wastewater based on the Enzyme standard test (APHA, 1998). Analyses of Enterococci were done according to Method B 7040, corresponding to the standard method ISO 7899-1: 1998 (ISO, 1998), also included in the Standard Methods for the Examination of Water and Wastewater (APHA, 1998). Conductivity was analyzed according to the Italian official standard Method 2030, based on electrodes with a surface of 1 cm^2 at 25 °C in a 200 ml water sample.

Uncertainties in flow rate measures can be assumed to be less than 10 % according to the considerations made by Madoux-Humery et al. (2013) and uncertainties in *E. coli* and Enterococci concentrations less than 25 %, according to Madoux-Humery et al. (2015).

Unfortunately, there were some events in which it was not possible to collect overflow samples and process them for the analytes of interest. These occurred in MD for the events of June 19th, June 26th, August 15th, and August 20th; and for S6 referring to the events of June 25th, July 24th, August 24th, and September 1st.

With regard to WWTP effluent quality, we prudently assumed that the treated effluent (chemically disinfected effluent) always had a content of *E. coli* equal to the maximum value allowed by the local control body authorization (5000 MPN/100 mL, according to the current law: D. Lgs 152/2006, reported in Table S4 in the Supplementary materials). This value corresponds to the 85° percentile of the measured values. Accordingly, for Enterococci, the assumed average concentration in the WWTP effluent was equal to the 85° percentile of the collected data and corresponds to 2,500 MPN/100 mL.

2.4 Data analysis

Collected data of *E. coli* and Enterococci concentrations in each CSO outfall were reported in terms of:

- box-plots;
- concentration profiles *vs*. event time for all the events in order to evaluate the intra-event variability at each CSO outfall and to compare the profiles of different CSO outfalls;
- event mean concentration *EMT* *vs*. event mean flow rate *EMF*,
- loads discharged by the different CSO outfalls in the studied period.

Moreover, the study evaluated and compared the percentage contribution of each CSO outfall and the WWTP with respect to the total discharged volume in the observation period on a monthly and seasonal basis.

2.4.1 Load of fecal indicator bacteria, event mean concentration and event mean flow rate

The bacterial loads for each event (*EL*) were calculated by eq. 1.

$$EL = \int_0^T C(t)Q(t)dt = \sum_{i=1}^{m} C_i Q_i \Delta t_i \qquad \text{(eq. 1)}$$

where *T* is the duration of each CSO event (s), *m* is the number of samples collected for each CSO event, *C(t)* and *Q(t)* are the pollutant concentration (MPN/100 mL) and outfall flow rate (L/s) as functions of time, and C_i and Q_i are the monitored pollutant concentration (MPN/100 mL) and outfall flow rate (L/s) at each time interval Δt_i (s).

The last sample concentration was also assigned to the total volume discharged until the end of the event, as proposed by other studies (Madoux 2015; Bach et al. 2010). In the case of events with only one value of concentration available, this concentration was assumed to be constant for the whole event. This was the case of the following events: July 13th in MD; June 14th, June 17th, June 30th in S6; June 14th and June 17th in S13; and June 14th and June 30th in S14.

If no concentration value was available for a CSO due to the brevity of the overflow, its modest entity or other technical reasons, we assumed the concentration value measured in another outfall referring to the same event, or occurring at the same outfall for an event with similar characteristics in terms of rainfall duration and intensity and antecedent dry days. This occurred for the following events: June 19th; June 26th, August 15th and August 20th in MD; June 25th, July 24th, August 24th, and September 1st in S6.

Event mean concentration (*EMC*) was calculated using equation 2 and event mean flow rate (*EMF*) using equation 3.

$$EMC = \frac{\int_0^T C(t)Q(t)dt}{\int_0^T Q(t)dt} = \frac{\sum_{i=1}^{m} C_i Q_i \Delta t_i}{\sum_{i=1}^{m} Q_i \Delta t_i} \qquad \text{(eq. 2)}$$

$$EMF = \frac{\int_0^T Q(t)dt}{T} \qquad \text{(eq. 3)}$$

3 Results and discussion

3.1 Water volume discharged by CSO outfalls and WWTPs

CSO outfalls were analyzed in terms of working frequency and discharged flow rates during the June-September 2014 period. Table S3 shows the operation days and the corresponding discharged flow (m^3/d) for each CSO outfall, as well as the WWTP daily volume (in terms of the completely treated effluent BIO_D and also the partially treated effluent BY) discharged into the receiving water body.

Data regarding CSO event duration and the average and maximum flow rates are compiled in Table 1. The CSO duration ranged between 0.4 min to 930 min (=15.5 h), with an average of 214 min and a 95° percentile of 611 min.

An analysis of the device (pumps and MD valve) operation time recorded during the observation period is reported in Table 2, on the basis of data reported in the third column of Table 1, in percentage with respect to each month and the whole observation period. As it was expected, the highest values were found for MD (ranging from 2.26 and 6.28 % on a monthly basis). If we consider each CSO outfall, the highest values were always in the month of July in the order: MD > S6 > S13 > S8 > S14.

Table 2

During the same rainfall event, not all CSO outfalls were in operation, and this is attributed to the spatial variability and the rain intensity associated with that event, and also according to the extension of the urban basin surface for which the outfall is responsible ~~and the nominal capacity of the pumps of each outfall~~.

An analysis of data reported in Table S3 shows that the working frequency of the different outfalls varied from event to event. In particular: overflow occurred 18 times in MD outfall, 13 times in S6, 5 times in S14 and 4 times in S8 and S13.

The MD outfall exhibited a greater number of CSO events with respect to the other outfalls, and this is due to its position and function. It receives urban wastewater from the whole catchment area through different collectors and is the last “hydraulic protection” for the WWTP. In particular, it receives all the wastewater coming from the north part of the study area, whose sewer network does not have any CSO outfall (Figure 1). Outfalls situated downstream the sewer network (S14 and S6) were in operation a greater number of times than S13 and S8 due to the larger drained surface area.

With regard to Table S3, overflow events are reported using a color code, each of which is also attributed to the rain event which causes the corresponding CSOs.

The analysis of the overflow events in terms of the percentage contributions of discharged water volume by each CSO outfall on a monthly basis with respect to the total flow in the sewer system is given in Fig. 2. The highest contribution of CSO outfalls for the total discharged overflow was observed in July (17%) and to a lesser extent in June (9 %), whereas the lowest one occurred in August (2%).

The overall volumes discharged from the outfall points during the study period were in the following order: MD (70,362 m^3) > S6 (60,538 m^3) > S13 (40,527 m^3) > S14 (33,502 m^3) > S8 (15,233 m^3), whereas the total volume discharged from the WWTP (that is BIO_D + BY) was 2.23×10^6 m^3. An analysis of the discharged water volume by each point is reported in Figure 3. For each specific outfall we evaluated the percentage of water volume discharged in each month (see Table S3) with regard to the total volume discharged by the point under evaluation in the four months (corresponding to the sum of the four discharged values of the point reported in Table S3). It emerges that the monthly percentage contribution to its total discharged water volume varies depending on the point and the month of July mostly contributed for all the points, with the exception of BIO_D, to the discharged water volume (Fig. 3). In particular: BIO_D equally contributed to the discharged volume over the observation period (as expected) and BY mostly contributed during the first two months. In S8 and S13, overflow events occurred only in two months, whereas in S14 occurred in three, and in MD and S6 in four months.

Figure 2.

Figure 3

Some overflows occurred during rainfall events on summer days with a lower tourist presence in the study area. This mainly happened for CSO outfalls draining large basin areas even when modest rain intensity occurred (e.g. June 17th, 25th and 30th, August 24th, and September 1st and 10th). On these days, however, the CSO flow rate was modest, with only one exception (June 17th) being the last day of a long and intense rain event which had started on June 14th.

Generally, overflow events of long duration occurred after prolonged rainfall events in terms of total cumulative precipitation depth (Table 1). Finally, the rainfall event of July 26thcaused flooding around the urban basin, in particular on the southern beaches, due to the intensity and duration of the event and the consequent fall of water on impervious surfaces.

3.2 Concentrations of investigated pollutants

Figure 4 represents the range of variability of the concentrations of both indicator bacteria observed during the monitoring campaign in all CSO outfalls, together with the Italian limits of *E. coli* and/or Enterococci for the direct discharge of WWTP effluents into surface water bodies and into inland (internal water) bodies, as well as marine bathing water. Table S4 provides details about these legal values as well as the definition of inland and marine water according to the current regulations.

The widest variability ranges for the two indicator bacteria were always observed for MD and the lowest for S13.

The corresponding median concentrations, reported in descending order were:

- *E coli* (MPN/100 mL): 2.40 x 10^6 (MD), 1.64 x 10^6 (S14), 1.49 x 10^6 (S8), 1.05 x 10^6 (S13) and 4.89 x 10^5 (S6).
- Enterococci (MPN/100 mL): 2.66 x 10^5 (S14), 2.06 x 10^5 (MD), 1.99 x 10^5 (S13), 1.48 x 10^5 (S6) and 1.18 x 10^5 (S8).

It is important to observe that the first quartile, median and third quartile of the measured concentrations were *always* above the reported legal limits. The only exception was observed for S6, where the minimum and first quartile concentrations were below the limit for the direct discharge of a WWTP effluent (limit A = 5 x 10^3 MPN/100 mL).

The interval of variability found in the studied area ranged between 10 and 1.3 x 10^7 MPN/100 mL for *E. coli* and 10 and 7.27 x 10^5 MPN/100 mL for Enterococci. Table S5 and S6 summarize minimum, maximum and median concentrations for each outfall.

Figure 4.

These ranges are in fairly good agreement with those reported in literature and, in particular, with Arnone and Walling (2006) (900-7 x 10^4 MPN/100 mL for *E. coli* and 1.1 x 10^4 –3 x 10^5 MP/100 mL for Enterococci), Marsalek et al.(1994) (*E coli* in the range 2.8 x 10^4-1.1 x 10^6 MPN/100 mL for) and Passerat et al. (2011) (3.8 x 10^5-6.4 x 10^6 MPN/100 mL for *E. coli* and 1.2 x 10^5-1.2 x 10^6 MPN/100 mL for Enterococci)

In a separate sewer system, a concentration of *E. coli* and Enterococci was found in the range of 10-4 x 10^4 CFU/100 mL and 10 -9 x 10^4 CFU/100 mL, respectively during rainfall events and between 10 and 5.7 x 10^4 CFU/100 mL and 10 and 8 x 10^3 CFU/100 mL, respectively in snowmelt periods (Galfi et al., 2016b).

Figure 5 reports all the measured concentrations for *E. coli* in CSOs *vs*. the corresponding sampling time. It confirms that *E. coli* concentrations depend on many factors (rain intensity and duration, moisture, temperature, nutrient availability, adsorption/desorption processes, hydrologic processes and predation). It also highlights the fact that although one could expect that during the night values should be lower due to the modest contribution of domestic wastewater, they are generally in the range of 10^5-10^7 MPN/100 mL.

Fig. 5.

Dry weather concentrations of *E. coli* and Enterococci in the raw WWTP influent measured by Local Water Managing Body CADF were on average 3.6 x 10^6 and 1.7 x 10^5, respectively. It emerges that for both *E. coli* and Enterococci, the median values found at the different outfalls are in the same order of magnitude of the average value measured in the raw influent WWTP in dry weather.

During the whole observation period, the average concentration of *E. coli* and Enterococci in the treated effluent (data not reported) were 2.5 x 10^3 MPN/100 mL and 1.12 x 10^3/100 mL, with only a few exceptions related to the occasional escapes of suspended solids from the secondary clarifier.

3.2.1 Intra-event variability of monitored parameters

Figures 6-10 present the profiles of *E. coli* and Enterococci concentrations as well as the conductivity for all CSO outfalls during the different rainfall events. The X-axis reports the sampling time for each outfall. Note that the Y-axis on the left is in a log scale and it refers to bacteria concentration, whereas for conductivity the scale is on the right side and is a normal scale.

Measured concentrations of *E. coli* and Enterococci showed similar variations in all CSO outfalls and they generally vary within one order of magnitude during each event, with only a few exceptions. Some events were characterized by lower concentrations, between 10 and 1000 MPN/100 mL for both indicators: this occurred in MD on June 17th and September 1st and in S6 on July 30th and August 3rd and S8 on July 30th. S6 and S8 lower values were due to the disinfection treatment by means of peracetic acid applied at these two outfall points by the local water management body in order to protect the receiving water body during the summer season and to guarantee adherence to marine bathing limits. This represents a strategy suggested and adopted in different countries, as it will be discussed in section 3.6. The concentration profiles found in the two events in MD seem to exhibit the occurrence of a first flush phenomenon in the investigated outfall that is a more polluted overflow discharged at the beginning of the CSO.

The maximum values of bacterial concentrations in CSOs were found at the beginning of the rain event in 60 % of cases for *E. coli* and 55 % for Enterococci. The concentration profiles (see Figures 6-10) are strictly related to rainfall duration and intensity and antecedent precipitations, as discussed by Pongmala et al. (2015).

It is important to know the maximum concentrations occurring for microbiological contaminants, as they represent the most critical situations for the receiving water body and could seriously affect and threaten its expected use and purpose (drinking needs, bathing, and recreational activities in general).

E. coli concentration profiles are in good agreement (variability ranges and trends) with the curves found by Madoux-Humery et al (2015) in summertime in a residential area with only 11 % of open lands.

With regard to conductivity, its variation over time during CSO generally shows a peak at the beginning of the event, then rapidly decreases and sometimes reaches a minimum before progressively increasing until the end of the overflow. This profile is in good agreement with that found by Passerat et al. (2011) for the CSO monitored in a French urban area.

As shown in Figures 6-10, the conductivity varied in the following ranges: 1782 - 5460 μS/cm in MD, 320 - 3010 μS/cm in S6, 1090-17,650 μS/cm in S8, 210 - 1727 μS/cm in S13 and 210-14200 μS/cm in S14. Based on the collected data, the corresponding median conductivity values for the different CSO discharges were 3550 μS/cm (MD), 1080 μS/cm (S6), 2655 μS/cm (S8), 650 μS/cm (S13) and 2430 μS/cm (S14). CSO conductivity is lower than the typical values found for raw wastewater. It was found that conductivity of the WWTP influent in the dry period was about 6210 μs/cm (CADF, Report 2013), highlighting the dilution effect of wastewater due to urban stormwater runoff (with a much lower conductivity). The values reported by Passerat et al. (2011) for the CSO are instead considerably lower (the maximum is equal to 500 μS/cm, the minimum to 150 μS/cm and the median to 200 μS/cm), and the same is true of the average conductivity in raw wastewater (1175 μS/cm). The differences are mainly due to the consistent apportionment of saline intrusion in the sewer network under study.

Figure 6.

Figure 7.

As reported in literature, there is a great inter-event and intra-event variation in the concentration of microorganisms in CSO depending on catchment characteristics, rainfall/runoff duration and intensity, stormwater quality, climate characteristics (namely air and water temperature), and the number of dry days before the event. The sources of bacteria in stormwater runoff are attributed to the presence of debris, human activities and animal feces in urbanized areas, and to wildlife feces, recreational activities and soil and vegetation in low-imperviousness surfaces (Galfi et al., 2016a).

Figure 8.

Figure 9.

Figure 10.

3.2.2 Event mean concentration

Most studies have presented and compared results on the basis of the *Event Mean Concentration* (*EMC*) (Madoux-Humery et al., 2015; Hathaway and Hunt, 2010). This parameter provides a macro-snapshot of the event under study, but does not consider the dynamics of microbial concentrations during the event. Tables S5 and S6 report the estimated *EMC* for each event and each outfall for *E. coli* and Enterococci respectively, whereas Figures 11 and 12 show the curves of *EMC*, for all the observed CSO events, *versus* the corresponding event mean flowrate *EMF*, for each CSO outfall.

It emerges that *EMCs* ranged from 5.45×10^2 to 9.69×10^6 MPN/100 mL for *E. coli* and from 7.56×10^2 to 6.58×10^5 MPN/100 mL for Enterococci.

EMCs of *E. coli* in all CSO outfalls were mostly in the range of 10^6 MPN/100 mL, with the exception of two events (July 30th at S6 and S8 and August 3rd at S6), where considerably lower *EMC* values were observed because the water managing body decided to disinfect this stream as it will be discussed later. A similar pattern was also observed for the Enterococci but with one order of magnitude less.

With regard to literature data, we found that *EMCs* of *E. coli* were one order of magnitude lower than *EMCs* found by Madoux-Houmery et al.(2015).

In Figures 11 and 12, the interpolating line of the data regarding MD outfall has a slightly positive slope for both *E. coli* and Enterococci. The low value of R^2 means that, on the basis of the collected data, the correlation is not clear. On the contrary, Hathaway and Hunt (2011) and Dickenson and Sansalone (2012) found a good correlation (slope < 1) between bacteria concentration and flow rate, indicating that there is a contribution of a *less concentrated* water stream (i.e. stormwater) to the total load of both bacteria (dilution effect). They found that the content of *E. coli* in stormwater was 2 orders of magnitude lower than in raw wastewater.

With regard to the other monitored CSO outfalls, due to the low quantity of data, concentration-discharge slopes were not considered.

Figure 11.

Figure 12.

3.3 Discharged bacterial load – Contribution of occasional and continuous points

The total discharged load of *E. coli* and Enterococci from each CSO outfall and the WWTP has been calculated and depicted in Figure 13 on a monthly basis in absolute terms (as the amount discharged from each point, see rectangles), and as a percentage of the discharged load with respect to the total load discharged into the receiving water body (see bold lines). The contribution of each point and the main sources in each month are immediately evident.

With regard to *E. coli*, the highest discharged amount in all months was due to MD outfall, with loads of 2.5 x 10^{14}, 8.6 x 10^{14}, 4.2 x 10^{14}, 9.4 x 10^{13} MPN/month from June to September. The second source varied: S6 in June and July, WWTP effluent in August and S14 in September.

With regard to Enterococci, the main contribution was due to S6 in June and September and to MD in July and August, followed by WWTP effluent in June and August, S8 in July, and MD in September.

The differences between the monthly load emitted by the main two sources were extremely high in August for *E. coli* and high in June for *E. coli* and Enterococci. In the other cases, the differences were quite modest.

Although the water flow discharged from CSO outfalls (Fig. 2) is much lower than that discharged from the WWTP into surface water (9% in June, 17% in July, 2% in August and 5% in September), the discharged load of bacteria from these points is consistently higher throughout the studied period (Fig. 13): on a monthly basis, they contribute more than 90 % for *E. coli* and more than 77 % on average for Enterocci.

Figure 13.

3.4 Fate of the released fecal indicator bacteria in the water environment

Once *E. coli* and Enterococci are released in the water environment (channels and then the Adriatic Sea) their elimination/survival is strictly correlated to the receiving water characteristics (mainly temperature, turbidity, salinity, residence time in the channel) and the environmental conditions (namely sunlight hours, UV irradiation, sunny/cloudy weather conditions). In addition the tide may also affect bacteria elimination processes. Enterococci can generally survive longer than *E. coli* in water (Byappanahalli et al., 2012). A brief presentation of the influence af the cited parameters is reported in Table 3 and an interesting discussion on their influence on the microbiological quality of the sea in all the Spanish beaches and in a Lake Michigan swimming beach are reported in Aragonés et al. (2016) and in Whitman et al. (2004) respectively.

Table 3

With regard to the study area, the channels receiving overflows are characterized by a turbidity equal to 40-90 mg/L SiO_2 in dry weather conditions and much higher after rain events, due to the induced turbulence leading to re-supspension of settled material. The water depth in these channels is between 2.5 and 4 m and water temperature after rainfall events in summer is between 17°C and 20 °C. The distance between the overflow release points and the Adriatic Sea varies between 1.26 km and 6.1 km. Assuming a water speed in the channels of 0.4 m/s, the residence time varies between 0.88 h and 4.2 h.

In this short period of time, in case of overflow, fecal bacteria elimination from the outfall release points to the final receiver (the Sea) is modest. In fact, after rainfall events, the sky is generally cloudy and thus the solar radiation is not able to efficienctly remove these microorganisms, even if there are many sunlight hours (from 15 h in June to 12 h in September). Moreover, water turbidity hinders light penetration. The really modest natural attenuation of the exceptionally high load/concentration of microorganisms released after an intense rainfall event in the channels is demonstrated by the fact that during each summer, soon after intense rain events, in the beaches near the immission of these channels in the Adriatic Sea, bathing is prohibited as bacteriological standards in sea water are exceeded. Unfortunately this is happening in many other coastal towns in Italy.

3.5 CSO management and treatment

In order to reduce and attenuate the pollutant load of intermittent CSOs in the receiving water body, correct management and treatment should be adopted.

Enlargement of the existing sewer network is possible but extremely expensive due to the wide extension of the sewer network and the necessary upgrading of the receiving WWTP in terms of an increment of the nominal hydraulic capacity and upgrading of the existing treatment capacity.

Lessons learned from recent experiences show that in combined sewer networks, adequate measures refer to a dedicated treatment of the occasional overflow rate. They must guarantee a high level of removal of suspended solids and bacteria and that in the vicinity of swimming beaches disinfection becomes a necessity.

Recently, technologies and/or treatment trains were tested in pilot and full scale plants. Of these, the most promising seem to be:

- chemical pre-treatments prior to UV disinfection. Investigations were carried out for alum ($Al_2(S0_4)_3 \bullet 12H_2O$), ferric chloride ($FeCl_3$) and cationic polymers. Higher UV light transmission (UVT) and suspended solid removal were observed with alum (20 mg/L increased the UVT of the raw CSO from 30 to 60% after settling; a dose of 100 mg/L of alum maximized UVT that reached approximately 85%). Flocculation, although not increasing UVT did improve the removal of total suspended solids. Cationic polymers worked quickly, compared to metal coagulants, but reached a maximum UVT of 60 % (Gibson et al., 2016).

  Interesting results have been achieved by treating CSO in a ballasted flocculation unit (BFU), that is a compartment employing microsand in order to favor bloc formation acting as a ballast agent, thus reducing hydraulic retention time and increasing the nominal overflow rate (Gasperi et al., 2012). The full scale BFU unit, equipped at the Seine Aval WWTP near Paris, showed that the treatment seems to be less sensitive to the influent concentration fluctuations and hydraulic peak load than to the control and adjustments of chemical doses and sand injection;
- vegetated and unvegetated horizontal subsurface flow beds as discussed by Pisoeiro et al. (2016). In a bed (size: 55.5 cm long, 36.1 cm wide and 40 cm high; filling material (35 cm height): 4-8 mm, 30 % porosity) fed with CSO (Enterococci concentration was on average 1,15 x 10^6 MPN/100 mL

(standard deviation 8.21 10^5), TSS 120 mg/L (standard deviation 48) and COD 233 mg/L (standard deviation 53)), with a hydraulic retention time of 1 d and 7 days, an average removal rate was found of 90-100 % for TSS, 60-90 % for COD and 2-6 log units for Enterococci; most of TSS and bacteria were removed in the first 24 hours. Moreover, plant species (*Phragmites australis*) did not influence the removal of TSS and bacteria;

- peracetic acid (PAA) disinfection: it was found that PAA concentration in the range of 5 - 15 mg/L and contact times from 2 to 10 mins are able to reduce the *E. coli* concentration from 10^5-10^6MPN/100 mL to below the limits posed by the Kentucky Administrative Regulation (KAR 2012) of 240 MPN/100 mL for the instantaneous samples and 130 MPN/100 mL for the geometric mean of samples taken over a 30-day period (see Table S2 in the Supplementary Data section) (Coyle et al., 2014);
- performic acid (PFA) disinfection: investigations on the disinfection of CSO using PFA in a sea-outfall pipe of a large WWTP in Copenaghen showed a removal of 1-3.5 log units for *E. coli* and 1.0-2.44 log removal for Enterococci at doses ranging in the interval 1-8 mg/L (Chhetry et al. 2015). These results, although interesting, are still at an early stage of development. (Chhetry et al., 2014).

On the basis of these findings and the characteristics of the area under study, attenuation measures have recently been discussed - in order to reduce the impact of the intermittent CSOs of the Comacchio area in the Adriatic Sea, the Local Water Management Body has planned to build a specific treatment plant for the MD CSO, consisting of a sedimentation tank (for the removal of suspended solids) and of a PAA disinfection step for a maximum flow rate of 350 L/s. This should guarantee respect of the Italian limits for bathing on beaches.

4 Conclusions

The analysis of the pollutant loads discharged by intermittent CSO outfalls compared to those released by the local WWTP highlights that although the CSO water volume is much lower than that released by the WWTP, the CSO microbiological load is much higher than that of the WWTP, particularly during periods of heavy rain in the summer. Once the overflow is released into the surface water network, auto-purification processes take place in the receiving system. Among these, UV irradiation is very effective in removing microorganisms in water environment. But after an intense rain event, this effect is modest due to different reasons: UV irradiation cannot well penetrate in water due to water turbidity and UV intensity is reduced by cloudy weather conditions. Moreover, released microorganisms stay 1-4.9 h in the channels before reaching the Sea and this period is not sufficient to guarantee a good removal under sunlight conditions. This fact could have an immediate acute negative impact on the quality of the receiving water body, and in the worst case scenario could lead to the prohibition of bathing as bacteriological limits in sea water are exceeded. Unfortunately, these events have frequently occurred during previous summer seasons in most of the Italian coastal area. The case study highlights that a correct measure could be disinfection for the effluent for the most critical CSO outfall in terms of discharged microbial load. This would greatly reduce the risk of compromising quality in recreational areas, mainly with regard to bathing.

5 Additional materials

Supplementary data to this article can be found at: ….

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References

American Public Health Association (APHA), American Water Work Association (AWWA) and Water Environment Federation (WEF). Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998. Washington DC, USA.

Aragonés L, López I, Palazón A, López-Ubeda R, García C. Evaluation of the quality of coastal bathing waters in Spain through fecal bacteria *Escherichia coli* and Enterococcus. Sci Total Environ 2016;566-567:288-97.

Arnone RD, Walling JP, Evaluating *Cryptosporidium* and *Giardia* concentrations in combined sewer overflow, J Water Health. 2006;4(2):157-65

Bach PM, McCarthy DT, Deletic A. Redefining the stormwater first flush phenomenon. Water Res 2010;44(8):2487-98.

Barco J, Papiri S, Stenstrom MK. First flush in a combined sewer system. Chemosphere 2008;71(5):827-33.

Bathing Water Committee, 2009. Bathing Water Profiles: Best Practice and Guidance. European Environment Agency (EC DG ENV 2009 URL: http://ec.europa.eu/environment/water/water-bathing/pdf/profiles_dec_2009.pdf).

Buerge IJ, Poiger T, Muller MD, Buser HR. Combined Sewer Overflows to Surface Waters Detected by the Anthropogenic Marker Caffeine. Environ Sci Technol 2006;40(13):4096-4102, doi:10.1021/es052553l

Burton GA, Pitt RE. Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists and Engineers. Lewis Publishers, Boca Raton. 2002

Byappanahalli MN, Nevers MB, Korajkic A, Staley ZR, Harwood VJ. Enterococci in the environment. Microbiol. Mol. Biol. Rev. 2012;76 (4), 685–706.

CADF, Piano di campionamento delle acque interne in prossimità del litorale Comacchiese, Report 2013, available at the web site: http://www.cadf.it/pdf/balneaz_settott2013.pdf (*in Italian*, last access May 2017).

Campos CJA, Avant J, Lowther J, Till D, Lees DN. Human Norovirus in untreated sewage and effluents from primary, secondary and tertiary treatment processes. Water Res 2016; 103:224-32.

Chèvre N, Coutu S, Margot J, Wynn HK, Bader H, Scheidegger R, Rossi L. Substance flow analysis as a tool for mitigating the impact of pharmaceuticals on the aquatic system. Water Res 2013;47(9):2995-3005.

Chhetri RK, Flagstad R, Munch ES, Hørning C, Berner J, Kolte-Olsen A, Thornberg D, Andersen HR. Full scale evaluation of combined sewer overflows disinfection using performic acid in a sea-outfall pipe, Chemical Engineering Journal 2015;270:133–139

Chhetri RK, Thornberg D, Berner J, Gramstad R, Ojstedt U, Sharma AK, Andersen HR, Chemical disinfection of combined sewer overflow waters using performic acid or peracetic acids, Sci. Total Environ. 2014;490:1065–1072.

Council Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment, Official Journal L 135, 30/05/1991.

Coyle EE, Ormsbee LE, Brion GM. Peracetic acid as an alternative disinfection technology for wet weather flows. Water Environ Res 2014;86(8), pp. 687-697

DGR-Deliberazione della Giunta Regionale 14 febbraio 2005, n. 286. Direttiva concernente indirizzi per la gestione delle acque di prima pioggia e di lavaggio da aree esterne (art. 39, DLgs 11 maggio 1999, n. 152)

Diaz-Fierros TF, Puerta J, Suarez J, Diaz-Fierros VF. Contaminant loads of CSOs at the wastewater treatment plant of a city in NW Spain. Urban Water 2002;4(3):291-9.

Dickenson JA, Sansalone JJ. Distribution and disinfection of bacterial loadings associated with particulate matter fractions transported in urban wet weather flows. Water Res 2012;46(20):6704-14.

Foundation for Water Rresearch, Urban Pollution Management Manual, 3rd edition, Marlow, 2012, document available at the web site: http://www.fwr.org/UPM3, last access June 12th 2017).

Galfi H, Haapala J, Nordqvist K, Westerlund C, Blecken G, Marsalek J, Viklander M. Inter-event and intra-event variations of indicator bacteria concentrations in the storm sewer system of the city of Östersund, Sweden. J Environ Eng 2016a;142(7).

Galfi H. Österlund H., Marsalek J, ViklanderM Indicator bacteria and associated water quality constituents in stormwater and snowmelt from four urban catchments, J Hydrol 2016b;539:125-140

Gasperi J, Laborie B, Rocher V. Treatment of combined sewer overflows by ballasted flocculation: Removal study of a large broad spectrum of pollutants. Chem Eng J 2012;211-212:293-301.

Gibson III CJ, Stadterman KL, States S, Sykora J. Combined sewer overflows: A source of *Cryptosporidium* and *Giardia*? Water Sci Technol 1998;38(12):67-72.

Gibson J, Farnood R., Seto P. Chemical pretreatment of combined sewer overflows for improved UV disinfection Water Sci Tecnol 2016;73(2): 375-381.

Hanner N, Mattsson A, Gruvberger C, Nyberg U, Aspegren H, Fredriksson O, Nordqvist A, Andersson B. Reducing the total discharge from a large WWTP by separate treatment of primary effluent overflow. Water Sci Technol 2004;50(7):157-62.

Hathaway JM, Hunt WF. Evaluation of first flush for indicator bacteria and total suspended solids in urban stormwater runoff. Water Air Soil Pollut 2011;217(1-4):135-47.

IRSA-APAT Metodi analitici per le acque, Istituto Poligrafico e Zecca dello Stato, 2003 ~~1994~~.

ISO International Standard Organization ISO 7899-1:199, Water quality -- Detection and enumeration of intestinal enterococci -- Part 1: Miniaturized method (Most Probable Number) for surface and waste water, 1998-11 (www.iso.org/standard/14852.html)

Jalliffier-Verne I, Heniche M, Madoux-Humery A, Galarneau M, Servais P, Prévost M, Dorner S. Cumulative effects of fecal contamination from combined sewer overflows: Management for source water protection. J Environ Manage 2016;174:62-70.

Kafi M, Gasperi J, Moilleron R, Gromaire MC, Chebbo G. Spatial variability of the characteristics of combined wet weather pollutant loads in Paris. Water Res 2008;42(3):539-49.

Kentucky Administrative Regulations KAR 2012. Surface Water Standards; 401 K.A.R. 10.031. http://lrc.ky.gov/kar/401/010/031.htm (last accessed April 2017).

Launay MA, Dittmer U, Steinmetz H Organic micropollutants discharged by combined sewer overflows – Characterisation of pollutant sources and stormwater-related processes. Water Res 2016;104:82-92

Line DE, White NM, Kirby-Smith WW, Potts JD. Fecal coliform export from four coastal north carolina areas. J Am Water Resour Assoc 2008;44(3):606-17.

Mac Kenzie WR, Hoxie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP. A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. New Engl J Med 1994;331(3):161-7.

Madoux-Humery AS, Dorner S, Sauvé S, Aboulfadl K, Galarneau M, Servais P, Prévost M. Temporal variability of combined sewer overflow contaminants: Evaluation of wastewater micropollutants as tracers of fecal contamination. Water Res 2013;47(13):4370-82

Madoux-Humery AS, Dorner S, Sauvé S, Aboulfadl K, Galarneau M, Servais P, Prevost M. Temporal analysis of microbiological and wastewater micropollutant loads from combined sewer overflows: implications for management. Environ Sci Process Impacts 2015;17(5):965-974.

Marsalek J, Dutka BJ, Tsanis IK. Urban impacts on microbiological pollution of the St. Clair river in Sarnia, Ontario. Water Sci Technol 1994;30:177-84.

McLellan SL, Hollis EJ, Depas MM, Van Dyke M, Harris J, Scopel CO. Distribution and fate of escherichia coli in lake michigan following contamination with urban stormwater and combined sewer overflows. J Great Lakes Res 2007;33(3):566-80.

McLellan SL, Hollis EJ, Depas MM, Van Dyke M, Harris J, Scopel CO. Distribution and fate of *Escherichia coli* in Lake Michigan following contamination with urban stormwater and combined sewer overflows. J Great Lakes Res 2007;33(3):566-80.

MetCalfe & Eddy, Wastewater Engineering : Treatment and Reuse, McGraw-Hill Education – Europe, 2003

NYC Global Partners. Best practice: redeveloping industrial harbor for public swimming [WWW Document]. URL http://www.nyc.gov/html/ia/gprb/downloads/pdf/Copenhagen_HarbourBath.pdf, 2011. [accessed April 2017]

Ouattara NK, Garcia-Armisen T, Anzil A, Brion N, Servais P. Impact of wastewater release on the faecal contamination of a small urban river: The Zenne river in Brussels (Belgium). Water Air Soil Pollut 2014;225(8):1-12.

Passerat, J., Ouattara, N.K., Mouchel, J.-M., Rocher, V., Servais, P., 2011. Impact of an intense combined sewer overflow event on the microbiological water quality ofthe Seine River. Water Res. 45 (2), 893-903.

Phillips PJ, Chalmers AT, Gray JL, Kolpin DW, Foreman WT, Wall GR. Combined sewer overflows: An environmental source of hormones and wastewater micropollutants. Environ Sci Technol 2012;46(10):5336-43.

Pisoeiro, J., Galvão, A., Ferreira, F., Matos, J. Potential for CSO treatment with horizontal flow constructed wetlands: influence of hydraulic load, plant presence and loading frequency, Environ Sci Pollution Res 2016;23(20): 20591-99

Pongmala K, Autixier L, Madoux-Humery A-, Fuamba M, Galarneau M, Sauvé S, Prévost M, Dorner S. Modelling total suspended solids, *E. coli* and carbamazepine, a tracer of wastewater contamination from combined sewer overflows. J Hydrol 2015;531:830-9.

Rechenburg A, Koch C, Claßen T, Kistemann T. Impact of sewage treatment plants and combined sewer overflow basins on the microbiological quality of surface water. Water Sci Technol 2006;54(3):95-9.

Sauvé S, Aboulfadl K, Dorner S, Payment P, Deschamps G, Prévost M. Fecal coliforms, caffeine and carbamazepine in stormwater collection systems in a large urban area. Chemosphere 2012;86(2):118-123.

Servais P, Garcia-Armisen T, George I, Billen G. Fecal bacteria in the rivers of the seine drainage network (France): Sources, fate and modelling. Sci Total Environ 2007;375(1-3):152-67

Tryland I, Surman S, Berg JD. Monitoring faecal contamination of the Thames estuary using a semi-automated early warning system. Water Sci Technol 2002;46(3):25-31.

U.S. Environmental Protection Agency (1993) Combined Sewer Overflow Control; EPA 625-R-93-007; U.S. Environmental Protection Agency, Office of Research and Development: Washington, D.C.

Whitman RL, Nevers MB, Korinek GC, Byappanahalli MN. Solar and temporal effects on *Escherichia coli* concentration at a Lake Michigan swimming beach. Appl. Environ. Microbiol. 2004;70 (7):4276–4285.

TABLES

Table 1: Characteristics of the studied rain events and CSO discharges. In the first column, in brackets, after the outfall name, the number of events occurring in summer 2014 at the specific site.

CSO point	Event	Overflow duration	Overflow discharged volume	Average Flowrate	Maximum Flowrate	Precipitation duration	Antecedent dry period (ADP)	Cumulative precipitations during the overflow event	Mean intensity of precipitation
		[min]	[m^3]	[L/s]	[L/s]	[min]	[d]	[mm]	[mm/h]
S6 (14)	14 June	167.4	5,550.3	553	754	202.7	0.7	16.2	4.9
	17 June	115.8	5,418.2	780	1300	117.6	2.9	14.8	7.5
	25 June	19.2	505.5	440	754	90.5	5.6	4.2	1.0
	30 June	75.3	1,871.4	414	754	232.4	3.4	10.6	3.6
	10 July	207.6	6760	543	754	733.0	0.4	25.8	2.1
	12 July	117.5	3,595	510	754	279.3	2.3	15.0	2.9
	24 July	34.8	845	405	405	126.6	2.9	3.01	1.4
	26 July	179.2	8967	834	1300	153.6	0.4	41.4	16.3
	30 July	253.0	11,078	730	1300	517.1	3.0	35.8	4.2
	3 August.	140.0	6943	826	1300	186.0	3.4	15.4	8.1
	24 August	47.8	1161	405	405	35.8	0.7	4.0	6.7
	1 September	24.4	593	405	405	108.3	7.0	6.4	3.5
	10 September	43.8	1063	405	405	207.0	6.7	9.0	2.6
	20 Sep.	202.2	5866	484	754	306.2	0.6	25.8	5.1
S8 (4)	12 July	134.1	3737	464	700	279.3	2.3	15.0	3.2
	26 July	226.0	6218	459	700	164.6	0.4	41.4	16.1
	30 July	96.4	2025	350	350	511.1	3.0	35.8	4.2
	20 September	154.9	3253	350	350	306.2	0.6	25.8	5.1
S13 (3)	14 June	367.9	16,299	738	1100	189.0	0.6	32.0	10.2
	17 June	0.4	24	1075	1100	153.0	2.9	6.6	2.6
	26-27 July	461.9	15,243	550	550	308.0	0.2	36.4	9.0

CSO point	Event	Overflow duration	Overflow discharged volume	Average Flowrate	Maximum Flowrate	Precipitation duration	Antecedent dry period (ADP)	Cumulative precipitations during the overflow event	Mean intensity of precipitation
S14 (5)		271.5	8961	550	550				
	14 June	240.0	12,748	885	1800	189.0	0.6	32.0	10.2
	30 June	1.9	73	650	650	269.0	3.7	14.6	3.1
	26 July	199.8	16,247	1355	1800	308.0	0.2	36.4	9.0
	10 September	94.4	3681	650	650	252.0	0.4	22.8	5.4
	20 September	19.3	753	650	650	279.0	0.6	16.8	3.6
MD (15)	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	11,618	430	651				
		135	966	119	321				
	30-31 July	735	30,383	689	2023				
		150	36	4	4				
	3-4 August	420	6710	266	775				
		30	29	16	16				
	15-16 August	450	2415	89	170				
		90	168	31	65				
	20 August	30	18	10	16				
	1 September	75	61	14	16				
	10 September	450	1669	62	240				
	20 September	450	4164	154	321				

Table 2. Percentage of time with CSO outfalls in operation with respect to each month and the whole period

	June	**July**	**August**	**September**	**Whole period**
MD	3.19	6.28	2.28	2.26	3.52
S6	0.87	1.77	0.42	0.63	0.93
S8	0	1.02	0	0.36	0.35
S13	0.85	1.64	0	0	0.63
S14	0.56	0.45	0.00	0.26	0.32

Table 3. Main parameters affecting the elimination/survival of fecal bacteria in water environment

Parameter	**Effect**
Water temperature	According to the Bathing Water Committee (2009), elimination of 90 % of *E. coli* and Entetococci requires respectively 35 h and 70 h in cloudy weather and 5 h and 15 h in sunny weather~~.~~
Turbidity	According to Whitman et al. (2004) water turbidity reduces the light penetration in the water column and thus it hinders the elimination of bacteria.
Salinity	High salinity waters are generally correlated to low microorganism concentration. Enterococci are more tolerant to higher values than *E. coli* (Aragonés et al., 2016).
Residence time in the water compartment	Bacteria elimination is proportional to their time spent in the channel before reaching the final receptor during which they may undergo to the different auto-purification processes.
Sunlight hours	Bacteria natural decay is associated to light exposure of microorganisms. During the night in fact, there is a replenishment (in terms of growth and or resuscitation) of bacteria (Withman et al., 2004)
UV irradiation	Light exposure and in particular the exposure to a light wavelength of 254 nm is responsible of a decay of the concentration of fecal bacteria. This is the working principle of UV reactors used for water and wastewater (=filtered biological effluent disinfection). The removal efficiency of bacteria by UV irradiation is higher in water with a high transmittance, that is with low turbidity: suspended particles shield microorganisms and radiation cannot reach them (Metcalf & Eddy, 2003).
Cloudy/sunny weather conditions	Clouds act as a shield for bacteria reducing the effect of the solar radiation that is responsible of their decay.
Tide	Tidal cycles may influence bacteria concentrations in water, depending on the tide height (Aragonés et al., 2016).

Captions

Figure 1. Schematics of the area under study with a focus on the combined sewer network, CSO outfalls, rain gauges and WWTP.

Figure 2. Volume of water discharged monthly into the receiving water body: percentage contribution of untreated CSOs and treated WWTP effluent (sum of BIO_D and BY).

Figure 3 Monthly discharged water volume (in percentage) by each point with respect to the corresponding total volume discharged in the four months. (BY is the effluent bypassing the secondary treatment and conveyed to the disinfection tank; BIO_D is the secondary effluent within the WWTP conveyed to the disinfection tank; MD is the combined sewage overflow outfall upstream the WWTP).

Figure 4. Box-plots of *E. coli* and *Enterococci* concentrations in the different CSOs. The dot lines refer to current Italian limits. In detail, A = suggested limit for release of a WWTP effluent (*E. coli)* into a surface water body; B = Inland bathing water limit (*E. coli*); C = Marine bathing water limit (*E. coli*) and Inland bathing water limit (*Enterococci*); D = Marine bathing water limit (*Enterococci*).

Figure 5. *E. coli* concentration in overflow *vs*. sampling time. The same symbol means a measurement referring to the same event in a specific CSO outfall.

Figure 6. Profiles of *E. coli*, Enterococci and conductivity during the overflows at MD outfall.

Figure 7. Profiles of *E. coli,* Enterococci and conductivity during the overflows at S6 outfall.

Figure 8: Profiles of *E. coli,* Enterococci and conductivity during the overflows at S8 outfall.

Figure 9. Profiles of *E. coli,* Enterococci and conductivity during the overflows at S13 outfall.

Figure 10: Profiles of *E. coli,* Enterococci and conductivity during the overflows at S14 outfall.

Figure 11. EMCs of *E. coli vs.* mean CSO event flowrate in log-log plots. Symbols with a star indicate disinfected events.

Figure 12. EMCs of *Enterococci vs.* mean CSO event flowrate in log-log plots. Symbols with a star indicate disinfected events.

Figure 13. Monthly discharged load of *E. coli* and *Enterococci* in the different CSO outfalls and released by the local WWTP effluent (dry and wet weather) as well as the cumulative percentage contribution to the total discharge (bold line).

Figures

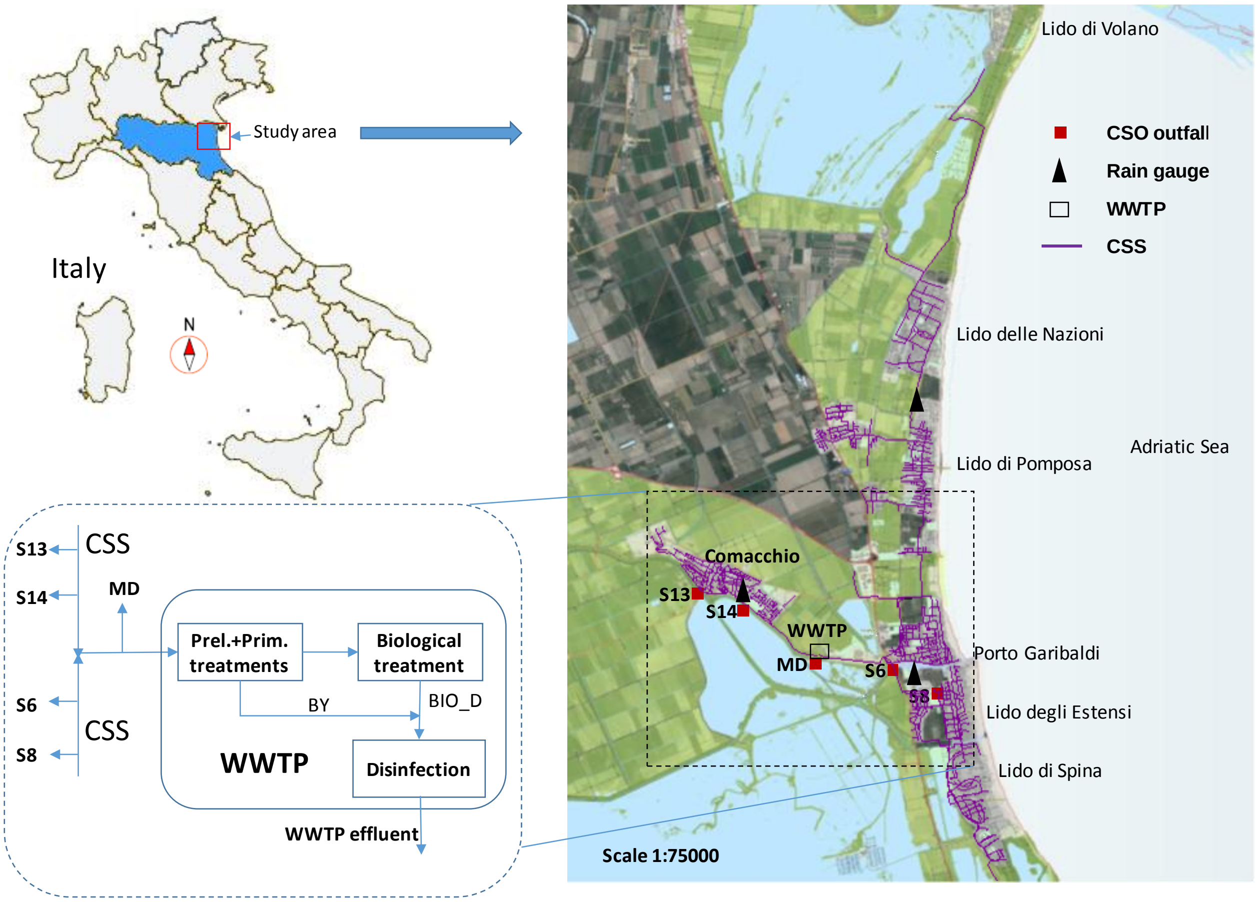

Figure 1.

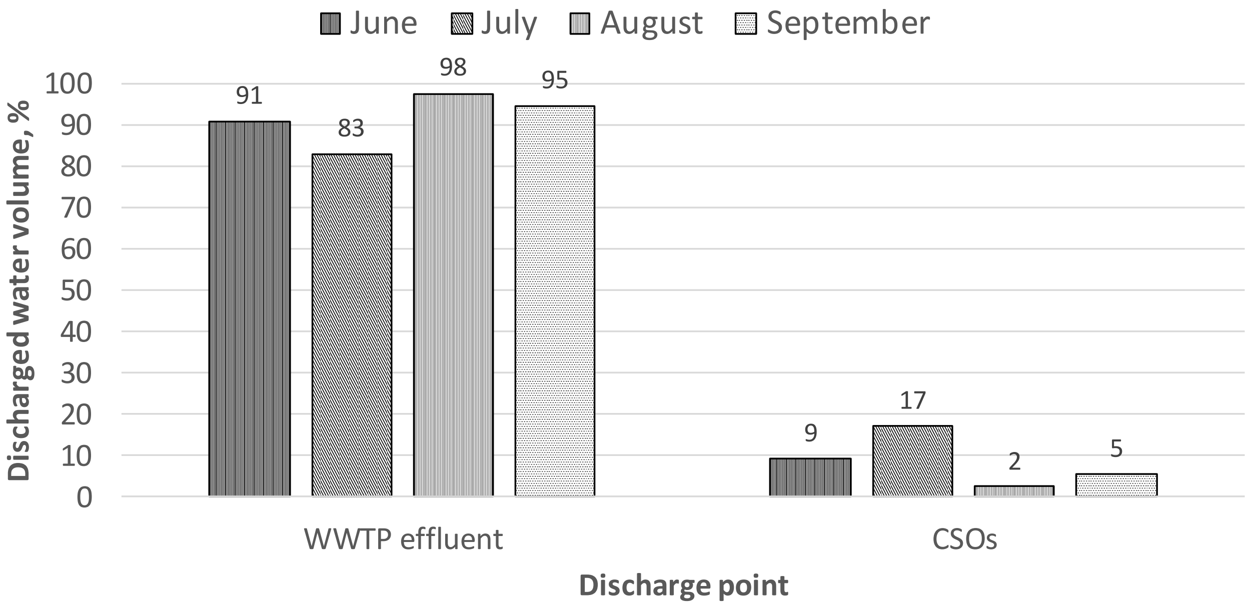
June
July
August
September
Discharged water volume, %
100
90
80
70
60
50
40
30
20
10
0
91
83
98
95
9
17
2
5
WWTP effluent
CSOs
Discharge point

Figure 2.

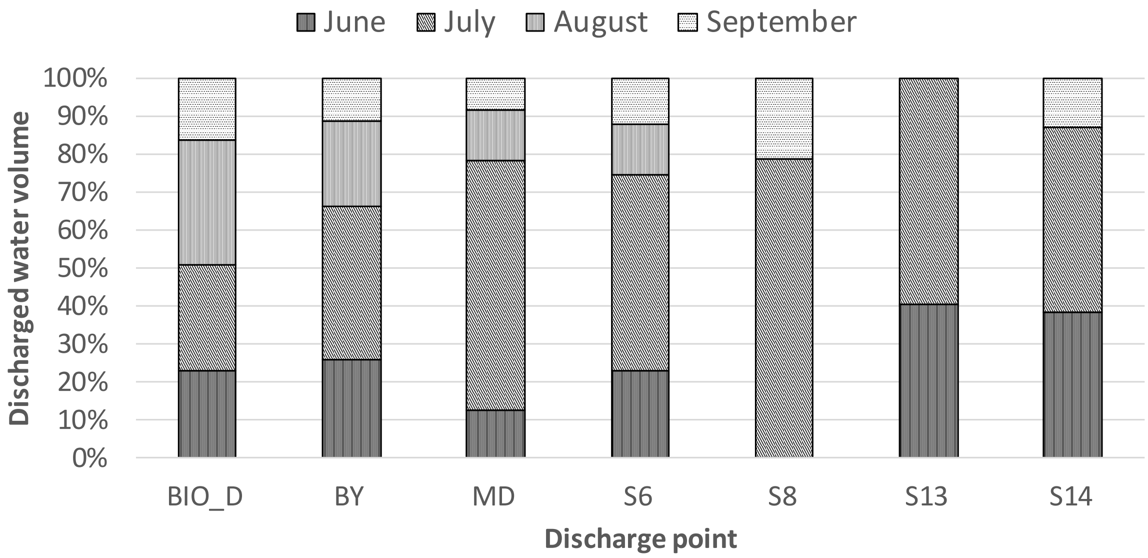
June
July
August
September
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Discharged water volume
BIO_D
BY
MD
S6
S8
S13
S14
Discharge point

Figure 3.

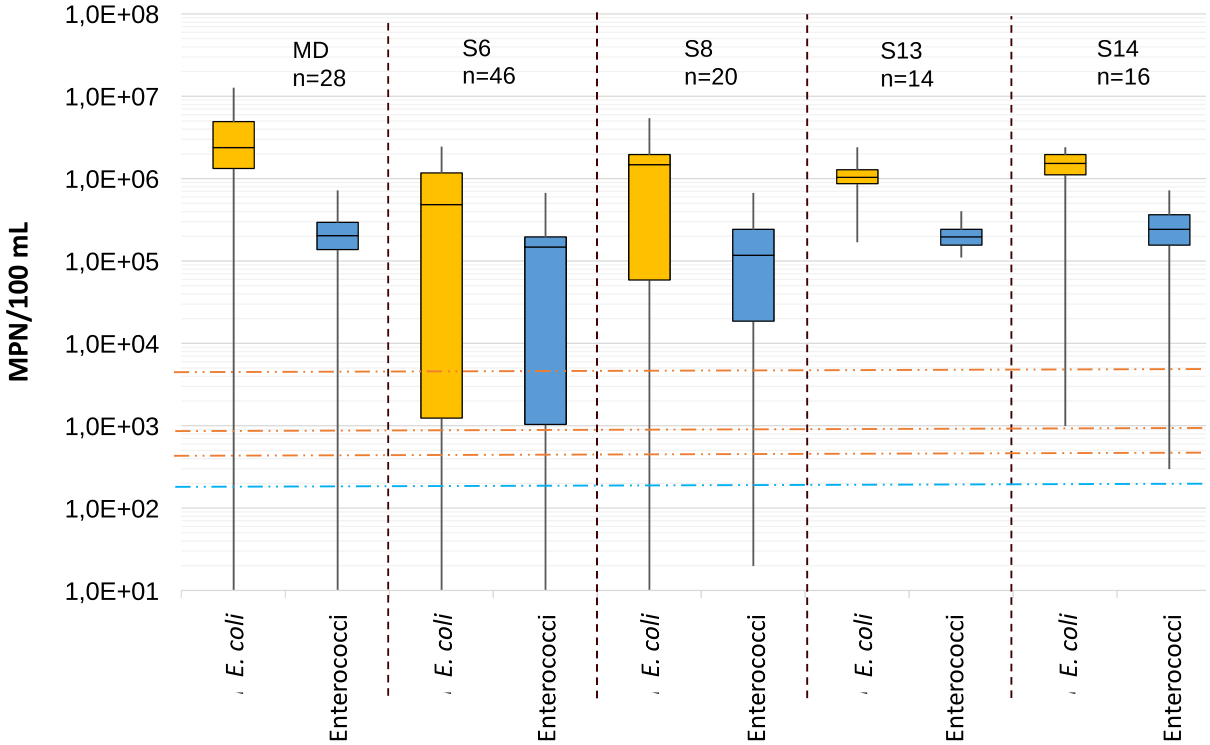
MPN/100 mL
1,0E+08
1,0E+07
1,0E+06
1,0E+05
1,0E+04
1,0E+03
1,0E+02
1,0E+01
MD
n=28
S6
n=46
S8
n=20
S13
n=14
S14
n=16
E. coli
Enterococci
E. coli
Enterococci
E. coli
Enterococci
E. coli
Enterococci
E. coli
Enterococci

Figure 4.

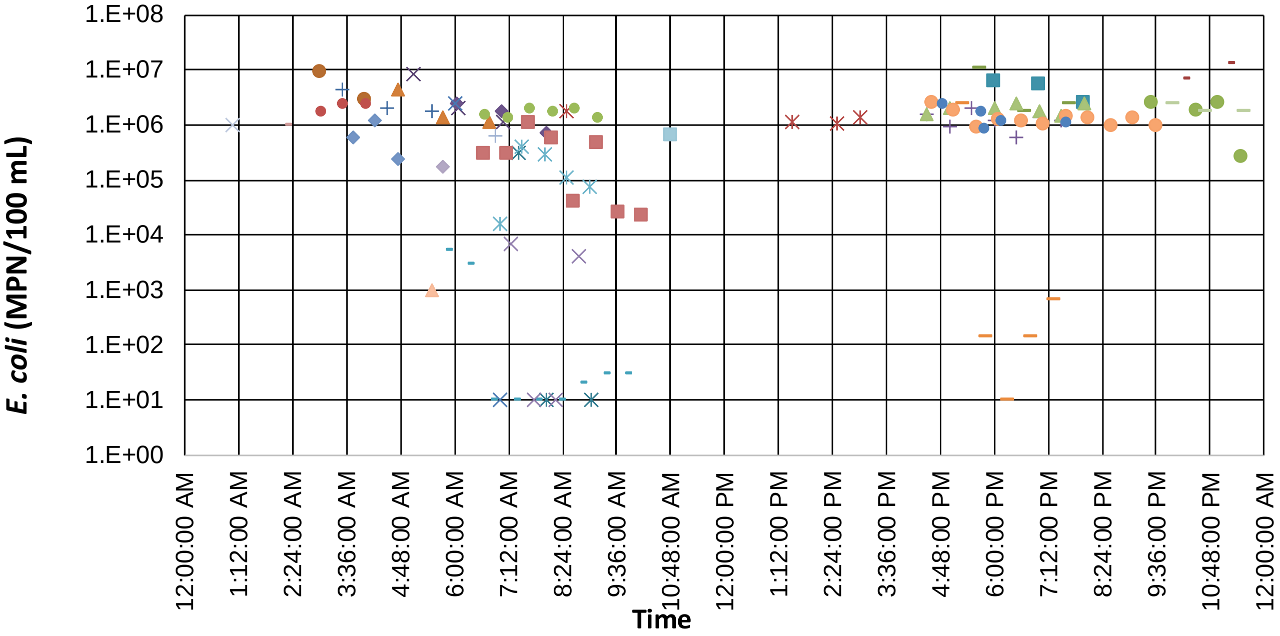
1.E+08
1.E+07
1.E+06
1.E+05
1.E+04
1.E+03
1.E+02
1.E+01
1.E+00
E. coli (MPN/100 mL)
12:00:00 AM
1:12:00 AM
2:24:00 AM
3:36:00 AM
4:48:00 AM
6:00:00 AM
7:12:00 AM
8:24:00 AM
9:36:00 AM
10:48:00 AM
12:00:00 PM
1:12:00 PM
2:24:00 PM
3:36:00 PM
4:48:00 PM
6:00:00 PM
7:12:00 PM
8:24:00 PM
9:36:00 PM
10:48:00 PM
12:00:00 AM
Time

Figure 5.

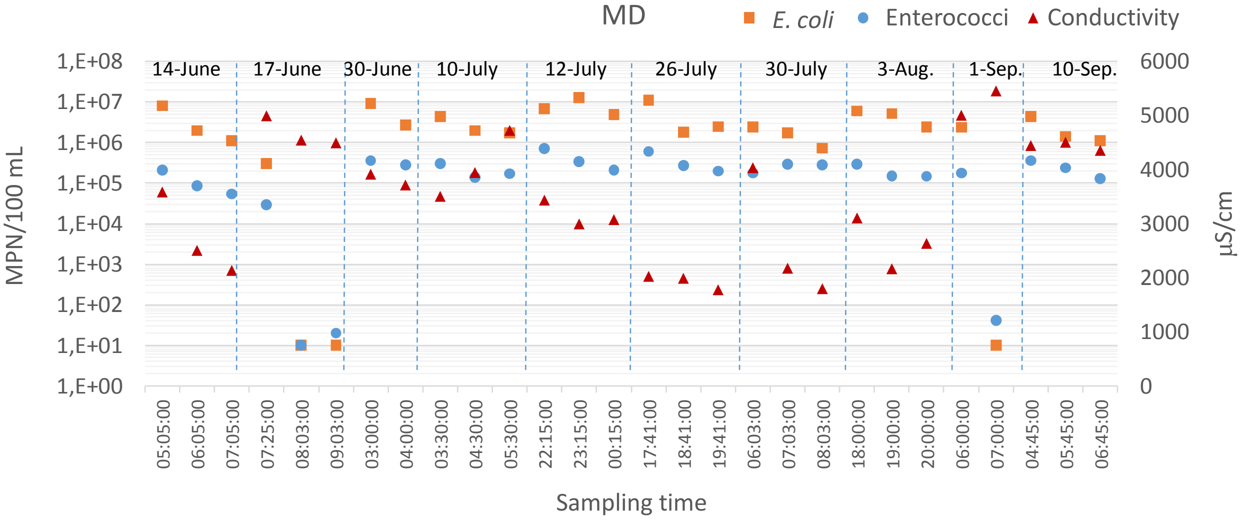

Figure 6

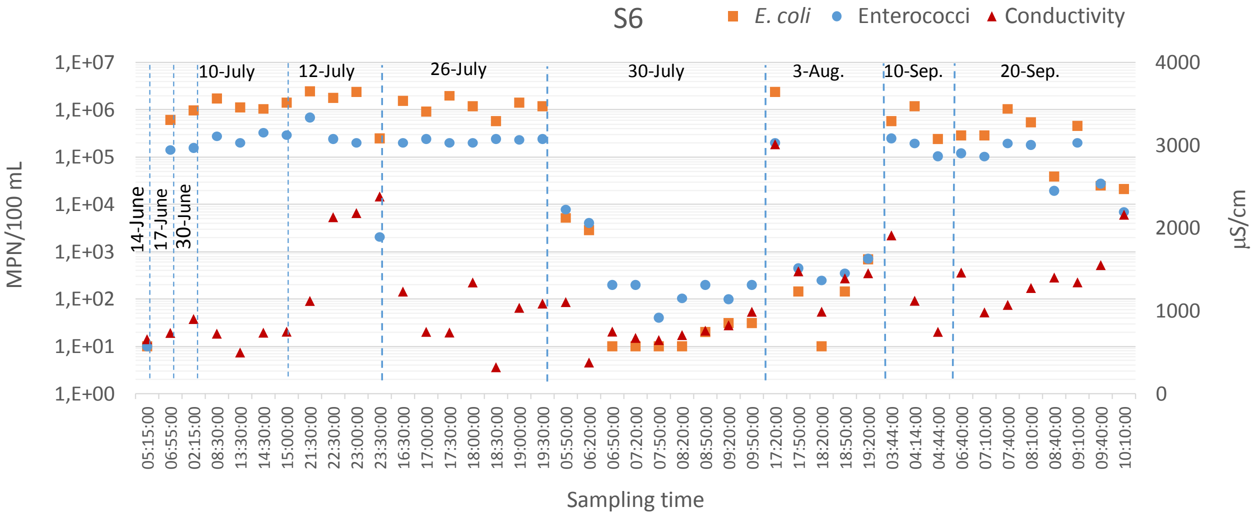

Figure 7

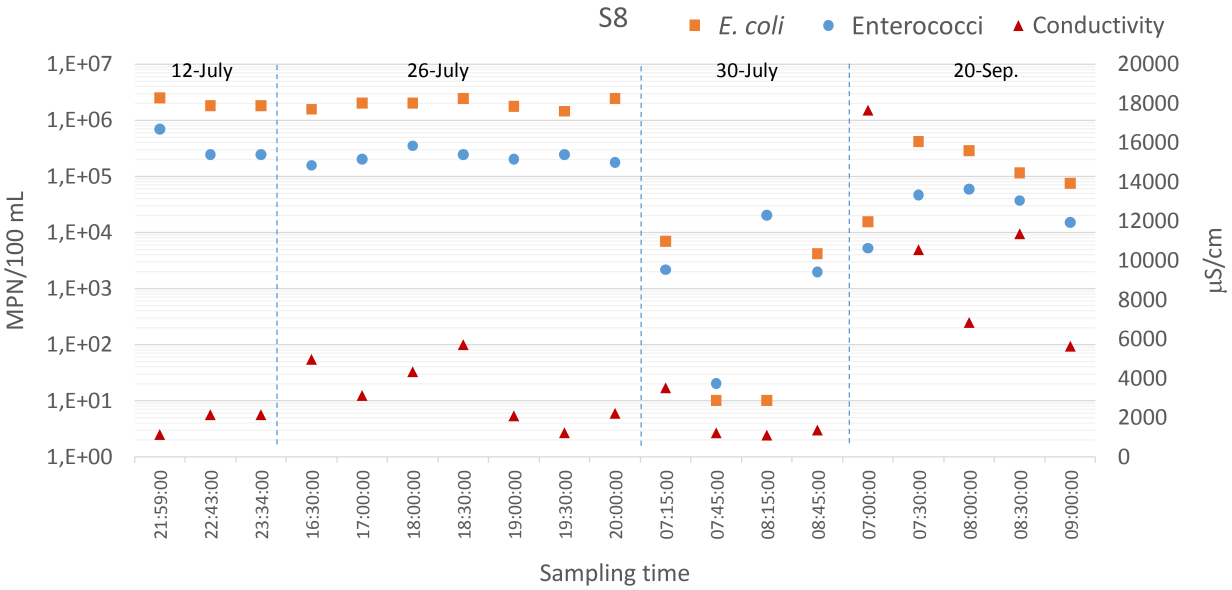

Figure 8

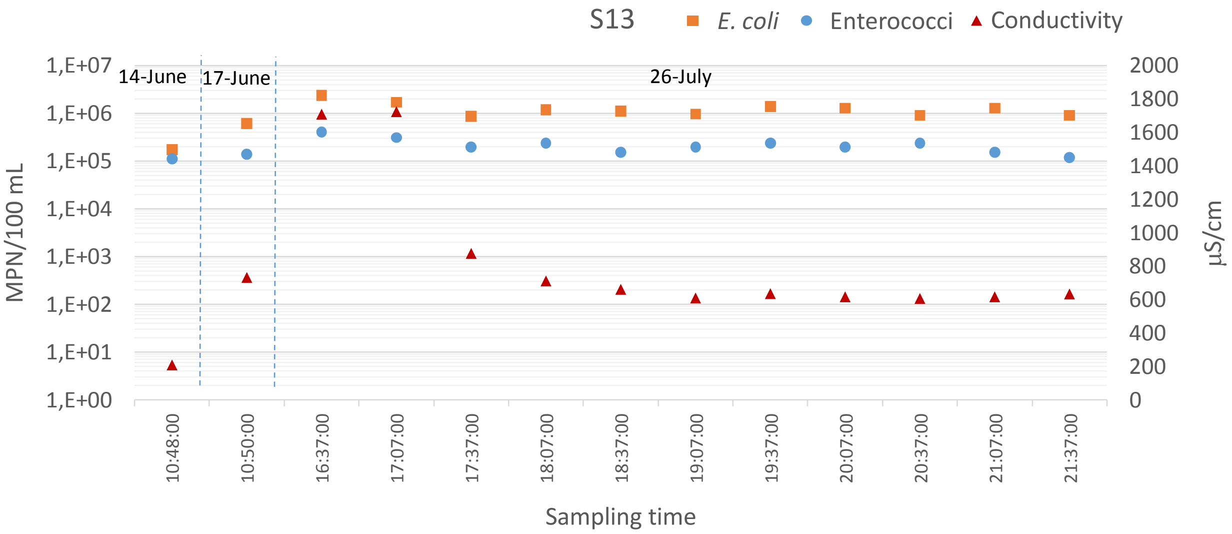

Figure 9

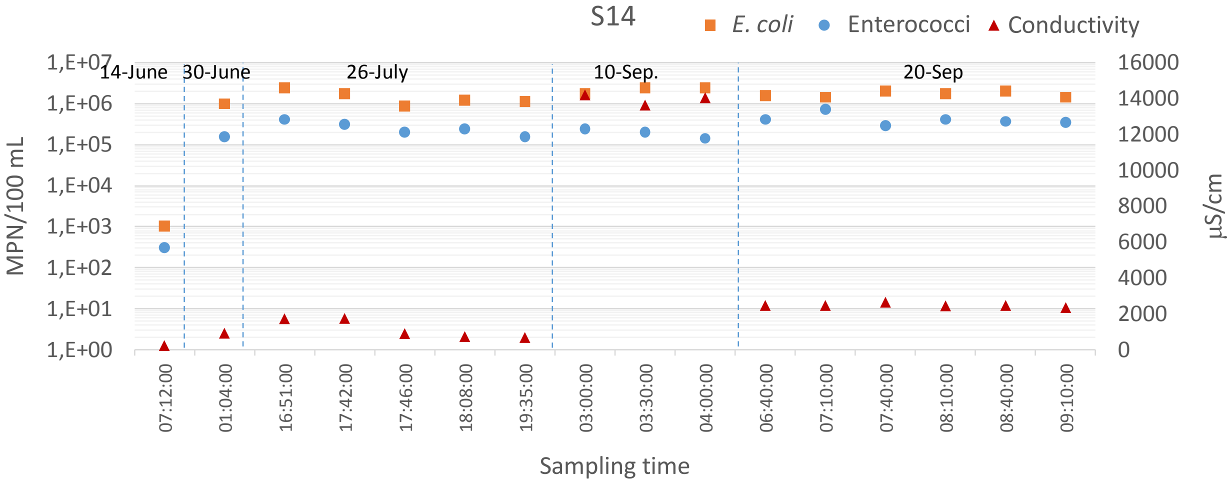
S14
E. coli
Enterococci
Conductivity
14-June
30-June
26-July
10-Sep.
20-Sep
1,E+07
1,E+06
1,E+05
1,E+04
1,E+03
1,E+02
1,E+01
1,E+00
MPN/100 mL
16000
14000
12000
10000
8000
6000
4000
2000
0
µS/cm
07:12:00
01:04:00
16:51:00
17:42:00
17:46:00
18:08:00
19:35:00
03:00:00
03:30:00
04:00:00
06:40:00
07:10:00
07:40:00
08:10:00
08:40:00
09:10:00
Sampling time

Figure 10

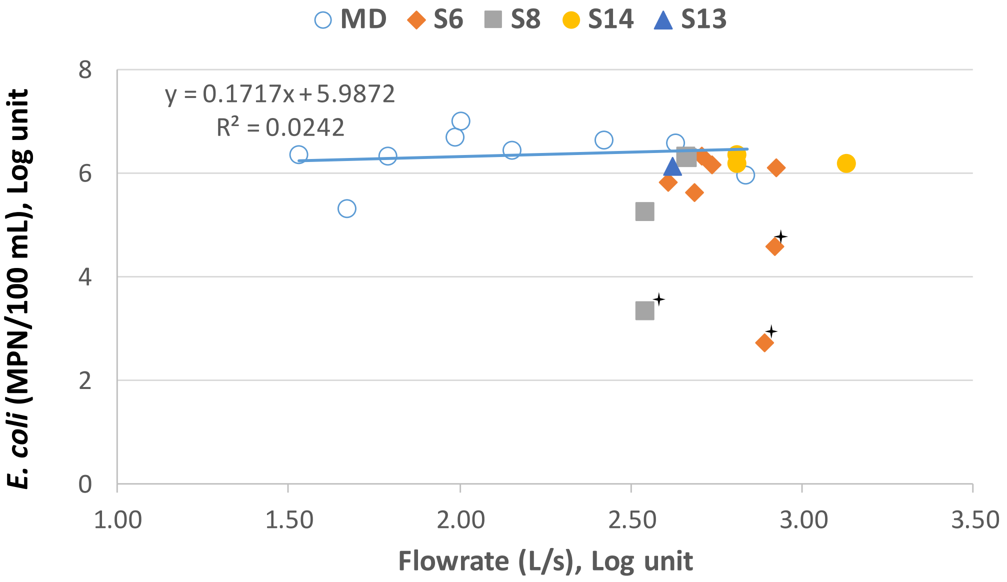
○ MD
◆ S6
■ S8
● S14
▲ S13
y = 0.1717x + 5.9872
R² = 0.0242
E. coli (MPN/100 mL), Log unit
0
2
4
6
8
1.00
1.50
2.00
2.50
3.00
3.50
Flowrate (L/s), Log unit

Figure 11.

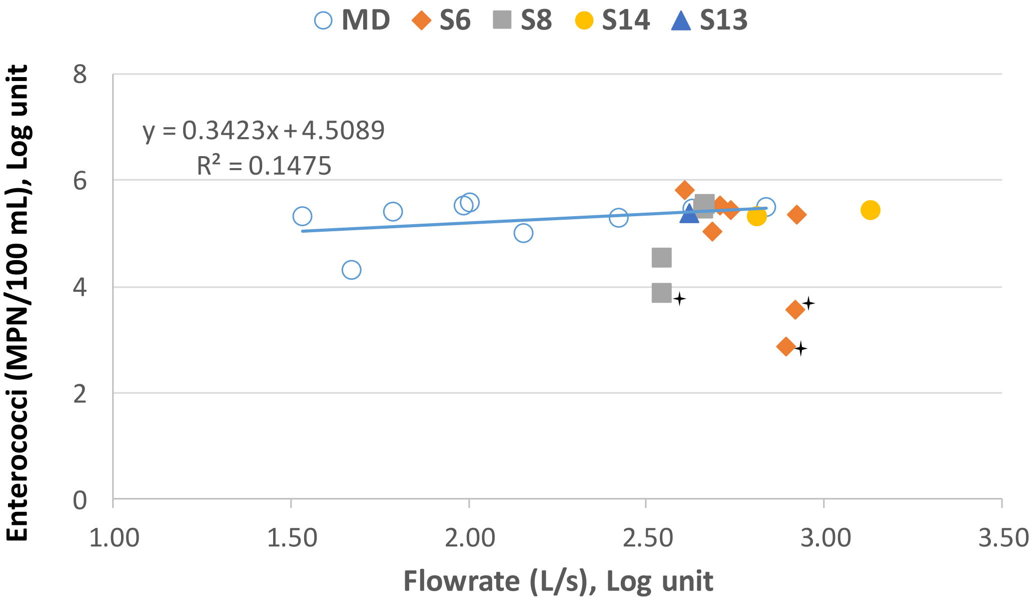
○ MD
◆ S6
■ S8
● S14
▲ S13
y = 0.3423x + 4.5089
R² = 0.1475
Enterococci (MPN/100 mL), Log unit
0
2
4
6
8
1.00
1.50
2.00
2.50
3.00
3.50
Flowrate (L/s), Log unit

Figure 12.

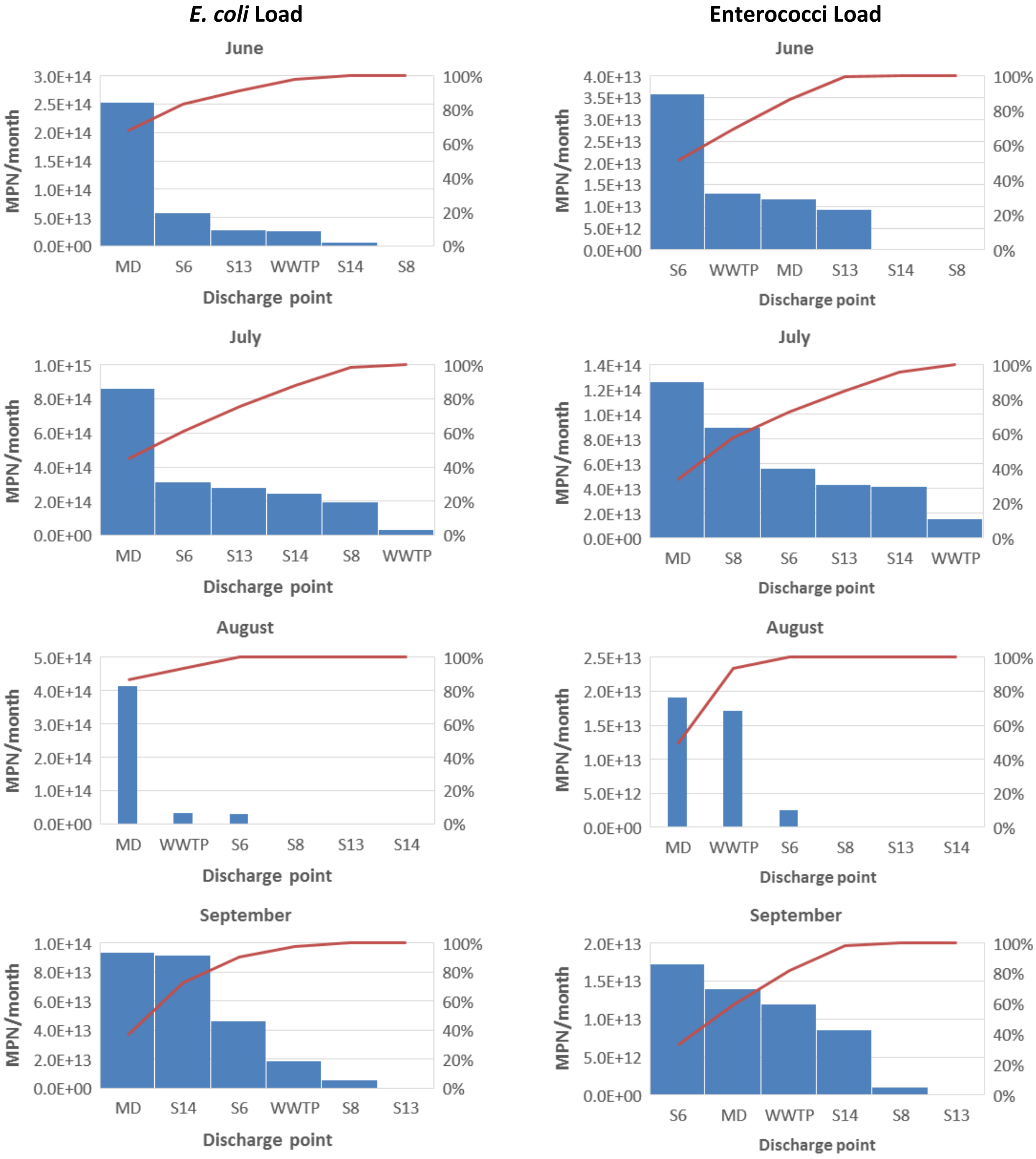
E. coli Load
Enterococci Load
June
MPN/month
3.0E+14
2.5E+14
2.0E+14
1.5E+14
1.0E+14
5.0E+13
0.0E+00
100%
80%
60%
40%
20%
0%
MD S6 S13 WWTP S14 S8
Discharge point
June
MPN/month
4.0E+13
3.5E+13
3.0E+13
2.5E+13
2.0E+13
1.5E+13
1.0E+13
5.0E+12
0.0E+00
S6 WWTP MD S13 S14 S8
Discharge point
July
1.0E+15
8.0E+14
6.0E+14
4.0E+14
2.0E+14
0.0E+00
MD S6 S13 S14 S8 WWTP
Discharge point
July
1.4E+14
1.2E+14
1.0E+14
8.0E+13
6.0E+13
4.0E+13
2.0E+13
0.0E+00
MD S8 S6 S13 S14 WWTP
Discharge point
August
5.0E+14
4.0E+14
3.0E+14
2.0E+14
1.0E+14
0.0E+00
MD WWTP S6 S8 S13 S14
Discharge point
August
2.5E+13
2.0E+13
1.5E+13
1.0E+13
5.0E+12
0.0E+00
MD WWTP S6 S8 S13 S14
Discharge point
September
1.0E+14
8.0E+13
6.0E+13
4.0E+13
2.0E+13
0.0E+00
MD S14 S6 WWTP S8 S13
Discharge point
September
2.0E+13
1.5E+13
1.0E+13
5.0E+12
0.0E+00
S6 MD WWTP S14 S8 S13
Discharge point

Figure 13.

Supplementary material for on-line publication only

The manuscript

Contributions of combined sewage overflows and treated effluents to the bacterial load released into a coastal area

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