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Multiannual Trend of Micro-Pollutants in Sediments and Benthic Community Response in a Mediterranean Lagoon (Sacca di Goro, Italy)

Valentina Pitacco [1], Michele Mistri [2,*], Carla Rita Ferrari [3], Adriano Sfriso [4], Andrea Augusto Sfriso [2] and Cristina Munari [2]

1 National Institute of Biology, Marine Biology Station, Fornače 41, 6330 Piran, Slovenia; v.pitacco@mbi.si
2 Department of Chemical and Pharmaceutical Sciences, University of Ferrara, Via Fossato di Mortara 17, 44121 Ferrara, Italy; sfrndr@unife.it (A.A.S.); mnc@unife.it (C.M.)
3 ARPAE, Struttura Oceanografica Daphne, Viale Vespucci 2, 47042 Cesenatico (FC), Italy; cferrari@arpae.it
4 Dept. of Environmental Sciences, Informatics and Statistics, University Ca' Foscari, Via Torino 155, 30127 Mestre, Venice, Italy; sfrisoad@unive.it
* Correspondence: msm@unife.it

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Abstract: Long-term variations of ecological status in a Mediterranean coastal lagoon (Sacca di Goro, Northern Adriatic) were investigated, combining data on the concentration of surface sediment contaminants and on the structure of the macrobenthic community. The aim was to assess any amount of chemical contamination and check the response of the macrobenthic community to sediment contamination. Over the studied period, the sediments of the lagoon showed contamination by trace metals and organochlorine pesticides, with most of them exceeding the thresholds indicated by the Italian legislation in many samples. Contamination by polychlorinated dibenzodioxins and dibenzofurans (PCDD/Fs) and polycyclic aromatic hydrocarbons (PAHs) instead never exceeded the threshold. The ecological status based on the macrobenthic community, evaluated through biotic indices (AMBI and M-AMBI), fell below the Good/Moderate threshold in most samples. The results indicate a possible influence of toxic compounds in sediment on benthic organisms, but most of the variability shown by the macrobenthic community is probably due to other factors. The difficulty in establishing a cause/effect relationship was due to the co-occurrence and variability of various stressors (both natural and anthropogenic) and their interactions. The methods currently used for monitoring transitional waters thus seem insufficient to disentangle the effect of pollutants and other environmental variables on the benthos. Integrated approaches (e.g., bioaccumulation and toxicity tests) are thus needed for a more precise identification of the risk posed by a high concentration of pollutants in such environments.

Keywords: coastal lagoon; macrobenthos; trace elements; organochlorine pesticides; PAHs; PCDD/Fs

1. Introduction

Contaminated sediments are a priority issue in aquatic systems impacted by anthropogenic use, industry, and urban development. Sediments act as a "sink" for contaminants, making the analytical determination of their concentrations easier with respect to the water column and providing time-integrated information about the ecosystem health. The evaluation of temporal changes in sediment contamination is crucial to answer questions about the state of the system, whether it is improving in time or to determine if remediation activities have benefited the system [1]. Unlike in the marine environment, where long-term studies are more frequent [2], coastal lagoon studies on the effect of contaminants on the biota are often a snapshot in time, with very few samples in a short study period.

In such studies, the influence of temporal variation cannot be evaluated. The knowledge on temporal variation in contaminant availability is desirable, as recommended by the Water Framework Directive [3], implemented in Italy through the National Act 260/10, which focuses mostly on trend analysis of time series data obtained in sediment monitoring programs.

Coastal lagoons are subjected to multiple stressors, from the high rate of natural dynamic changes (fluctuating temperature, salinity, and instability of sediment surface) to degradation due to human population growth and economic development [4]. Together with climatic changes, the major threats are related to the increase in pollutants produced by anthropogenic activities that influence both the geomorphology and geochemical backgrounds [5]. Coastal lagoons receive large amounts of pollutants derived from urban, agricultural, and industrial effluents, and can accumulate them, temporarily or permanently [6]. Interpreting the effects of anthropogenic contamination in coastal lagoons is often complex and confounding, since dynamic physical, chemical, and geologic conditions may interfere with the assessment of anthropogenic impacts on lagoon biotic integrity [7]. Macrobenthic communities play an important functional role in lagoons and other aquatic ecosystems. They alter geochemical conditions at the sediment–water interface, promote decomposition and nutrient cycling, and transfer energy to other food web components. Community composition and structure are influenced by an array of anthropogenic and non-anthropogenic stressors, and direct causal relationships are not evident [7]. The evaluation of the relative influence and interaction of different factors, including the effects of sediment-associated contaminants, poses a major challenge. The analysis of long-term data series could be a powerful tool to analyze those dynamics, and to assess the ecological risk posed by sediment pollutants in transitional waters.

The Sacca di Goro, a large Mediterranean lagoon, provides a good case study for examining spatial/temporal trends of contaminated sediments. It is a natural heritage (wetland of international importance according to the Ramsar Convention, UNESCO heritage site, and the Site of Interest of the European Union) and is part of one of the largest Mediterranean deltaic systems (Po River Delta). This gives the study a supra-local perspective: the need to better understand field-observed contaminant effect on the biota in coastal lagoons is relevant for many places on the globe. Chemical contamination from industry, urban, and agricultural runoff of the Po Valley are known to affect coastal lagoon ecosystems at the Po Delta [8,9]. Many of those micro-pollutants are known to have adverse biological effects on benthic organisms [10,11]. In the present work, the ecological status (ES) of the lagoon was assessed through the combined analysis of chemical elements (the presence of anthropogenic pollutants in sediments) and biological elements (the structure and diversity of macrobenthic community as a proxy for healthy ecosystem functioning). To assess the level of chemical pollution, we targeted major anthropogenic micro-pollutants required by Italian legislation (National Act 260/10), such as: organochlorine pesticides (OCPs), trace elements (TEs), polychlorinated dibenzodioxins (PCDDs), dibenzofurans (PCDFs), and polycyclic aromatic hydrocarbons (PAHs). For the assessment of the integrity of the macrobenthic community, structural (richness and diversity) and biotic indices (AMBI and M-AMBI) were used (as required by Act 260/10). The aim of the present work was threefold: (i) to evaluate the level and temporal trend of sediment contamination of the lagoon and to compare contaminants' concentration with national and international thresholds; (ii) to test if the macrobenthic community showed a response to multiannual sediment contamination; and (iii) to combine the temporal trend of the ecological quality based on chemical and biological indices of the Sacca di Goro.

2. Materials and Methods

2.1. Study Area

The Sacca di Goro is a shallow coastal lagoon located in the southern part of the Po River Delta, with a surface area of 2600 ha and an average depth of about 1.2–1.5 m. Freshwater inputs come mainly from the Po di Volano river (about 3.5×10^8 m^3/y) and from canals with similar flows (2.0–5.5 $\times 10^7$ m^3/y), named Giralda, Romanina, and Canal Bianco, that flow directly into the western part of the lagoon, and from the Po di Goro deltaic branch, which is artificially regulated through a dam in its eastern part. Marine inflows, varying according to the tidal dynamics, come from two mouths connecting the lagoon to the Northern Adriatic Sea. Consequently, the area is characterized by large daily fluctuations, linked to the height of the tides, and seasonal fluctuations in the physicochemical parameters of the lagoon's water (temperatures: 2–33°C, salinity: 6–30 psu, and pH: 7–8.8; [12]. Most of the lagoon's floor consists of silty-clay sediments, carried to the sea by rivers, but there are also sandy areas, particularly near the lagoon mouths and behind the spit and the barrier island [12]. Sampling was overall performed from 2004 to 2010 at 4 sampling sites (Figure 1), representative of the different hydrological characteristics of the lagoon. Site GOR1 (44°49'39.21'' N, 12°16'54.68'' E) was located in the western part. It was influenced mainly by freshwater discharged from Po di Volano and Giralda and was therefore characterized by variable salinity. Sites GOR2 (44°49'07.19'' N, 12°19'11.93'' E) and GOR4 (44°49'47.11'' N, 12°18'18.30'' E) were located in the central area and were influenced more by tidal exchange, and showed higher salinity. Site GOR3 (44°48'43.67'' N, 12°20'20.09'' E) was located in the eastern part, at the edge of the Valle di Gorino, the shallower and most confined part of the lagoon.

Figure 1. Map of the studied area with sampling sites.

2.2. Sample Collection and Chemical Analysis Methods

One sediment sample (top 5 cm) was collected seasonally at each site by means of a box corer, distributed into separate pre-labeled, acid-washed, and solvent-rinsed glass jars, stored on board a ship in coolers filled with ice and then stored at −20 °C immediately upon return to the laboratory. All samples were analyzed for grain size and organic matter. Particle size distribution was determined by ASTM D 422–63 method. Organic matter was estimated by loss on ignition (LOI: 105 °C for 24 h followed by ashing at 550 °C for 4 h). Trace elements (Cr, Ni, Pb, Hg, Cd, and As) were analyzed by inductively coupled plasma mass spectrometry (ICP-MS) according to the EPA method 6020B [13]. Organochlorine pesticides (OCPs) were extracted and cleaned using a QuEChERS method [14], followed by gas chromatography mass spectrometry (GC-MS) analysis. This QuEChERS extraction method involves mixing the sample with acetonitrile and permits the salt out liquid–liquid partitioning step using anhydrous $MgSO_4$ and sodium acetate. After shaking and centrifugation, cleanup is done by dispersive solid phase extraction (d-SPE). Sulfur cleanup followed the EPA method 3660B (USEPA, 1996). Analyses of PCDD/Fs were performed following the EPA method 1613B and quantification through high-resolution gas chromatography high-resolution mass spectrometer (HRGC-HRMS). Briefly, sediment samples were extracted in a Soxhlet apparatus using 300 mL of 10% acetone in toluene for 20 h. The extract was divided into portions prior to the purification for PCDD/Fs, dioxin-like PCBs, and PBDEs. The extracts for PCDD/F, PCBs, and PBDEs analysis were cleaned by passing through a multi-layer silica gel column. Analytical accuracy was achieved by the use of blanks and certified reference material for sediment included in all series of analysis. The analyses of polycyclic aromatic hydrocarbons (PAHs) was performed following EPA methods 8270D [15], and quantification was performed through high-resolution gas chromatography high-resolution mass spectrometer (HRGC-HRMS).

Three replicates were collected seasonally for macrofaunal community analysis using a 4.0 L Van Veen grab at three sampling sites (GOR1, GOR3, and GOR4; the site GOR2 was not sampled for macrofauna). These samples were sieved at 0.5 mm and preserved in 8% formalin. Benthic organisms were sorted and identified to the species level.

2.3. Sediment and Benthic Data Analysis

In order to obtain a global picture and a temporal trend of the general status of the lagoon, sampling sites were used as replicates. Given the hydro-morphological differences within the lagoon, Trellis graphs, using Lattice package for R [16], were used as explorative analysis to check whether the trend of each variable was consistent among sampling sites. If the prerequisite of no differences among sampling sites was not met, the temporal trend was analysed separately for each site. To illustrate the main characteristics of sediments in Sacca di Goro, descriptive statistics were computed. In order to define the chemical status of the lagoon, chemical data were confronted to the existing national thresholds, when available, according to the Italian Law (National Act 260/10). This law fixes the threshold concentration of harmful elements and compounds to discriminate between Good and Moderate status, as requested by the Water Framework Directive (WFD). For trace metals, local background values should be taken into consideration, together with national thresholds, so values calculated from Holocene lagoon deposits sampled from several deep cores drilled in the Po river coastal plain [17,18] were considered as well, as suggested by the WFD. Moreover, in order to evaluate the potential risk for benthic organisms, chemical concentrations were compared with thresholds defined by Sediment quality guidelines [19], which define two thresholds for a chemical: Effects Range-Low (ERL) and Effects Range-Median (ERM). These values defined three ranges, rarely (below the ERL), occasionally (above the ERL, but below ERM), and frequently (above the ERM) associated with adverse effects on acquatic organisms. To test for differences in sediment characteristics and contaminant concentrations among years, a non-parametric Friedman test [20] was used. When significant differences were encountered, a post-hoc test according to Nemenyi [21] was also carried out. If a linear increase or decrease was observed, its significance was tested with Spearman rank correlation coefficient (r_s).

For benthic communities, richness (S), Shannon index (H), AMBI index [22], M-AMBI index [23], and their Ecological Quality Ratios (EQR) were calculated for each sample using the free software (http://www.azti.es, v.5.0). Then, their annual averages were calculated. AMBI and its multivariate version M-AMBI are probably the most widely used biotic indices all over the world [24]. The percentage of invertebrates belonging to the different ecological groups (EGs) according to AMBI library was also calculated for each sample and then annual averages were calculated. The reference values ($H_{log2} = 3.4$, $S = 28$ and AMBI = 2.14) and thresholds for ecological status (ES) classification were those reported by Italian legislation (National Act 260/10), for the typology "M-AT-2, oligo/meso/poly microtidal lagoon".

Differences in macrofaunal richness, diversity, AMBI and M-AMBI values, and EGs among the different years, were investigated through permutational multivariate analysis of variance

(PERMANOVA) [25], using a single factor design with 7 levels (corresponding to years of sampling) and the "unrestricted permutation of row data", as recommended in [25].

In order to explore the relations between contaminants and benthic indices, the sites were considered separately. The Spearman rank correlation coefficient (r_s) was used in order to check for correlations: (i) among different pollutants, (ii) among sediment texture and pollutants, and (iii) among biotic index and abiotic parameters (sediment texture and pollutants). Distance-based linear models (DISTLM) were used to test and quantify the variation in macrobenthic community explained by abiotic variables. DISTLM does a partitioning of variation in a data cloud described by a resemblance matrix, according to a multiple regression model [26]. First, the marginal test shows the amount of variation explained by each variable when taken alone, ignoring all other variables; then, with the sequential test, individual variables are selected with a forward procedure (R^2 selection criteria), and the proportion of explained variation attributed to each variable that is added to the model is a function of other variables already present in the model [25]. Chemical and sediment variables were analysed separately and selected in order to meet the assumption required by DISTLM analysis: the number of samples higher than the number of variables [25]. Abiotic variables were chosen in order to avoid multi-collinearity, keeping variables that showed a significant relation with biotic variables. Matrices with (i) sediment and (ii) chemical variables for year for site were used as predictor variables. As response variables, the following matrices were used: (i) 'taxa/abundance' matrix; (ii) 'richness' matrix; (iii) 'diversity' matrix; (iv) 'AMBI' matrix; (v) 'M-AMBI' matrix; and (vi) 'EGs' matrix. Bray Curtis similarity was used for 'taxa' and 'EGs' matrices, while Euclidean distance was used for 'richness', 'diversity', 'AMBI', and 'M-AMBI' matrices. All those calculations were performed with PRIMER v6 + PERMANOVA software package [25,27]. For a more detailed analysis of the relationships between chemical pollutants and macrobenthic community, factors showing a significant correlation according to the marginal test (DISTLM analysis) where analysed with the Spearman rank correlation coefficient (r_s) and plotted to check differences among sites. A p-value < 0.05 was chosen as a significant threshold. Those analyses were run using R version 2.4.0 [28].

3. Results

3.1. Sediment Characteristics

Sediments of Sacca di Goro lagoon at all four sampling sites were typically sandy mud (from 50% to 75% of silt). Not significant differences were observed among years in the percentage of OM, sand, silt, and clay in the sediment (Friedman test, $p < 0.05$) (Figure 2). Organic matter (OM) ranged between 37.6 ± 5.4 mg kg^{-1} in 2007 and 50.4 ± 8.6 mg kg^{-1} in 2009 (Figure 2).

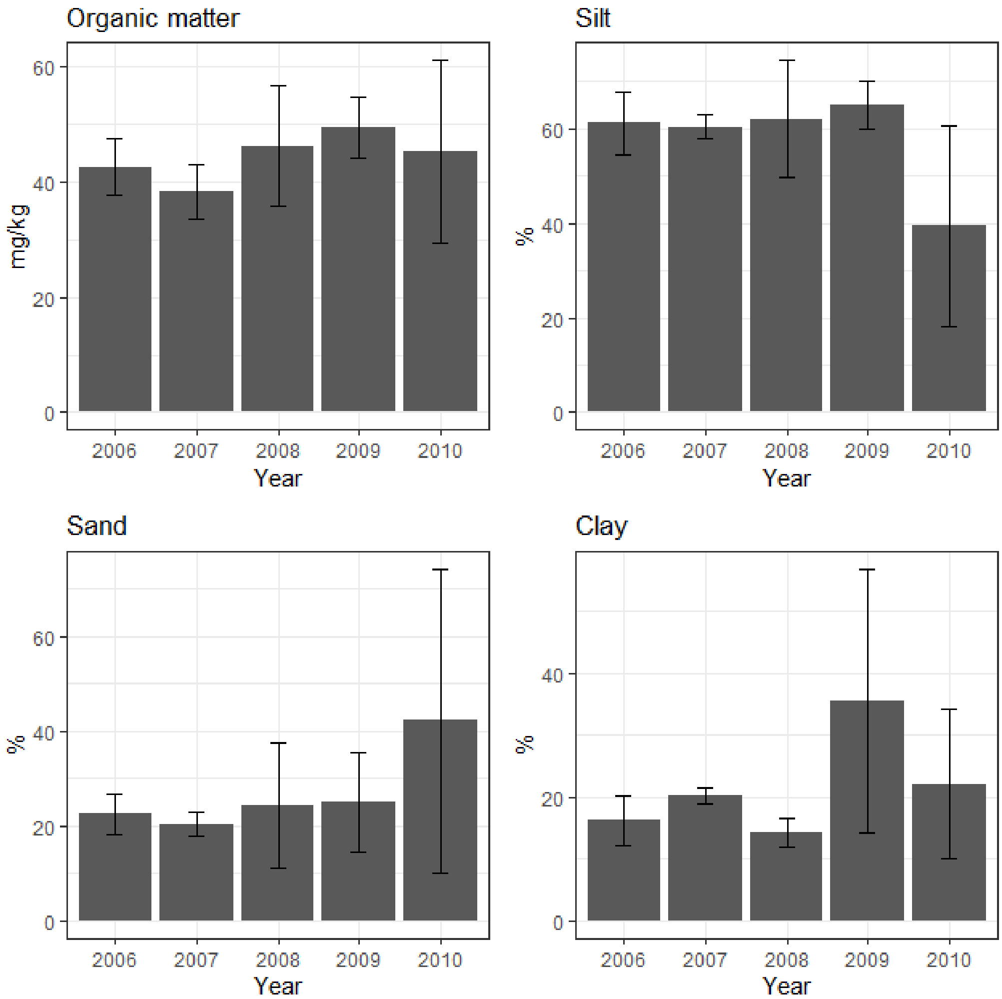

Figure 2. Sediment parameters of samples collected from 2006 to 2010 (n = 4). The average annual values (±SD) of sediment classification (sand > 50µm, silt from 50 to 2 µm, clay < 2µm). The values of organic matter are expressed in mg/kg, values of sand, silt, and clay are expressed in %.

3.2. Trace Elements

Sacca di Goro lagoon showed signs of contamination by TEs from 2004 to 2010 (Table 1), particularly by Pb and Ni (Table 1), with average concentrations exceeding both their EQS and their background value in most of the studied years (Table 1). Concentrations of Cr and Cd exceeded their EQS almost every year as well, but Cr concentration was always below its background level (Table 1). Average concentrations of Hg reached its EQS only once in 2008, but exceeded its background values in most samples, while mean annual concentrations of As were always below the EQS values (Table 1). Mean annual concentrations of Ni exceeded both the ERL and ERM threshold every year (Table 1). As and Cr exceeded their ERL but not their ERM thresholds (Table 1). The concentrations of Pb and Cd were always below their ERL threshold, and Hg exceeded its ERL only in 2006, 2008, and 2009. No linear trend was observed during the studied period (r_s, $p > 0.05$ for all TEs), but Ni, Cr, and Hg showed significant differences among the studied years (Friedman test, $p < 0.05$). The lowest values of Ni and Cr were observed in 2004 and 2005 and the highest values in 2007 (Figure 3). The highest value of Hg was observed in 2008, whereas in 2004 it was under the limit of detection. The concentration of As, Pb, and Cd did not differ among years (Friedman test, $p > 0.05$).

Table 1. Yearly average (±SD), maximum and minimum yearly concentrations of trace elements (TEs) from 2004 to 2010 in sediments of the Sacca di Goro (n = 4), threshold limits, and the number of years from 2004 to 2010 in which TEs' concentrations exceeded those limits expressed in percentage. EQS = limits for ecological quality status (Good/Moderate) according to the Water Framework Directive (WFD); Ref = background levels of Ni, Pb, Cr and Hg for Po River Delta [18]; ERM = Effects Range-Low values [19]; ERL = Effects Range-Low values [19].

	Ni (mg/kg)	Cr (mg/kg)	Hg (mg/kg)	Pb (mg/kg)	Cd (mg/kg)	As (mg/kg)
2004	95.5 ± 7.8	80.6 ± 8.9	0.0 ± 0.0	29.6 ± 7.2	0.4 ± 0.1	10.0 ± 3.3
2005	83.9 ± 2.4	77.4 ± 9.5	0.1 ± 0.2	28.8 ± 10.1	0.3 ± 0.2	9.3 ± 1.2
2006	110.3 ± 14.2	149.9 ± 17.7	0.2 ± 0.1	36.0 ± 14.6	0.4 ± 0.2	9.6 ± 3.5
2007	113.9 ± 7.2	157.5 ± 8.3	0.1 ± 0.1	43.5 ± 13.9	0.5 ± 0.2	9.7 ± 3.6
2008	93.4 ± 7.5	143.6 ± 13.1	0.3 ± 0.1	33.6 ± 8.4	0.4 ± 0.1	6.7 ± 1.5
2009	100.1 ± 16.0	129.6 ± 28.4	0.2 ± 0.1	32.0 ± 10.2	0.4 ± 0.1	8.3 ± 2.8
2010	106.7 ± 10.3	137.2 ± 14.6	0.1 ± 0.1	28.6 ± 13.9	0.4 ± 0.1	9.2 ± 3.5
Max	113.9 ± 7.2	157.5 ± 8.3	0.0 ± 0.0	43.5 ± 13.9	0.5 ± 0.2	10.0 ± 3.3
Min	83.9 ± 2.4	77.4 ± 9.6	0.3 ± 0.1	28.6 ± 13.9	0.3 ± 0.2	6.7 ± 1.5
EQS (mg/kg)	30	50	0.3	30	0.3	12
>EQS	100%	100%	14%	57%	100%	0%
Ref (mg/kg)	97	158	0.12	24	-	-
>Ref	71%	0%	43%	100%	-	-
ERL (mg/kg)	20.9	81	0.15	46.7	1.2	8.2
>ERL	1	0.71	0.43	0	0	0.86
ERM (mg/kg)	51.6	370	0.71	218	9.6	70
>ERM	100%	0%	0%	0%	0%	0%

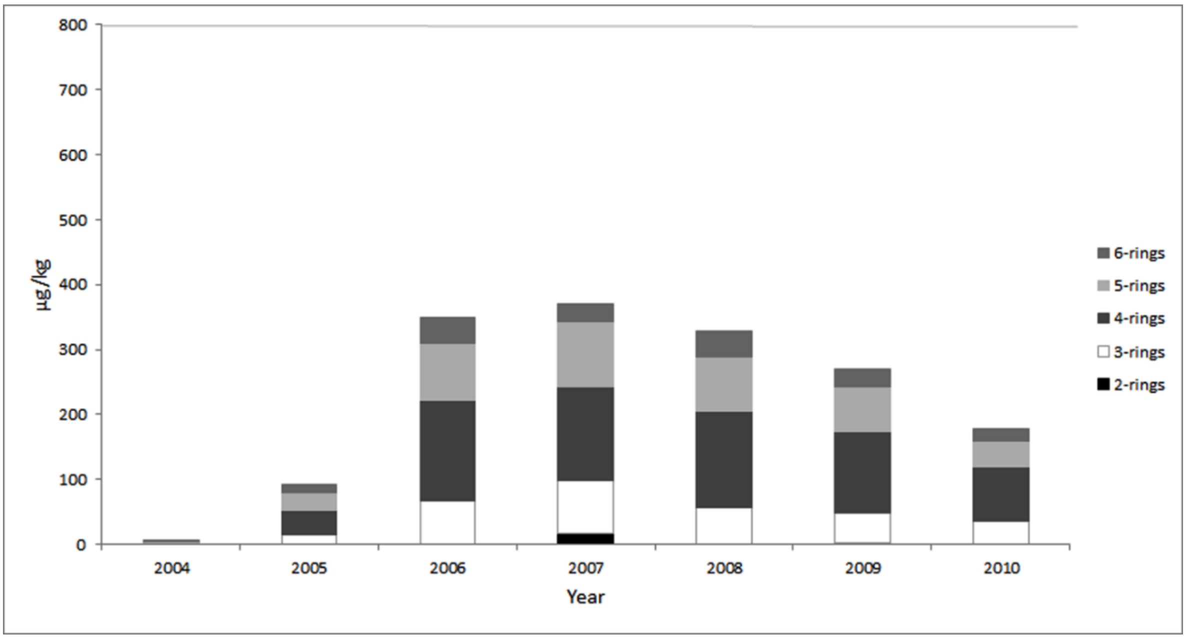

Figure 3. Annual average of polycyclic aromatic hydrocarbons (PAHs) (Σ16PAHs in μg/kg) for 2004 to 2010 (n = 4) and group profiles of LPAHs (two or three-fused rings) and of HPAHs (four to six-fused rings) in the sediments from Sacca di Goro. The EQS limit (Good/Moderate) for the sum of PAHs according to the WFD is indicated.

3.3. Organochlorine Pesticides (OCPs)

A moderate contamination by OCPs was observed, mainly related to dichlorodiphenyltrichloroethane (DDT) metabolites. Total concentrations of OCPs ranged from 0.09 ± 0.18 μg/kg in 2004 to 4.14 ± 2.44 μg/kg in 2007, showing two main peaks in 2007 and 2010 (ΣOCPs,

Table 2). DDT metabolites (DDE and DDD), were recorded almost every year (Table 2). DDE was the metabolite present with the highest concentrations almost every year and exceeded its national threshold in 2007 (1.91 ± 1.04 μg/kg) and 2010 (2.47 ± 1.34 μg/kg). The only exception was in 2008 when DDD concentration was higher (1.35 ± 2.20 μg/kg) and exceeded its threshold value. The concentration of DDE in 2010 and DDD in 2008 exceeded even the 20% of tolerance permitted by Italian legislation. Some samples exceeded the ERL value for DDE, particularly in 2010, but not the ERM value (Table 2). DDE also showed a slight increase ($r_s = 0.37$, $p < 0.05$) during the studied period. Conversely, concentrations of DDT were under the limit of detection every year, with the exception of 2007, when it was recorded with values not exceeding the national EQS. Other OCPs, namely Hexachlorobenzene, Simazine, Terbuthylazine, and Alaclor, were under the level of detection every year except in 2005 and 2007, with concentrations not exceeding their national EQS. Therefore, DDT metabolites were the only OCPs preventing the achievement of Good chemical status in 2007, 2008, and 2010.

Table 2. Mean (±SD) annual organochlorine pesticides (OCPs) concentrations (μg/kg) in sediments of Sacca di Goro (n = 4). The EQS according to the WFD and D.M. 260/2010.

	Hexachlorobenzene	**DDE**	**DDD**	**DDT**	**Simazine**	**Terbuthylazine**	**Alaclor**	**ΣOCPs**
2004	<0.1	0.06 ± 0.13	0.03 ± 0.05	<0.1	<0.1	<0.1	<0.1	0.09 ± 0.18
2005	0.15 ± 0.30	1.04 ± 1.29	0.40 ± 0.80	<0.1	<0.1	<0.1	<0.1	1.59 ± 1.88
2006	<0.1	1.41 ± 1.21	<0.1	<0.1	<0.1	<0.1	<0.1	1.41 ± 1.21
2007	0.09 ± 0.07	1.91 ± 1.04	0.28 ± 0.49	0.08 ± 0.23	0.15 ± 0.42	1.25 ± 1.67	0.38 ± 0.60	4.14 ± 2.44
2008	<0.1	1.09 ± 0.44	1.35 ± 2.20	<0.1	<0.1	<0.1	<0.1	2.44 ± 2.19
2009	<0.1	1.51 ± 0.75	0.35 ± 0.60	<0.1	<0.1	<0.1	<0.1	1.86 ± 1.29
2010	<0.1	2.47 ± 1.34	0.69 ± 0.88	<0.1	<0.1	<0.1	<0.1	3.16 ± 2.07
EQS	0.4	1.8	0.8	1				
ERL		2.2	1.58					
ERM		27	46.1					

3.4. Polychlorinated Dibenzodioxins (PCDDs) and Dibenzofurans (PCDFs)

PCDDs and PCDFs were measured from 2007 to 2010 (Table 3). Total PCDD/Fs ranged from 128.15 ng/kg in 2009 to 178.46 ng/kg in 2007. Concentrations of PCDD/Fs did not vary significantly during the period (Friedman testKW $p > 0.05$). Octachlorodibenzo-p-dioxin (O8CDD) was predominant every year, with concentrations between 88.4 ng/kg in 2009 and 123.9 ng/kg in 2007. The mean annual toxic equivalents (TEQs) for PCDD/Fs varied from 0.32 ng/kg in 2009 to 0.37 ng/kg in 2007, never exceeding the threshold (2 ng/kg) imposed by Italian legislation (Table 3).

Table 3. Mean (±SD) annual concentrations (ng/kg) PCCDs and dibenzofurans (PCDFs) (n = 4), and TEQs (toxic equivalents) calculated for total PCCD/Fs, based on Toxic Equivalency Factors (TEFs) according to the WHO (World Health Organization).

		H7CDD	O8CDD	H7CDF	O8CDF	ΣPCDD/Fs	ΣTEQ
2007	Mean ± SD	19.58 ± 22.60	123.91 ± 91.51	13.30 ± 7.53	21.67 ± 16.71	178.46	0.47
	TEQ	0.20	0.12	0.13	0.02		
2008	Mean ± SD	18.83 ± 16.89	89.14 ± 82.67	6.66 ± 4.78	22.14 ± 22.92	136.76	0.37
	TEQ	0.19	0.09	0.07	0.02		
2009	Mean ± SD	12.74 ± 9.41	88.40 ± 58.45	8.59 ± 6.43	18.41 ± 13.68	128.15	0.32
	TEQ	0.13	0.09	0.09	0.02		
2010	Mean ± SD	17.23 ± 11.10	94.18 ± 72.88	8.58 ± 4.79	21.15 ± 10.62	141.13	0.37
	TEQ	0.17	0.09	0.09	0.02		

3.5. Polycyclic Aromatic Hydrocarbons (PAHs)

Contamination by PAHs was observed every year (Figure 3). PAHs' concentrations varied during the period (Friedman test $p < 0.05$). The lowest values of the sum of PAH (Σ16PAHs) were observed in 2004 (4.05 ± 5.7 μg/kg) and 2005 (4.05 ± 52.6 μg/kg). PAH concentrations markedly increased in 2006 (349.92 ± 144.4 μg/kg), reaching a peak (370.8 ± 151.1 μg/kg) in 2007 (Figure 3). The sum of PAHs never exceeded the threshold imposed by Italian legislation (Figure 3) for a Good chemical status, and was markedly below the threshold established by the Sediment Quality Guidelines (ERL = 4022 ng/g; ERM = 44,792 ng/g). Four-ring PAHs were the most abundant every year (38.9%–54.3% of total PAHs), followed by five-ring PAHs (9.8%–29.7%) and three-rings PAHs (15.7%–23.6%). Four-ring PAHs were mainly represented by pyrene (Py) and benzofluoranthene (BFl), five-rings were mainly benzopyrene (BaP) and indenopyrene (InPy), and three-ring PAHs were mainly fluoranthene (Fl) and phenanthrene (Phe). Two-ring PAHs (0.2%–9.9%) and six-ring PAHs (2.5%–13.5%) accounted for small percentages of the total PAHs. Concentrations of high molecular weight (HMW) PAHs (4- to 6-ring), clearly predominated along the studied period (from 66.5% to 83% of total PAHs) compared to those of low molecular weight (LMW) PAHs (2- to 3-ring). The LMW/HMW ratio ranged from 0.2 to 0.5, the Phe/Ant ratio was low (always <30), and the Flu/Py ratio was high (always >0.8, except in 2005 and 2009).

3.6. Ecological Status Based on Macrobenthic Community

The macrobenthic community was dominated by tolerant species (EGIII) along the entire studied period (Figure 4), with percentages ranging from 61.6% in 2008 to 73.4% in 2006. Among them, the most abundant species was the polychaete *Streblospio shrubsolii* (32.6%–75.2%). The percentages of opportunistic species were more variable but generally quite high. Second order opportunistic species (EGIV) ranged from 3.9% in 2006 to 29.3% in 2008, and were mainly represented by the polychaete *Polydora ciliata* (2.4%–32.4%). First order opportunistic species (EGV) ranged from 0.8% in 2006 to 23.4% in 2004 and were almost entirely represented by the polychaete *Capitella capitata* (0.04%–6.6%) and by oligochaetes (0%–17.2%). Contrastingly, the percentages of sensitive species (EGI) were generally low, ranging from 1.1% in 2008 to 10.9% in 2010, and they were mainly represented by amphipods, such as *Ampelisca sarsi* (0%–1.5%), *Gammarus aequicauda* (0%–4%), and *Melita palmata* (0.1%–2.3%). The percentages of indifferent species (EGII) were generally even lower (0.5%–4.1%), with the only exception of the high percentage observed in 2006 (18.3%), mainly due to the polychaete *Salvatoria clavata* (13.6%).

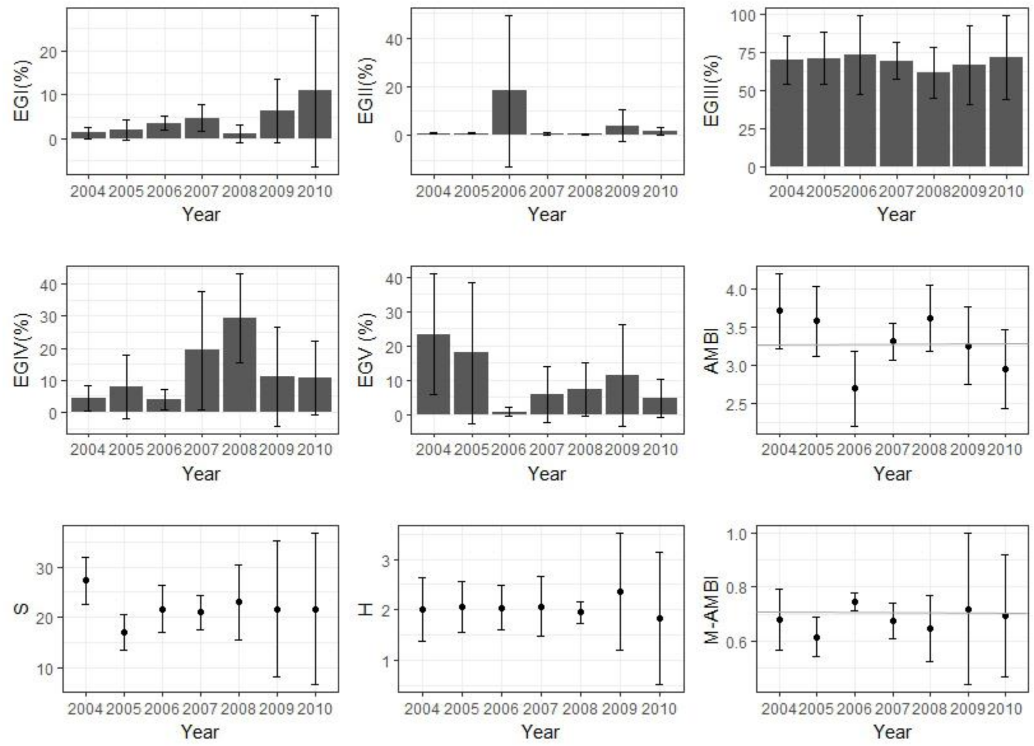

Figure 4. Annual mean (±SD) of percentage of Ecological Groups (sensitive species = EGI, indifferent = EGII, tolerant = EGIII, second order opportunistic = EGIV, first order opportunistic = EGV), structural indices (richness = S, Shannon index = H) and ecological indices (AMBI and M-AMBI) for macrobenthic community in Sacca di Goro from 2004 to 2010 (n = 3). Grey line = Good/Moderate threshold.

According to the AMBI index, ecological status was Good/High only in 2006, 2009 and 2010 (AMBI < 3.3), while M-AMBI index exceeded the threshold for Good Ecological status (M-AMBI > 0.71) only in 2006 and 2009. Taxa/abundance matrix varied significantly among years (PERMANOVA, $p > 0.05$). The percentage of each ecological group (EG), richness, diversity, AMBI and M-AMBI average values did not showed significant differences among years (PERMANOVA, $p > 0.05$). The percentage of sensitive species (EGI), S, H and M-AMBI showed an increase of within-year variation along the studied period (increasing SD, Figure 4), because the temporal trend of biotic indices varied among sites, as well. Oscillations between Good and Moderate ecological statuses were observed at each site, for both the AMBI and M-AMBI index. The temporal trend deviated more or less markedly from linearity in all cases and differed among sites. The M-AMBI index showed a general improving trend of ecological condition with increasing M-AMBI values at sites GOR3 and GOR4 but a general worsening trend, with decreasing M-AMBI values, at site GOR1. The AMBI index showed a similar trend: a general increasing in AMBI values (corresponding to worsening ecological conditions) at GOR1, and a decreasing at GOR3 (corresponding to improving ecological conditions), whereas, at site GOR4, values did not vary so much around the Good/Moderate threshold. S showed an increasing trend at site GOR4, but a decreasing one at sites GOR1 and GOR3. H values were generally stable at site GOR3, while a marked non-linear trend was observed at sites GOR1, with the highest values in 2007, and at GOR4, with the highest value in 2009.

3.7. Combined Trend of Abiotic and Biotic Variables

Collinearity was observed between some pollutants: Ni was positively correlated with Cr and As and negatively correlated with Hg (Table 4). A positive correlation was observed also between Cr and Pb, between PCDFs and PCDDs (Table 4). Few pollutants showed a correlation with sediment texture (Table 4). Organic matter (OM) showed a significant negative relationship with concentrations of Ni and Cr, and silt was negatively correlated with DDE (Table 4).

Table 4. Collinearity (r_s) among abiotic and biotic variables (n = 3). Only variables showing significant correlations were displayed. *NS* = $p > 0.05$.

	Ni	Cr	As	Pb	PCDFs	OM	DDE	Clay	M-AMBI	AMBI	H
Ni											
Cr	0.56										
As	0.69	*NS*									
Hg	−0.54	*NS*	*NS*								
Pb	*NS*	0.62	*NS*								
PCDFs	*NS*	*NS*	*NS*	0.7							
PCDDs	*NS*	*NS*	*NS*	0.9	0.89						
OM	−0.61	−0.58	*NS*	*NS*	*NS*						
DDE	0.69	NS	0.89	*NS*	*NS*	−0.54					
Sand	*NS*	*NS*	*NS*	*NS*	*NS*	0.55	*NS*				
Silt	0.55	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*				
AMBI	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	−0.69	−0.59		
H	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	*NS*	0.89	−0.64	
S	*NS*	*NS*	−0.67	*NS*	*NS*	*NS*	−0.62	*NS*	0.71	*NS*	*NS*

Collinearity was observed also among biotic indices, with a positive correlation between M-AMBI and H, M-AMBI and S, and a negative correlation between M-AMBI and AMBI, and between AMBI and H (Table 4). Among the biotic indices, only AMBI values were significantly related with sediment texture, and only with clay (Table 4). Among the pollutants, only As and DDE were negatively related with species richness (S) (Table 4). DISTLM analysis showed no correlation of macrobenthic community ('taxa/abundance' matrix) and biotic indices with sediment variables, i.e. sand, silt, clay and OM (DISTLM, $p > 0.05$, marginal test). Ten variables (PCDD/Fs, PAH tot, DDE, DDD, Ni, Hg, Pb, Cd, Cr, As) were chosen among chemical pollutants, to meet the assumptions required by DISTLIM analysis. Those chemical variables did not show significant relationships with macrobenthic community in terms of the 'taxa/abundance' matrix (DISTLM, $p > 0.05$, marginal test). Considering biotic indices, species richness (S) was the only one showing a correlation with pollutants, namely As and DDE (DISTLM, $p < 0.05$, marginal test). The highest percentage of variability within the S index was explained by As alone (52% of variability, sequential test). Site GOR1 was the most contaminated, with concentrations of both As and DDE exceeding the threshold for good chemical status in most samples, and with the lowest values of S (Figure 5A). Conversely, in most samples at sites GOR3 and GOR4, concentrations of As and DDE did not exceed their threshold and S values were higher (Figure 5B).

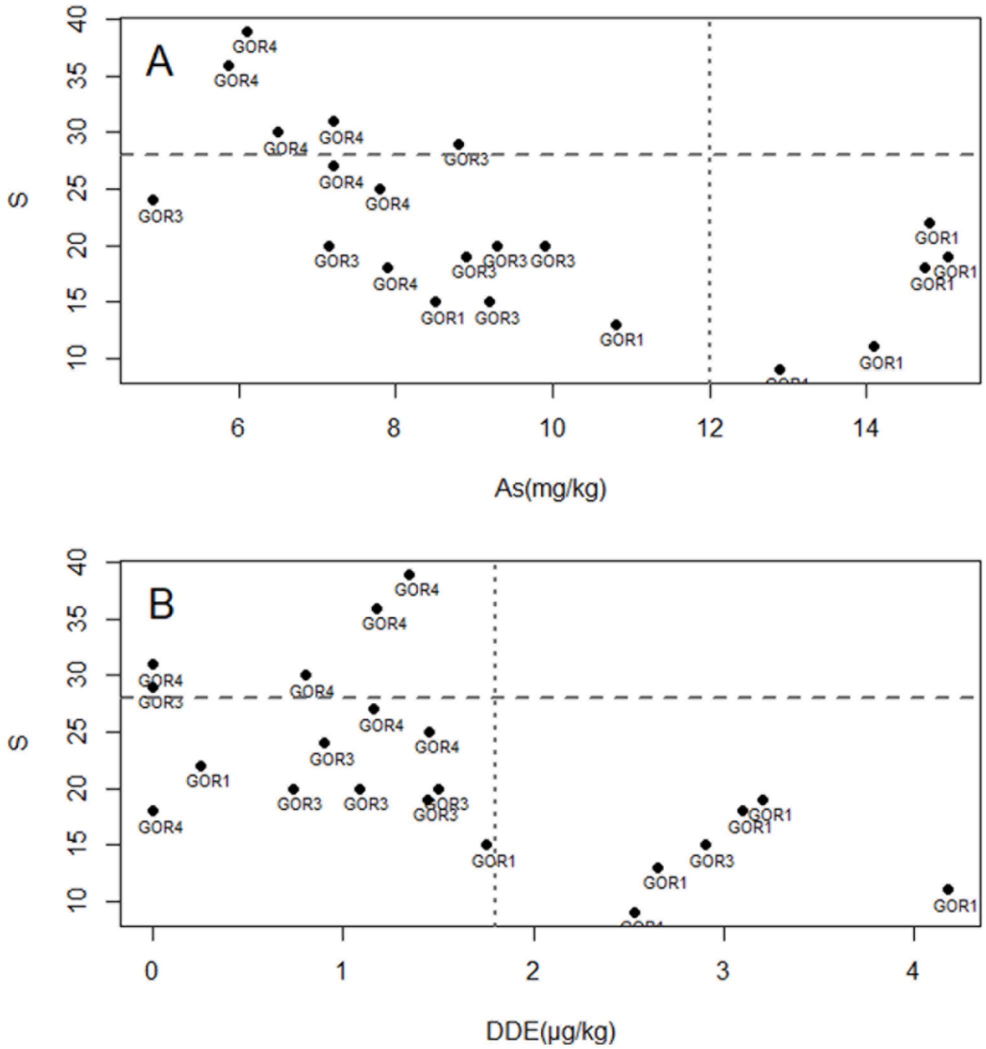

Figure 5. Values of species richness (S) against the concentration of As (**A**) and DDE (**B**), with threshold values for S (high/good, horizontal dashed line), As, and DDE (good/moderate, vertical dotted line), defined by National Law (n = 3).

4. Discussion

4.1. Trace Elements Contamination

Evidences of contamination by TEs were observed in sediments of Sacca di Goro from 2004 to 2010. Concentrations of most TEs exceeded their national EQS in many samples, with the exception of Hg and As, showing annual means always below their EQS values. According to the WFD and Italian Legislation, for a correct estimation of the chemical status, the local background levels of trace metals of the area should be considered as well. Cr and Ni, even exceeding their national EQS values in most samples, were below background levels [17,18] in most samples. Similar situations with high values of Cr and Ni, were reported also from other lagoons of the Po River Delta: Pialassa Baiona (Ni: 35–57 mg/kg; Cr: 53–142 mg/kg; [18] and Comacchio lagoon (Ni: 59–75.4 mg/kg; Cr: 72.5–97.5 mg/kg [28]. Sediments of the Po River Delta are known to be enriched in Cr and Ni, due to geological reasons [9,17], and such concentrations are attributed to sediment provenance from ultramorfic source rocks of the Po catchment basin [29]. Therefore, such an enrichment of Cr and Ni in Sacca di Goro lagoon was likely of natural origin, as already pointed out by previous works [5]. Conversely, the high levels of Pb recorded in Sacca di Goro sediments exceeded both its national EQS and its background level, suggesting an anthropogenic enrichment. Concentrations of Pb were higher than those reported for other areas of the Po River Delta, such as Comacchio lagoon (21–28 mg/kg, [30], Pialassa Baiona lagoon (17 ± 9.1 mg/kg, [18], and Pialassa dei Piomboni lagoon (mean 18 mg/kg [17]). High levels of Pb in Sacca di Goro, were reported also by [5]. Concentrations of Hg also suggest an anthropogenic enrichment, even if this element did not exceeded threshold for Good chemical status. In Sacca di Goro lagoon, the mean annual concentrations of Pb, As, and Cr exceeded the ERL but not the ERM threshold defined by the Sediment quality guidelines, indicating a "Possible-effects" within which effects on benthic organisms would occasionally occur [19]. Cd and Hg concentrations were mostly below their ERL threshold, so an effect on benthic organisms was unlikely, whereas Ni exceeded its ERM threshold every year, indicating potentially adverse biological effects.

4.2. Organochlorine Pesticides Contamination

We found the presence of OCP in the Sacca di Goro: DDE was the dominant metabolite, persistent with increasing concentration along the entire studied period. Its mean concentration was comparable with values reported for other Mediterranean lagoons, such as Comacchio lagoon (0.68 ± 0.42 SD, [30], and Berre lagoon (DDE: 1.7 μg/kg; [31], but it exceeded its EQS in some samples. The watershed of Sacca di Goro is predominantly agricultural, with only a few main crop typologies: corn and wheat, rice, sugar beets, soybean, and vegetables [8]. The main freshwater input and therefore the major input of pollutants of agricultural origin in the lagoon comes from Po di Volano canal. The use of dichlorodiphenyltrichloroethane (DDT) in agriculture was banned by the European Union in the 1970s but is still very common in many ecosystems because of its high persistence [32]. Both DDE and DDD existed as by-products in commercial DDT formulations, and both may be formed by environmental degradation of DDT [33]. The dominance of DDE, with low concentrations of DDT, in Sacca di Goro indicates that the contamination was likely not recent. One among the probable causes for the persistence of those contaminants is the storage in the watershed soils and/or aquatic sediments and delayed delivery [8]. DDT compounds are of particular concerned, since their toxicity for benthic organisms has been proved by several studies (e.g., for amphipods) [34,35]. According to the Sediment quality guidelines [31], some samples in Sacca di Goro exceeded the ERL value for DDE, even if not the ERM value; therefore, an effect on benthic organisms was probable.

4.3. Polychlorinated Dibenzodioxins and Dibenzofurans Contamination

Concentrations of PCDDs and PCDFs in Sacca di Goro were comparable with those recorded for other Adriatic lagoons, such as Pialassa Baiona (PCDDs: 96–19,641 ng/kg and PCDFs: 16–1,313 ng/kg) [18], Venice lagoon (PCDDs: 16–13,642 ng/kg and PCDFs: 49–126,561 ng/kg) [36], Comacchio lagoon (PCDD/Fs: 15.31 ng/kg–52.76 ng/kg) [30], and other locations, such as the Spanish northern Atlantic coast (PCDD/Fs: 0.15–3.99 ng/kg) [37], and the intertidal zone of the North Sea (PCDD/Fs: 0.124–3.156 ng/kg) [38]. Differences in concentration could be explained with the different distances from industrial and/or thermal power plants. In fact, PCDD/Fs do not occur naturally, but they are formed as by-products during the production of other chemicals or during combustion and incineration processes [39]. They are highly toxic, and very persistent, so they are now globally distributed. These compounds have low solubility and are highly hydrophobic; thus, they tend to accumulate in organisms and to adsorb to particles [39]. In Sacca di Goro lagoon, the mean annual TEQs for PCDD/Fs was always well below the threshold imposed by Italian legislation and therefore no adverse effects on benthic organisms were expected.

4.4. Polycyclic Aromatic Hydrocarbons Contamination

Sediments of Sacca di Goro were contaminated by PAHs along the studied period, but total PAHs concentrations (range Σ16PAHs: 4.05–370.8 μg/kg) never exceeded the threshold for a Good chemical status. Total concentration of PAHs was higher than those reported from other Adriatic lagoons, such as Lesina (8.51–70.41 ng/g) and Varano (6.61–55.06 ng/g) lagoons [40] but lower than Comacchio lagoon (9.1–2100.2 μg/kg) [30], Pialassa Baiona lagoon (2500–120000 ng/g) [41], Grado and Marano lagoon (<limit of detection-1056 mg/kg) [42], and other Mediterranean lagoons, such as Faro and Ganzirri lake (74–5755 ng/g) [43] and Stagnone lagoon (72–18381 ng/g) [44]. PAHs are an

important class of persistent organic pollutants, introduced into the environment mainly via anthropogenic input [45]. Their potential sources could be estimated analysing the relative concentrations of PAHs by aromatic groups (two-ring, three-ring, four-ring, five-ring, and six-ring PAHs). The dominance of High Molecular Weight PAHs (=low LMW/HMW ratio), together with the low Phe/Ant ratio and high Flu/Py ratio observed suggested that the main sources of PAHs in Sacca di Goro were combustion processes. The predominant pyrolitic origin of PAHs in sediments has also been commonly observed in other Mediterranean lagoons [6,42]. PAHs could result from a large number of possible sources, such as industrial wastewater, sewage, road runoff/street dust, and petroleum-related activities [6], but a more accurate identification of the origins of PAH is beyond the scope of this work. Some PAHs and their metabolites are recognized to be carcinogenic; therefore, different tools have been proposed in order to predict their potential toxicity. Total PAHs' concentrations in Sacca di Goro were markedly below thresholds established by the Sediment Quality Guidelines [19] and therefore no adverse effects on benthic organisms were expected.

4.5. Ecological Status Based on Macrobenthic Community

The macrobenthic community in Sacca di Goro was dominated by tolerant species, and values of M-AMBI and AMBI indices resulted below the threshold for Good ecological status in most samples. Low diversity and high abundances of tolerant species is a common condition of transitional waters, which are known to be harsh environments because of shallow waters, limited water renewal and marked seasonal variations of oxygen, temperature, and salinity. Those naturally stressed conditions coincide with anthropogenic disturbance according to the models based on Pearson–Rosenberg paradigm [46], potentially reducing the robustness of the AMBI index in transitional ecosystems [47,48]. Annual averages of EGs, richness, and diversity did not show a general clear pattern along the studied period, because of the marked differences among sampling sites. Those differences lead to the increase in within-year variation along the studied period (SD). The general trend of most biotic indices differed among sampling sites, suggesting a general tendency towards an improvement of ecological conditions at sites GOR3 and GOR4 (increasing M-AMBI), and a tendency towards a deterioration at site GOR1 (decreasing M-AMBI). At site GOR4, the improvement of ecological conditions was mainly related to an increase in richness and diversity, whereas, at site GOR3, it was mainly related to a decrease of AMBI index and therefore to a proportional decrease in tolerant and opportunistic species. At GOR1, the worsening of ecological condition was related to the decrease in richness and abundance and an increase in AMBI values. Those differences represent a potential impediment for a correct evaluation of the ES, since the positive trend of two sites could mask the negative trend in one site. An incorrect estimation of the temporal trend of ecological status could limit the possibility of identifying drivers of changes and consequently plan appropriate restoration measures.

4.6. Coupling Abiotic and Biotic Variables

The fluctuations observed in the present work in terms of both pollutant concentrations and biotic indices reflected the marked fluctuations of environmental parameters characterizing those ecosystems. In this framework, the relationship between biotic and physico-chemical variables is always complex, and disentangling the effect of natural and anthropogenic disturbances is a major challenge [49,50].

It is well known that salinity and confinement (defined as the degree of connection to the sea) [51] are the main structuring factors of the biotic lagoon communities; however, this study suggests that other factors, such as some contaminants, may also play a role. Macrobenthic community responded, in terms of species richness, to high levels of two micro-pollutants: As and DDE. The highest concentration of As and DDE was observed at the site strongly influenced by the watershed through the Po di Volano and minor canals. This influence of watershed and irrigation canals was reported also by [8]: peaks of pesticides were observed in areas of the lagoon coinciding with freshwater plumes, whereas water exchange with the adjacent sea favour herbicide dilution and export, keeping their concentrations at lower levels in other parts of the lagoon. The problem is the difficulty of separating the effect of contaminants from other factors, especially salinity, which is typically lower close to river mouths. Salinity itself can directly influence species' richness: a decrease in richness with decreasing salinity has been reported as a common pattern from many transitional areas, even if the relationship is not necessarily strong and linear [51,52]. Moreover, salinity is also recognised as an important driving factor in metal toxicity, since free metal ion are more concentrated, with related bioavailability and toxicity, in conditions of low salinity [53]. Therefore, it is not possible to disentangle the effects of these two pollutants and salinity on the macrobenthic community, but it is likely that salinity and pollutants played a synergic role. Pollutants themselves can act synergistically when they co-occur. Organisms in the aquatic environment are commonly exposed to chemical mixtures, and the toxicity of a mixture is usually higher than each of the individual substances [54]. Toxicological investigations on the polychaete *Laeonereis acuta* showed that prolonged exposure to high levels of As resulted in a conspicuous reduction in the activity of an enzyme involved in the detoxification of several compounds, including organophosphorus pesticides [55]. The effects of contaminated freshwater released in wetlands was observed also in other lagoons, such as the Yellow River Delta [56], where heavy metal contamination of sediments and macrobenthic organisms, together with a dominance of pollution-resistant species were recorded in the affected area.

Interestingly, there was a lack of response of benthic organisms to other metals (namely Ni, Cr, Pb), present in even higher concentrations in relation to their EQS and their ERL/ERM thresholds [19]. The complexity of the response of the macrobenthic community to metal pollution reflects the fact that the measurement of contaminant levels alone does not directly indicate the bioavailability of pollutants to organisms [57,58]. The way in which high levels of pollutants in sediments affect the macrobenthic community, could vary in relation to abiotic processes, causing metal mobilizations, and to the physiological or genetic response of benthic organisms. Recent field and laboratory studies have documented the fact that environmental history can change the sensitivity of benthic organisms to stressful conditions [54,59]. The chronic exposure of animals to trace metals can considerably affect their physiological and biochemical responses, leading to increased tolerance to chronic contamination [60]. This adaptation ability was observed in different acquatic species, such as the bivalves *R. philippinarum* [61], and *Macoma balthica* [62], and the isopod *Platynympha longicaudata* [63].

Our results show that the methods currently used for monitoring transitional waters are insufficient to disentangle the effect of pollutants and other environmental variables, such as salinity or confinement, on benthic organisms and community structures. Integrated interdisciplinary approaches with new additional analyses, for instance, focusing on bioaccumulation and toxicity tests, are needed for a more precise identification of the risk posed by high concentrations of pollutants in transitional waters.

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