Relationships between plastic litter and chemical pollutants on benthic biodiversity☆

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ABSTRACT

Five Descriptors (D) of Marine Strategy Framework Directive (MSFD): marine litter (D10), non-indigenous species (D2) and organic and inorganic pollutants (D8), were estimated in a coastal area of GSA 16 (Augusta harbour, Central Mediterranean Sea) in order to study their effects on the biodiversity (D1) of the benthic community D6) and to improve data for the MSFD. Investigation of plastic debris had led to the identification of 38 fragments divided into four categories, among which microplastics resulted as the most abundant. Six non-indigenous species, belonging to Polychaeta (*Kirkegaardia dorsobranchialis*, *Notomastus aberans*, *Pista unibranchia*, *Pseudonereis anomala*, *Branchiomma bairdi*) and Mollusca (*Brachidontes pharaonis*) were found. Biodiversity and benthic indices suggested a generalised, slightly disturbed ecological status. Anthracene, Zinc and Chrome were the most abundant chemical compounds in analysed sediments. Significant correlations were found between the abundance of trace elements *vs* biotic indices and between plastic debris *vs* biodiversity and benthic indices. This study represents the first report about the abundance of plastic debris and its relationship to contaminants and infauna in Augusta harbour. Our results can provide useful information for national and international laws and directives.

1. Introduction

Human impacts can change the natural status of physical, chemical and biological components of marine ecosystems causing overexploitation, changes of environmental interactions and loss of habitat and biodiversity (Goulletquer et al., 2014; Reiss et al., 2014). Coastal areas are particularly subjected to human pressure since most of the anthropogenic activities (*i.e.*: harbour, refinery, shipyard) are located in this these zones (D'Alessandro et al., 2016; Romeo et al., 2015). In this view, a regular monitoring of sediment quality is an essential tool to assess the possible influence of anthropogenic pressure on ecosystem quality (Romeo et al., 2015) giving information to support management in the Marine Strategy Framework Directive (MSDF) perspective. This directive was created with the purpose of reaching and archiving the "Good Environmental Status" (GES) of European marine waters by 2020 and to protect the resources related to economic and social activities. Marine sediments are also essential in the evaluation of the emerging pollutants such as marine litter, that tend to accumulate on the sea floor (Cozar et al., 2014; Nuelle et al., 2014). At present, this type of pollution represents one of the major concerns affecting all world oceans, defined in the MSFD as descriptor D10 into

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different categories, including microplastics. Plastics in the environment are subjected to a combination of physical, biological and chemical processes that reduce their structural integrity (Cole et al., 2011), producing high densities of smaller debris as microplastics. This material is considered a threat to biodiversity and a risk for human safety, indeed due to its ability to adsorb environmental pollutants, it can produce numerous physical and toxicological damages to organisms belonging to all trophic levels (Fossi et al., 2017; Thompson et al., 2004). In addition, numerous studies report that plastic litter can become a vector of introduction of NIS (Derraik, 2002; Winston, 1982). As reported in the Directive, S. F. (2013), the monitoring of litter on the sea floor cannot consider all coastal areas because of limited resources, for this reason opportunistic approaches (*i.e.* data from other research activity in the harbour) could be used to improve the existing monitoring plans.

Quality of the sea floor (D6, MSFD) reflects characteristics of the sea bottom influencing, in particular, the structure and functionality of communities living on the sea floor (benthic ecosystems). Disturbance of the bottom caused by pollutants may change the benthos community, damaging mainly sensitive species and causing loss of biodiversity. Macroinvertebrates, due to their skill in modifying their community patterns in response to natural and anthropogenic stress (Warwick, 1988), are considered excellent bioindicators of marine ecosystems (Warwick, 1993; Romeo et al., 2015). To date, a lot of benthic indices based on the structure of macrofaunal communities were created to assess the ecological quality status (EcoQ) to support data for MSFD, *e.g.* AMBI (Borja et al., 2000), M-AMBI (Muxika et al., 2007), BENTIX (Simboura and Zenetos, 2002) and BOPA (Dauvin and Ruellet, 2007). These indices, based on the subdivision of species in different ecological groups, give indication on the environmental quality/disturbance status.

This paper, through a multidisciplinary approach that takes into account biotic and abiotic parameters, aims to study the distribution of the main pollutants and their relationships with biodiversity and benthic communities. It represents the first study reporting both the abundance of plastic debris in the coastal GSA 16 and their relationship to biodiversity indices. All this data could be useful for providing information for national and international laws and directives, before the start of the monitoring programme of MSFD.

2. Material and methods

2.1. Study area

The Augusta site is located in the MSFD Ionian sub-region of the central Mediterranean Sea, in a harbour area with high marine traffic activity. This area hosted a variety of different chemical and petrochemical refining plants, a commercial harbour and bases of the Italian Navy and NATO activities (Sprovieri et al., 2007). The port is closed to the South and East by artificial dams. Two main inlets connect the harbour with the open sea: the south-east and the east inlet. Three different circulation systems characterise the basin: the eastern inlet, dominated by a tidal current with a northward flow, the south-eastern inlet, characterised by flowing parallel to the coast and the northern portion of the basin, characterised by a shallow seabed and scarcely affected by active currents (Sprovieri et al., 2007; Romano et al., 2013). Three small rivers flow in the area, Mulinello in the North and Marcellino and Cantera in the central part of the bay (Fig. 1). Due to the dangerous contamination of air, seawater, and marine biota documented in this area, Augusta coastal area has been included by the Italian Government in the national remediation plan (G.U.R.I., L. 426/1998)

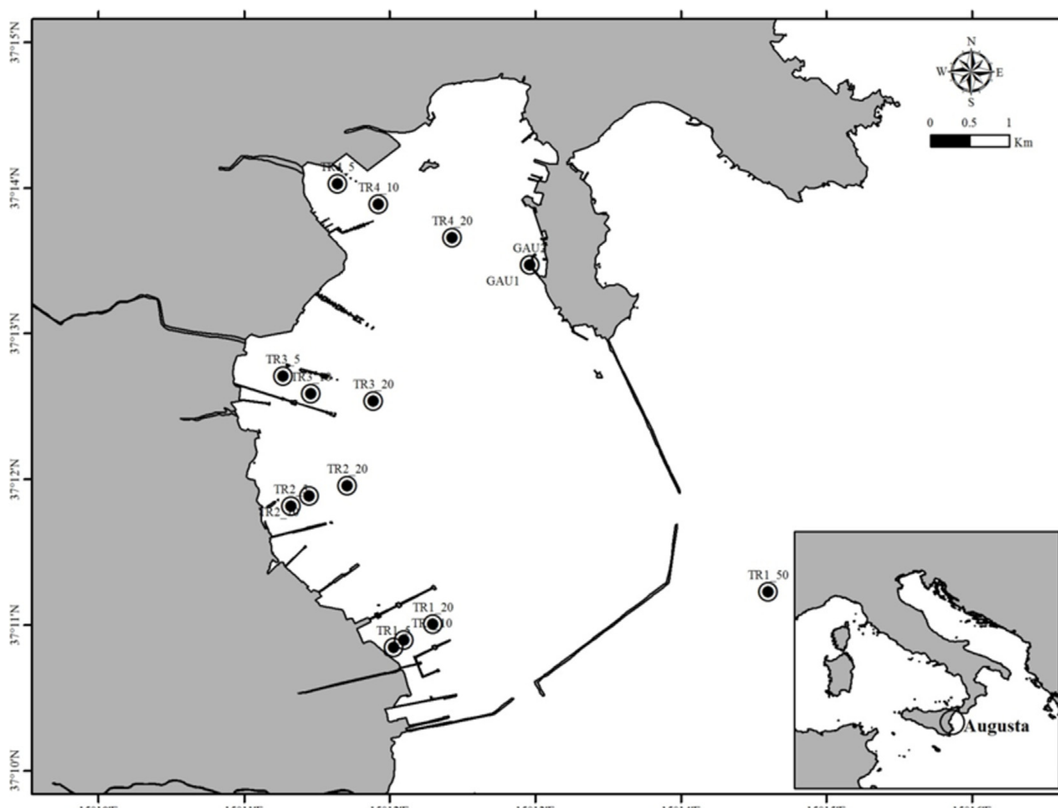

Fig. 1. Augusta sampling points of hard and soft bottom.

and evaluated by the World Health Organization as causing a high environmental risk.

2.2. Sampling activities and laboratory analyses

Samples were collected from hard and soft bottoms during the summer of 2013 (Table 1, Fig. 1). Soft bottom samples were collected by means of a Van Veen grab (0.1 m^2) along four transects perpendicular to the coastline at three different depths (5, 10 and 20 m). For each sampling site, four replicates were carried out, three of which were used for biological analysis, and one for the environmental characterisation following the methods described in D'Alessandro et al. (2016) and Romeo et al. (2015). Grains greater then 0.063 mm were analysed by means of a sieve series with intervals of 1 Φ, while the smallest fraction (silt and clay) was analysed using the column dispersion method (Buchanan and Kain, 1971). The percentage of pebble, gravel, sand, silt and clay was determined according to the ternary Wentworth scale (Wentworth, 1922).

Plastic debris were classified according to Guidance on Monitoring of Marine Litter in European Seas (Joint Research Centre, EC, 2013) adapted. Four size classes were identified: microplastics (1–5 mm), macroplastics (5–10 mm), megaplastics (10–20 mm) and plastics (>20 mm) (Claessens et al., 2011). Microplastics were extracted according to Alomar et al. (2016) with some modifications. For each sample, 1 kg of sediment was dried at 50 °C for 48 h and then sieved for 15 min by means of stainless steel sieves with a mesh diameter of 20, 10, 5, 0.5 and 0.1 mm. For each fraction, plastics were extracted by density separation method and then the sediments and particles extracted were observed under Stereomicroscope (Zeiss Discovery.V8) with optical enhancement with a maximum magnification of 80×. Accurate precautions have been used to prevent contamination during all phases of this study in accordance with Woodall et al. (2015). Hard bottom samples were collected by SCUBA diving, scraping a surface of 400 cm^2 from two pillars within the refinery. Three replicates were collected at three different depths (0, 3.0 and 6.0 m), for a total amount of 9 samples per pillar.

Chemical analyses were conducted taking into account contaminants included in the MSFD monitoring plan and other contaminants of interest on the basis of the different typologies of anthropogenic impacts reported in literature for a similar study area (Falandysz et al., 2006; Fang et al., 2003; Relić et al., 2005). Prior to each analysis, sediment samples were treated as reported in D'Alessandro et al. (2016) and Romeo et al. (2015), then Inductively Coupled Plasma-Mass Spectrometry (ICP-MS; mod. Agilent Technologies) was used to assess concentrations of the trace elements (Cd, Hg, Pb, As, Cr, Cu, Ni, and Zn) according to the US-EPA 6020A method. Quantifications of PCBs (congeners 28, 52, 77, 81, 101, 118, 126, 128, 138, 153, 156, 169, 180) were conducted following the US-EPA 8082A/2007 standard method. PAHs (acenaphthene, acenaphthylene, anthracene, dibenzo(a,h)anthracene, benzo(b)-fluoranthene, benzo(a)pyrene, benzo(ghi)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, indeno(1,2,3)pyrene, naphthalene, phenanthrene, pyrene, perylene, acenaphthene) were determined according to the US-EPA Method 8270D. Finally, a modified method from Binato et al. (1998) and Morabito et al. (1995) was used in the chemical analyses of butyltins (BT) in the surface sediments. Tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT) and total butyltins (ΣBT) were determined as described in Romeo et al. (2015) and D'Alessandro et al. (2016). All results of chemical analysis were calculated on dry weight (d.w.), trace elements were expressed in μg/kg, persistent organic pollutants in mg/kg, while the butyltins concentrations were expressed as ng Sn g^{-1}.

In order to characterise the benthic communities, the main biodiversity indices were calculated: number of species (S), Shannon's index (H′), and Pielou's evenness (J) (Magurran, 1988). Benthic indices (*i.e.* AMBI, M-AMBI, Bentix and BOPA) were calculated on the abundance of each species to evaluate the environmental status of soft bottom fauna. AMBI and M-AMBI were calculated by means of AMBI index software (version 4.0, available at www.azti.es); BOPA index and its relative environmental quality were calculated using the revisited formula proposed by Dauvin and Ruellet (2007).

2.3. Statistical analysis

A data exploratory test for univariate analysis was assessed evaluating collinearity between variables in order to avoid type II error rates (Zuur et al., 2010). Thereafter, to highlight the potential correlation between the biotic indices, environmental variables,

Table 1
Coordinates, grain-size fraction (%) and plastic abundance (kg-1 dry sediment) at each sampling site in Augusta harbour.

	Coordinates		Grain size					Plastic abundance			
	Latitude	Longitude	Pebble	Gravel	Sand	Silt	Clay	micro	macro	mega	plastic
TR1_5	37°10.850′N	15°12.030′E	1.71	4.55	93.74	0	0	0	0	0	0
TR1_10	37°10.900′N	15°12.100′E	0.95	1.63	41.07	51.47	4.87	6	1	0	0
TR1_20	37°11.010′N	15°12.300′E	0.00	0.34	8.24	87.93	3.49	2	1	2	2
Total abundance								**8**	**2**	**2**	**2**
TR2_5	37°11.820′N	15°11.324′E	0.05	0.24	36.89	50.61	12.21	0	0	0	0
TR2_10	37°11.890′N	15°11.451′E	4.59	4.04	32.15	53.51	5.71	1	0	0	0
TR2_20	37°11.960′N	15°11.693′E	1.04	0.78	16.74	76.96	4.49	1	0	1	1
Total abundance								**2**	**0**	**1**	**1**
TR3_5	37°12.710′N	15°11.270′E	0.57	0.54	29.49	66.05	3.36	2	0	0	0
TR3_10	37°12.590′N	15°11.460′E	0.11	0.67	50.33	46.38	2.51	0	0	1	0
TR3_20	37°12.551′N	15°11.892′E	0.26	1.06	8.45	82.58	7.62	1	0	0	1
Total abundance								**3**	**0**	**1**	**1**
TR4_5	37°14.030′N	15°11.643′E	0.00	0.00	0.00	95.17	4.83	4	3	1	4
TR4_10	37°14.000′N	15°11.780′E	0.19	0.13	6.77	88.45	4.46	2	0	0	0
TR4_20	37°13.662′N	15°12.401′E	3.50	3.01	6.32	81.37	5.80	1	0	0	0
Total abundance								**7**	**3**	**1**	**4**
GAU1	37°13.475′N	15°12.972′E									
GAU2	37°13.475′N	15°12.972′E									

trace elements and organic contaminants, a Pearson correlation matrix was produced (Feld et al., 2016). Subsequently, the Generalised Variance Inflation Factors (GVIF) was calculated, retaining those variables with negligible collinearity (a GVIF of 2 or less; Zuur et al., 2010).

To detect the relationships between potentially explanatory environmental variables and the diversity and biotic indices, linear regression models were applied. Moreover, as an important factor shaping the biodiversity, depth was considered as a fixed factor in the regression analysis. Prior to analysis, the normality of the distribution of each diversity and biotic indices was tested before applying parametric statistical tests (Shapiro and Wilk, 1965). Afterwards, Akaike's Information Criterion (AIC; Akaike, 1974) was used to select the best model among all classes of competing models after applying backwards and forwards stepwise variable elimination (Czado et al., 2007; Famoye and Rothe, 2001). Finally, the statistical assumptions of independence, normality and homogeneity of variances were tested using the gvlma function in the gvlma package in R (Pena and Slate, 2014). All statistical univariate analyses were performed using the software program R, version 3.4.2, R packages: car, gvlma, PerformanceAnalytics (R Development Core Team, 2017).

Multivariate analyses were carried out using the software PRIMER6 and PERMANOVA+ (Anderson et al., 2008; Clarke and Warwick, 2001). Potential variations in the abundance of the 4 plastic dimensional categories by factor depth were evaluated through a non-parametric multivariate analysis of variance (PERMANOVA). Data were square root transformed and the analyses were carried out on the basis of Bray-Curtis similarity (4999 permutations). When significant differences ($p < 0.05$) among factor levels were highlighted, pairwise comparisons were computed.

The potential differences among the composition of the macrobenthic community, in depth x transect, were assessed through a two-way crossed PERMANOVA analysis. Prior to analysis, abundance data were square root transformed and analysed based on Gower distance. When significant differences ($p < 0.05$) among factor levels were detected, pairwise comparisons were computed. SIMPER analysis was performed to assess the contribution of the different *taxa* to the average dissimilarity between groups. The square root transformed abundance matrix was analysed on the basis of Bray-Curtis similarity index (Bray and Curtis, 1957) to assess the spatial pattern of macrobenthic community composition. Then, to group stations with similar (*i.e.* branch with $p > 0.05$) contaminant data, a not agglomerative hierarchical clustering (routine CLUSTER), coupled with a similarity profile test (SIMPROF) based on permutation, was applied on a Euclidean distance resemblance matrix created for normalised abiotics. The contaminant data that contributed the most to variation among the identified groups were evidenced in a Principal Coordinates (PCO) analysis.

3. Results

With regard to the soft bottom sediment analysis, Augusta harbour resulted as being mainly characterised by a fine fraction with the highest values in the northern side (TR4_5, 95.17% of silt), while the highest percentages of sand were recorded along TR1 (TR1_5 and TR1_10 with 93.74% and 41.07% of sand, respectively). In TR2_5 the major percentage of clay was recorded (12.21%). Pebble and gravel represent the less abundant fractions with percentages of 4.59% and 4.55% respectively, in TR1_5 (Table 1).

Regarding the plastic analysis, in Augusta sediments were found in a total of 38 fragments kg^{-1} of dry sediment. Microplastics was the most abundant group (52.63% of total abundance) followed by plastics (21.5% of total abundance), macro and megaplastics both had 13.16 of total abundance. Microplastics were found in 9 stations, megaplastics and plastics in 4 stations while macroplastics in 3. Considering all the dimensional ranges, the highest number of plastic debris was found in TR4_5 (12 particles kg^{-1} dry sediment), while in TR1_5 and TR2_5 sediments, the presence of debris was not detected. Microplastics showed highest abundance ($n = 6$ particles kg^{-1} dry sediment) in TR1_10, macroplastics and megaplastics in TR4_5 (both with 3 particles kg^{-1} dry sediment), megaplastics in TR1_20 (2 particles kg^{-1} dry sediment) and plastics in TR4_5 (4 particles kg^{-1} dry sediment) (Table 1).

Regarding the hard bottom communities, a total of 4191 specimens were found in Augusta harbour. Mollusca represented the most abundant group (76.54% of total abundance). Three non-indigenous species were recorded: the bivalve *Brachidontes pharaonis* (57.27% of total abundance) and the polychaetes *Pseudonereis anomala* (0.84% of total abundance) and *Branchiomma bairdi* (0.21% of total abundance). *B. pharaonis* dominated the hard bottom community, followed by the native crustacean *Elasmopus rapax* (6.54% of total abundance). The highest value of species richness was recorded in GAU2_0 ($S = 24$), the number of individuals showed the highest value in GAU1_0 ($N = 1232$), whereas J was highest in GAU2_6 ($J = 0.92$), while H′ resulted highest in GAU1_3 and GAU2_3 ($H' = 2.26$) (Table 2).

The faunistic soft bottom analysis highlighted the presence of a total of 2125 specimens of which 64.33% belonging to Polychaeta, 29.22% to Mollusca, 1.88% to Crustacea and 4.56% to other minor

Table 2
Values of biodiversity (S, J, H′) and biotic indices (AMBI, M-AMBI, BOPA, BENTIX) and corresponding disturbance/EcoQ values of soft bottom station in Augusta harbour.

	S	J	H′	BOPA	EcoQ	BENTIX	Classification	AMBI	Disturbance Classification	M-AMBI	Status
TR1_5	17.7	0.8	2.2	0.0000	High	2.84	Moderate	2.68	Slightly disturbed	0.89395	High
TR1_10	7.3	0.8	1.6	0.0000	High	2.74	Moderate	3.083	Slightly disturbed	0.55466	Good
TR1_20	17.7	0.7	2.1	0.0000	High	2.95	Moderate	1.13	Undisturbed	0.8554	High
TR2_5	18.0	0.8	2.4	0.0000	High	2.72	Moderate	1.533	Slightly disturbed	0.91924	High
TR2_10	17.7	0.8	2.1	0.0000	High	3.10	Moderate	2.29	Slightly disturbed	0.8638	High
TR2_20	12.0	0.7	1.7	0.1761	High	3.35	Moderate	0.942	Undisturbed	0.78908	High
TR3_5	18.7	0.7	2.0	0.1761	Good	3.56	Good	2.795	Slightly disturbed	0.8146	High
TR3_10	16.0	0.8	2.3	0.0000	Good	3.31	Moderate	3.202	Slightly disturbed	0.79392	High
TR3_20	12.3	0.7	1.7	0.1761	High	2.86	Moderate	1.14	Undisturbed	0.75693	Good
TR4_5	16.3	0.6	1.8	0.0000	Good	2.72	Moderate	1.421	Slightly disturbed	0.78326	High
TR4_10	14.7	0.8	2.1	0.0000	High	3.18	Moderate	1.772	Slightly disturbed	0.80407	High
TR4_20	10.7	0.9	2.1	0.0000	High	3.82	Good	1.79	Slightly disturbed	0.76087	Good
GAU1_0	20	0.43	1.26								
GAU1_3	17	0.83	2.26								
GAU1_6	11	0.77	1.82								
GAU2_0	24	0.61	1.95								
GAU2_3	14	0.85	2.26								
GAU2_6	12	0.92	2.25								

groups (Nematoda, Nemertea, Echinodermata, Sipuncula and Chordata). The most abundant species was the polychaete *Aricidea (Aricidea) pseudoarticulata* (Hobson, 1972), representing 24.99% of total benthic assemblage. Among Polychaeta, three non-indigenous species *Kirkegaardia dorsobranchialis* (Kirkegaard, 1959), *Notomastus aberans* (Day, 1957) and *Pista unibranchia* (Day, 1963) were also found. Among Crustacea, the Decapoda *Alpheus glaber* (0.33% of total abundance) resulted the most represented species while the most abundant Mollusca was the bivalve *Corbula gibba* (11.58% of total abundance). The average values of the diversity indices for each sampling station are reported in Table 2. The species richness showed the highest value (S = 18.7) in TR3_5 with a negative peak in TR1_10 (S = 7.3); in the latter station, the lowest value of H' (1.6) was recorded. The highest values of H' (2.4) was observed in TR2_5, while lowest values of J (0.7) were recorded in TR1_20, TR2_20, TR3_5 and TR3_20 (Table 2). BOPA index showed highest values in TR2_20, TR3_5 and TR3_20 (0.1761), Bentix index in TR4_20 (3.82), AMBI in TR3_10 (3.202) and M-AMBI in TR2_5 (0.91924). BOPA and M-AMBI indices showed a generalised High/Good ecological status, AMBI assigned levels of disturbance that ranged between undisturbed and slightly disturbed, while Bentix provided a classification that ranged between high and moderate (Table 2).

Concerning the chemical analysis, anthracene was the most abundant compound (158 μg/Kg in TR1_10), followed by: benzo(*b*) fluoranthene (107 μg/kg in TR1_10), fluoranthene (56 μg/kg in TR3_20), benzo(*a*)pyrene (91 μg/kg in TR1_10), naphtalene (86 μg/kg in TR1_10), benzo(*k*)fluoranthene (34 μg/kg in TR1_10) and benzo(*g,h,i*)perylene (<10 μg/kg in all stations). The abundances of indenopyrene and PCB_{tot} were <5 μg/kg in all the sampled stations. Among trace elements, Zn showed the highest abundance values in the whole study area, reaching a value of 50.6 mg/kg in TR1_10, followed by: Cr_{tot} (38.4 mg/kg in TR3_5), Cu (18.8 mg/kg in TR2_10), Pb (24.3 mg/kg in TR1_10), Ni (15.4 mg/kg in TR3_5), As (8.0 mg/kg in TR1_10); Hg (9.49 mg/kg in TR3_20) and Cd that showed abundance <0.3 mg/kg in all analysed stations. TBT, DBT and MBT showed the highest abundance in TR3_20 with values of 522 and 22 ng Sn g^{-1} respectively (Table 3).

3.1. Statistical analysis

Regarding the univariate analysis, Pearson correlation coefficients and their P-values of the entire dataset were reported in Fig. 2. This analysis highlighted that the grain-size composition is highly correlated with the AMBI index, in particular sand (0.7) and silt (−0.6). Regarding the plastic analysis, macro and plastic dimensional categories are highly related with J diversity index, with values of −0.6 and −0.8 respectively. Moreover, the microplastic result negatively correlated with the H′ and with the M-AMBI (−0.7 and −0.8). Only the TBT, DBT and MBT results intra-correlated. Concerning the trace elements, Cu resulted negatively correlated with S, H' and M-AMBI, with values of −0.6, −0.6 and −0.7 respectively. M-AMBI resulted negatively related also with Zn (−0.6) and Pb (−0.7).

The best linear models chosen after the model selection analysis were reported in Table 4. The variables entered in the S model were sand, clay, microplastic and depth factor, while the important variables for J resulted as sand, clay, megaplastic, TBT and the factor depth. The model of M.AMBI was formed by four variables, sand, microplastic, Hg and the depth factor. The first model resulted significant ($R^2 = 0.70$) and S was negatively related to the presence of microplastics showing a decrease with the depth factor. Regarding Pielou's evenness index, the model was highly significant ($R^2 = 0.89$) and J resulted as positively related to sand, clay and the depth factor, while negatively correlated to TBT. With

Table 3
Values of trace elements (mg/kg), PHAs (μg/kg), TBT, DBT and MBT (ng Sn g-1) measured at each sampling site in Augusta harbour.

	Naphtalene	Anthracene	Fluoranthene	Benzo(b) fluoranthene	Benzo(k) fluoranthene	Benzo(a) pyrene	Indenopyrene	Benzo(g,h,i) perylene	PHA tot	PCB tot	TBT	DBT	MBT	Cu	Zn	Cd	Ni	Pb	As	Cr tot	Hg
TR1_5	–	–	–	–	–	–	–	–	–	–	16	10	<4	–	–	–	–	–	–	–	–
TR1_10	86	158	53	107	34	91	<10	<10	–	<5	48	8	<4	34	50.6	<0.3	10.8	24.3	8	23.1	0.98
TR1_20	<10	12	<10	<10	<10	<10	<10	<10	–	<5	21	17	13	8.8	13.2	<0.3	4.2	7.9	2.7	9.6	0.44
TR2_5	–	–	–	–	–	–	–	–	–	–	39	33	19	–	–	–	–	–	–	–	–
TR2_10	<10	<10	<10	<10	<10	<10	<10	<10	–	<5	18	22	19	18.8	36.1	<0.3	11.4	12.1	8	20.1	1.4
TR2_20	<10	<10	<10	<10	<10	<10	<10	<10	–	<5	34	24	18	14.8	28.6	<0.3	10.4	9.6	6.8	16.6	3.14
TR3_5	<10	<10	<10	<10	<10	<10	<10	<10	–	<5	6	5	6	17	34.3	<0.3	15.4	17.4	6.2	38.4	1.92
TR3_10	<10	<10	<10	<10	<10	<10	<10	<10	–	<5	22	21	18	10.5	21	<0.3	10.6	14	5.6	18.7	1.67
TR3_20	<10	<10	56	<10	<10	<10	<10	<10	–	<5	522	36	22	16	32.9	<0.3	12.9	12	6.3	19.3	9.49
TR4_5	–	–	–	–	–	–	–	–	–	<5	–	–	–	–	–	–	–	–	–	–	–
TR4_10	–	–	–	–	–	–	–	–	–	<5	–	–	–	–	–	–	–	–	–	–	–
TR4_20	<10	<10	18.44	27.6	<10	15	<10	12.45	158.5	<5	–	–	–	12.9	23.1	<0.3	6.2	19.1	4.1	12.9	0.9

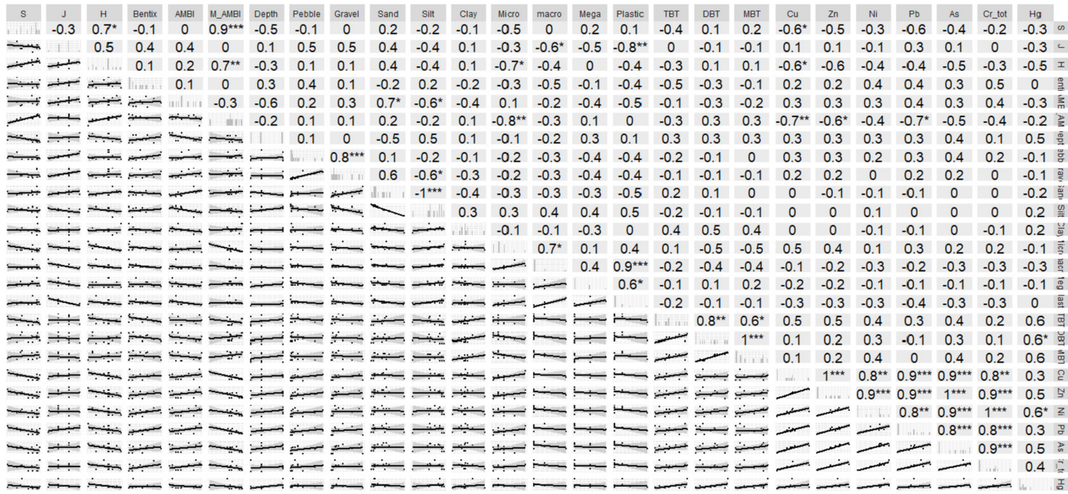

Fig. 2. Pearson correlation matrix plot of the diversity (S, J, H′) and biotic indices (BENTIX, AMBI, MAMBI), environmental variables (Depth and grain size sediment composition in terms of pebble, gravel, sand, silt and clay), plastic debris dimension (micro, macro, mega, plastic), chemical parameters (TBT, DBT, MBT, Cu, Zn, Ni, Pb, As, Cr total and Hg). Bold data represent P < 0.05; *, P < 0.01; **, P < 0.001; ***.

regard to the M-AMBI biotic index, the model resulted highly significant ($R^2 = 0.84$) and a significant negative correlation was found only with the microplastic abundance.

Multivariate analysis, performed by PERMANOVA, evidenced no significant differences between the plastic size categories and the depth. Results of the two-way multivariate analysis conducted on the species abundance revealed significant differences in macrobenthic community composition between investigated depths ($F_{1,24} = 2.89$; $p < 0.01$) and between transects ($F_{1,24} = 1.72$; $p < 0.01$). Pairwise comparison showed significant differences among all the levels of the factor depth, except for 5 m *vs* 10 m, and among all the levels of factor transect, except for TR3 *vs* TR4. SIMPER tests showed the highest average dissimilarity between the macrobenthic composition at 5 and 20 m ($\delta = 75.31$), ascribable to the bivalve *C. gibba*, more abundant at 5 m of depth, and the polychaetes *Aricidea (Aricidea) pseudoarticulata*, more abundant at 20 m. Moreover, the highest average abundances of the polychaetes *Pseudoleiocapitella fauveli*, at the depth of 10 m were responsible for the dissimilarities among this and the other investigated depths. With regard to transect, SIMPER tests showed the highest average dissimilarity between the macrobenthic composition at TR1 and TR2 ($\delta = 78.92$), ascribable to the higher average abundances of the two polychaetes *A. pseudoarticulata* and *P. fauveli* recorded in TR2. While *C. gibba* is the species that most contributed to the dissimilarities between TR3 and the other investigated transects (TR1 and TR2).

Table 4
Linear model results of biodiversity and biotic indices and environmental variables. Significant codes: 0 '***' 0.001 '**' 0.01 '*'.

Model			
S = Sand + Clay + microplastic + factor (Depth)			$R^2 = 0.70$
	Estimate	Standard Error	P value
Intercept	24.31	3.60	**0.000514** ***
Sand	−0.07	0.04	0.190538
Clay	−0.37	0.31	0.284959
microplastic	−1.42	0.54	**0.038371** *
Depth (10)	−3.44	1.95	0.128410
Depth (20)	−6.74	2.34	**0.027994** *
J = Sand + Clay + megaplastic + TBT + factor (Depth)			$R^2 = 0.89$
	Estimate	Standard Error	P value
Intercept	0.54	0.06	**0.000241** ***
Sand	0.003	0.0008	**0.006710** **
Clay	0.022	0.007	**0.021571** *
megaplastic	−0.03	0.022	0.216174
TBT	−0.003	0.001	**0.012936** *
Depth (10)	0.14	0.03	**0.006413** **
Depth (20)	0.18	0.04	**0.006006** **
M.AMBI = Sand + microplastic + Hg + factor (Depth)			$R^2 = 0.84$
	Estimate	Standard Error	P value
Intercept	0.96	0.05	**7.94e-07*** **
Sand	−0.0009	0.0007	0.26
microplastic	−0.05	0.007	**0.004** **
Hg	−0.009	0.007	0.21
Depth (10)	−0.07	0.04	0.12
Depth (20)	−0.07	0.05	0.17

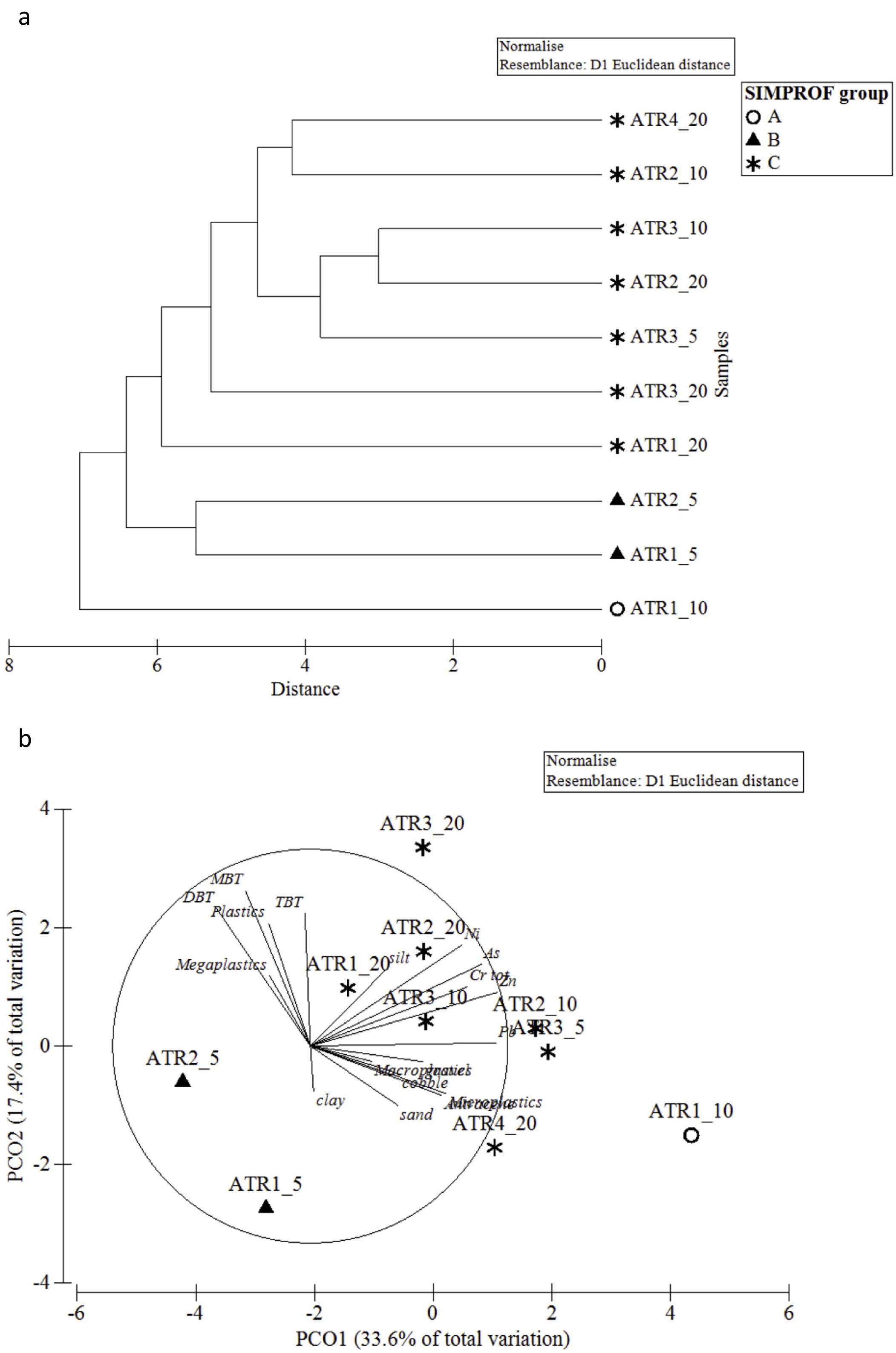

Fig. 3. SIMPROF dendrogram based on the environmental data (a); Principal Coordinates (PCO) analysis biplot representing the spatial distribution of the sampling stations in the Augusta harbour on the basis of the Euclidean distance matrix and showing the contaminant concentrations and grain-size composition (superimposed vectors) characterizing each of the group identified by the SIMPROF procedure (b).

The SIMPROF analysis of the environmental variables highlighted the presence of 3 different groups (A, B and C; $p < 0.05$; Table 4), also highlighted by the hierarchical cluster analysis (Fig. 3a). Similarly, the PCO results confirmed the presence of three different groups, with the PCO1 and PCO2 axes explaining the 33.6% and 17.4% respectively of the total variation (Fig. 3b). Two sets of contaminants were evidenced from the overlaid vectors related to A and C groups, while no contaminants resulted as being related to group B.

The characteristics of each group are reported in Table 5 and Fig. 3. In detail, group A resulted as being composed of only one station (TR1_10), characterised by silt sediments, high concentrations of PAHs and trace elements and the highest abundance of micro- and macroplastics. Moreover, a total of 14 species were recorded, among which the non-indigenous *Kirkegaardia dorsobranchialis*. While, the highest concentrations of DBT and the presence of 59 species, three of which non-indigenous, characterise group B, composed of TR1_5 and TR2_5 stations. Finally, the remaining 7 stations constituted group C, characterised by the highest concentrations of TBT and MBT, and the highest total number of species (88), three of which were non-indigenous.

4. Discussion

Augusta harbour, located in the MSFD Ionian sub-region in the Central Mediterranean Sea, represents a strategic site to improve starting data for the Monitoring Plane related to the National Subprogramme Module 5T and to provide data about other descriptors of MSFD such as D1 (biodiversity), D2 (non-indigenous), D6 (seafloor integrity), D8 (contaminants) and D10 (marine litter).

Sediment grain size analysis testified that the area is mainly characterised by fine texture, especially in the northern and southern part, this may be due to its particular conformation close to artificial dams that make the area scarcely affected by active currents (Romano et al. 2013; Sprovieri et al., 2007). This particular hydrodinamism seems to also influence the distribution of plastic litter, showing the highest abundances in the most southern (Transect 1) and most northern (Transect 4) areas. Indeed, as reported in several studies, the distribution of plastic debris is closely linked with geomorphological and hydrodynamic characteristics of the area, and, with the exception of some accumulation zones in the open sea, is more abundant in shallow waters or bays than areas nearer the continental shelf (Alomar et al., 2016; Auta et al., 2017). Understanding the main causes, the distribution and the sources of microplastic pollution at different spatial scales is therefore essential to develop appropriate policies and laws, in order to carry out sustainable management of marine resources, especially in coastal environments (Pasquini et al., 2016). At present, microplastics in the marine environments represent one of the emerging problems in the world. This type of pollution, creating growing global concerns among governments, scientists and organisations, poses a significant threat to environmental protection and human security (Seltenrich, 2015). The importance of increasing knowledge about this current issue, was also highlighted by indicator 10.1.3 of MSFD, that aims to find information on trends in the quantity, distribution and, if possible, in the composition of microparticles, in particular microplastics (Auta et al., 2017; Galgani et al., 2013, 2014). Microplastic abundance found in this study area resulted as being lower than that recorded in other studies conducted in shallow water, such as the Aeolian archipelago where a maximum abundance of 1037 debris kg^{-1} dry sediment; was reported (Fastelli et al., 2016). Moreover, Augusta's plastics were also lower than those reported in three ports of the Balearic Islands (*i.e.* Andratx, Es Port and Santa Maria) where a maximum abundance of 250, 160 and 100 debris kg^{-1} dry sediment was detected (Alomar et al., 2016). Similarly, in Grand Harbour of La Valletta, the abundance of plastics found (59 debris kg^{-1} dry, 35 of which belonging to microplastics) was higher than in Augusta harbour (Romeo et al., 2015). Nevertheless, microplastics found in Augusta was also higher than the values reported in other similar studies conducted in the United Kingdom (Thompson et al., 2009) and Singapore (Ng and Obbard, 2006), where 3 and 8 plastic debris fragments per kg of dried sediments were respectively recorded. Significant negative correlations were found in this study among plastic debris *vs* biodiversity and benthic indices. This could be due to the coexistence of plastic with other contaminants that are both added during production and adsorbed from sea water (Auta et al., 2017). Even if numerous studies have underlined the effects of microplastics on invertebrates, more studies are necessary to understand in depth the effects of plastics on biodiversity (Avio et al.,

Table 5
SIMPROF affinity groups characterisation in Augusta harbour.

		A	B	C
Trace elements	Ni (mg/kg d.w.)	10.8	0	10.1
	Pb (mg/kg d.w.)	24.3	0	12.5
	As (mg/kg d.w.)	8	0	5.80
	Cr tot (mg/kg d.w.)	23.1	0	18.79
	Zn (mg/kg d.w.)	50.6	0	25.8
Persistent contaminants	Anthracene (μg/kg d.w.)	158	0	9.4
	TBT (ng Sn/g d.w.)	48.1	27.4	77.8
	DBT (ng Sn/g d.w.)	7.9	21.4	15.6
	MBT (ng Sn/g d.w.)	3.0	11.1	12.0
Plastic debris	Microplastic (n/kg)	6	0.0	0.8
	Macroplastic (n/kg)	1.0	0.0	0.1
	Megaplastic (n/kg)	0.0	0.0	0.5
	Plastic (n/kg)	0	0.0	0.5
Sediment characteristics	Gravel (%)	1.6	0.1	1.4
	Sand (%)	41.1	18.4	22.8
	Silt (%)	51.5	25.3	69.9
	Clay (%)	4.9	6.1	4.6
Biotic characteristics	Total number of species	14	59	105
	N. of non-indigenous species	1	3	3
	Non-indigenous species	*Monticellina dorsobranchialis*	*Notomastus aberans, M. dorsobranchialis, Pista unibranchia*	*N. aberans, M. dorsobranchialis, P. unibranchia*

2016; Green, 2016; Van Cauwenberghe et al., 2015). Despite growing international attention, the accumulation of these materials in the environment continues to be high, both because of the increasing world production of plastics and the continued improper disposal of plastic waste. In the examined sediments, all plastic dimensional categories were found, among these, microplastics resulted as the most abundant. This could derive from both an indiscriminate release into the sea of any typology of plastic and from the lack of appropriate national laws that limit its consumption and release (Browne et al., 2011; Dubaish and Liebezeit, 2013; Fendall and Sewell, 2009; Thompson et al., 2004).

Biotic indices are widely used in ecological studies to understand the relationships between biodiversity and environmental disturbance and to assess the ecological status of waters (Fisher et al., 2001; Siddig et al., 2016; Simboura and Zenetos, 2002; van der Linden et al., 2016). Their recognised importance is underlined, also, in MSFD, in which D6 is focused on the integrity of the seafloor and the safeguarding of benthic ecosystems. The dominance in the harbour of opportunistic (e.g., *C. gibba*, *P. fauveli* and *N. aberans*) and tolerant (*e.g.*, *A. pseudoarticolata*) species suggests a slight degree of environmental degradation. These findings agree with previous studies that reported similar communities and which could be ascribable to "heterogeneous communities" described by Pérès and Picard (1964), Crocetta et al. (2009), Romano et al. (2013). However, our study testifies a general situation of stability of the benthos community, also confirmed by values of biodiversity indices that resulted as high if confronted with other Mediterranean harbours subjected to similar anthropogenic pressure, such as Malta (Deidun et al., 2003; Romeo et al., 2015), Trieste (Solis-Weiss et al., 2004) and Napoli (Bergamin et al., 2009). The presence of six non-indigenous species underlines the peculiar role of the harbour in hot-pointing alien species (D'Alessandro et al., 2015; Occhipinti-Ambrogi et al., 2011). There are many vectors for the introduction of non-indigenous species such as marine traffic (either fouling or ballast water), aquaculture and interoceanic canals. Species found in Augusta harbour are linked to shipping: *B. bairdi*, *B. pharaonis*, *P. anomala* and *K. dorsobranchialis* (D'Alessandro et al., 2015; Katsanevakis et al., 2012; Sarà et al., 2018; Streftaris et al., 2005) and to natural expansion from Canals*: P. unibranchia* and *N. aberans* (Çinar et al., 2014). Among Polychaeta, the first three records for the Ionian Sea of *L. geldiaij* (Carrera-Parra et al., 2011), *A. bidentata* (D'Alessandro et al., 2014) and *P. anomala* (Gravier, 1900) were also recorded. These findings could be related to the intense marine traffic in this area, being one of the most important petrol-chemical poles of the Ionian Sea.

Our study testifies a correlation between the structure of the benthic community and the environmental status of the harbour (Gray and Elliott, 2009; Simboura et al., 2000). Despite the high concentration of human activities in the area, the benthic indices calculated, highlighted a slightly disturbed classification. However, a general, lesser stressed status was recorded with the increase of depth, as confirmed by statistical analysis that ascribes the difference between depths to the major abundance of opportunistic species at a lower depth (*C. gibba* and *P. fauveli*) and of sensitive species (*A. pseudoarticolata*) in deeper stations. In general, the benthic community seems to be adapted to environmental stress, showing a well-structured assemblage (Romano et al., 2013).

Marine sediments represent the main source of organic and metallic pollutants (Cabrini et al., 2017). The MSFD considers the concentration of contaminants in D8 and imposes that their levels must not cause polluting effects. The abundance of heavy metals in Augusta harbour seems to be related to human activities and to the presence of rivers, which receive industrial effluents, municipal wastewater effluents and stack emissions from smelting operations and fossil-fuel combustion (Nriagu and Davidson, 1980). Zn, Cr and Cu were trace elements that showed the highest abundance in this study area. The main contributors to global emanations of these metals are the fossil fuel incineration, fabrication of industrial metal and urban drainage and wastes (Cabrini et al., 2017; Christensen and Guinn, 1979). Benthic invertebrates, due to their poor locomotion, are strongly subject to heavy metals present in the sediments (Qu et al., 2017). Moreover, being among the main organisms responsible for the recycling of metals, they constitute the main export route of heavy metals and pollutants for terrestrial trophic webs causing human health risks (Fowler, 1982). The results of our statistical analysis, also highlighted as heavy metals, explicate their negative effects on biodiversity indices and macrobenthos assemblages, facilitating this increase of opportunistic and alien species (Gray and Delaney, 2008; Qu et al., 2010; Takarina and Adiwibowo, 2011).

Organic pollutants, anthracene, benzo(b)fluoranthene and fluoranthene were the most abundant PAHs. These hydrocarbons can become dangerous for human safety, especially if they enter in food chain since some of PAHs and their metabolites can form DNA adducts and thus induce mutations (Readman et al., 2002). However, PAH compounds showed no worrying concentrations in the investigated area, even if an increment of values was recorded in the southern part of the Rade. The distribution of trace elements and contaminants within the study area, showing highest abundance along Transect 1, testifies that their main input is attributable to the industrial pole located in the southern sector. Another distinct point of source is located in the northern part of the Rade, while the central part is the least influenced by contamination. In general, trace elements and PAHs values resulted lower compared to those highlighted in Trieste (Solis-Weiss et al., 2004), Malta (Romeo et al., 2015) and Napoli (Bergamin et al., 2009; Romano et al., 2004) harbours. Different results were recorded for the organotin contaminants (TBT, MBT, DBT), which showed the highest abundances in the central area of Augusta harbour. This peculiar situation may be due to the presence of high maritime traffic and the small neighbouring rivers that enter into the Augusta Rade. Indeed, despite the restriction of TBT (Reg. EC 782/2003) and the decrement of contamination level, the contaminated sediments continue to act as a source (Ritsema, 1994; Stäb et al., 1995), and the release of BTs persists into terrestrial and aquatic environments at levels considered chronically toxic for most organisms (Stäb et al., 1995). The high concentrations of BT found in this study resulted similar to those measured in sediments of heavily industrialised areas and harbours around the world (Berto et al., 2007; Hoch et al., 2003; Romeo et al., 2015). Moreover, considering the EQS value reported by the European Directives (2000/60/EC, 2008/105/EC and 2013/39/EU) for the priority of hazardous substances, TBT concentrations found in the Augusta Rade sediments exceeds most sampling sites.

5. Conclusion

The complexity of marine ecosystems has led the scientific community to produce approaches for integrating information from a broad range of indicators (Fisher et al., 2001). In this paper, five of the main important descriptors used to assess the ecological status of marine waters were studied: seafloor integrity, biodiversity, marine litter, contaminant concentrations and biodiversity. Results of the analysis conducted in Augusta harbour evidenced two hot spots of pollutants in the northern and southern parts of the Rade. This peculiar situation may be linked to close anthropogenic activities and the conformation of the area. This study confirms the validity of the multidisciplinary approach to conducting an environmental assessment, which, despite to an exclusive focus on one type of analysis, provides a more realistic and accurate summary of the ecological status of the investigated area. Furthermore, data provided by this study could be applied within a regulatory framework of scientific advisory values and to the Marine Strategy Framework Directive.

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