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Ecological assessment of a heavily human-stressed area in the Gulf of Milazzo, Central Mediterranean Sea: an integrated study of biological, physical and chemical indicators

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ABSTRACT

Marine environmental disturbance can be assessed directly from physical and chemical parameters, or indirectly by the study of indicator species. In this study, an integrated approach to monitor the Gulf of Milazzo, labeled as a highly contaminated site, is presented. A total of 83 samples were collected from hard and soft bottoms in 2010. In sum, 2739 specimens belonging to 246 taxa, two first records for the Tyrrhenian Sea (*Micronephthys stammeri* and *Nicomache lumbricalis*) and three nonindigenous species (*Brachidontes pharaonis*, *Crassostrea gigas* and *Notomastus aberans*) were recorded. Biodiversity and biotic indices and their relationship with sediment parameters and the level of pollutants were assessed to describe faunal assemblage and evaluate environmental quality. Pearson tests evidenced significant negative correlation between polychlorinated biphenyls (PCBs) and specific richness ($p < 0.10$). A comparison of the standard and recorded biotic values showed that M-AMBI seems to be the index more representative of ecological quality status (EcoQ) in the Gulf of Milazzo. No evident signs were highlighted on the complex.

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1. Introduction

Coastal waters are sensitive habitats that support high levels of biodiversity and provide many marine raw materials and services (Costanza et al., 1998; de Groot et al., 2002; Beaumont et al., 2008). Excessive human use of marine ecosystems (Mason, 2002; Matthiessen and Law, 2002) has led to local different degrees of environmental disturbance such as loss of habitat, change of nutrient status and cycling, loss of food supplies, erosion, reduced sediment supply, change of sea level and consequent inundations, and increased exposures to natural disturbances (Lotze et al., 2005; Worm et al., 2006; McCauley et al., 2015).

Other focal threats to ecological health are represented by bioinvasions of nonindigenous species that, by different vectors such as marine traffic (hull fouling or ballast water), aquaculture and inter-oceanic canals, are able to colonize new locations, causing loss of biodiversity of autochthon species (Vitousek et al., 1996; Ojaveer et al., 2014; D'Alessandro et al., 2015). Nonindigenous species, because of lack of

natural prey, tend to supply autochthon species, causing loss of biodiversity (Bax et al., 2003; Occhipinti-Ambrogi, 2007).

Environmental disturbance, caused by natural or human causes, can be assessed directly from physical and chemical parameters (Daskalakis and O'Connor, 1995) or indirectly by the study of indicators as macrozoobenthic species (Pearson and Rosenberg, 1987; Borja et al., 2000; Lindegarth and Hoskin, 2001). The latter method is found on the paradigm of Pearson and Rosenberg (1987), which showed that macrobenthic organisms change their community structure according to natural conditions and anthropogenic environmental impacts in three progressive steps: abundance increases, species diversity increases and dominant species change from pollution-tolerant to pollution-sensitive ones (Grigg, 1994; Pearson and Rosenberg, 1987; Otway, 1995; Borja et al., 2000). On the basis of this assumption, by the study of benthic community, it is possible to establish an ecological quality status (EcoQ) of studied areas (Borja et al., 2000; Cruz-Motta and Collins, 2004; Currie and Isaacs, 2005).

The implementation of the European Water Framework Directive 2000/60/EC and 2008/56/CE has developed several biotic indices based on the classification of species (or groups of species) in several ecological groups representing specific sensitivity levels to disturbance. In this study, the applicability of BOPA (benthic opportunistic polychaetes amphipods) index, AMBI (AZTI marine biotic index) and M-

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AMBI (multivariate AMBI) for monitoring the impact of pollution on soft-bottom macrobenthic communities of the Gulf of Milazzo was tested. The first index is easier to use than the other two indices. However, it needs the maximum taxonomic effort. For using BOPA index, it is necessary to recognize amphipods, distinguishing the opportunistic genera of amphipods *Jassa* from others and a reduced list of opportunistic polychaetes. M-AMBI is a multimetric index based on benthic macroinvertebrates, which integrates AMBI values with values of diversity and richness. Aims of this study are to assess the ecological status of the highly polluted Milazzo shelf through a multidisciplinary study, including chemical, physical and biological features, to examine relationships between biotic and abiotic compounds (seafloor features and contaminant distribution) and investigate the presence of nonindigenous species.

2. Materials and methods

2.1. Study area and sampling activity

Sampling was carried out along the narrow continental shelf of the Gulf of Milazzo, north-eastern coast of Sicily, Central Mediterranean Sea (Fig. 1). The Gulf is exposed to high anthropogenic pressure because of the presence of an international harbor, oil refineries, thermal power plant and shipbuilding industry. According to a census on the marine traffic associated with refinery activities (ISPRA, 2009), it was estimated that approximately 3256 vessels (66% chemical/oil product tanker; 15.86% crude oil tanker; 12.56% LGT tanker and 5.48% oil product tanker) have transited between 2006 and 2010 through the Gulf of Milazzo. The area is also subject to marine traffic associated with fishing and tourism (recreational ships, cruise ships and hydrofoils daily to the Aeolian Islands). In 2005, the industrial area of Milazzo was included in the list of Contaminated Sites of National Interest (Directive 23 December 2005 n. 266, art. 1 com. 561, national law in Italian language).

The study area is characterized by low water circulation, mainly driven by NW winds. The surface water circulation is defined by a branch of Modified Atlantic Water (MAW), which, flowing eastward, forms a near-coast anticyclonic gyre and generates an accumulation area in the northern part of the Gulf (Sitran et al., 2009). This area is also subject to the influence of three small rivers with irregular flows (Corriolo, Niceto, Muto and Saya Archi/Rio Cucugliata Streams).

Undisturbed soft-bottom samples were collected during the summer of 2010 by a 0.1-m² van Veen grab in 16 sampling sites, two of which, at a distance of about 10 km from the refinery, were chosen as control.

Sampling sites were located along two bathymetries, at depths of 20 and 50 m (Table 1). Four replicates were collected for each sampling site, for 64 samples, of which three were used for macrofaunal analysis and one for grain size and chemical analyses.

Sampling from hard bottom was carried out by scuba diving, scraping a surface of 400 cm² from three pillars of refinery, of which two were functional (P1 and P2) and one was disused (P3). Three samples were collected from each station at three different depths (0.5, 6.0 and 15.0 m), for a total of 35 samples (Table 1).

Benthic samples from soft and hard bottoms were sieved on board through a 0.5-mm mesh, and the retained material was fixed with 4% buffered formalin. After 48 h, the samples were transferred for a long-term storage to 70% ethanol.

2.2. Laboratory analyses

Grain size analysis was performed according to Buchanan and Kain (1971), the fraction above 63 μm was examined by American Society for Testing Materials (ASTM) series sieves with an interval of 1 Φ ($\Phi = -\log_2 \Phi$, mm), while the fraction below 63 μm (silt and clay) was analyzed by column dispersion method. Sediment types were classified according to the ternary Wentworth scale (Wentworth, 1922).

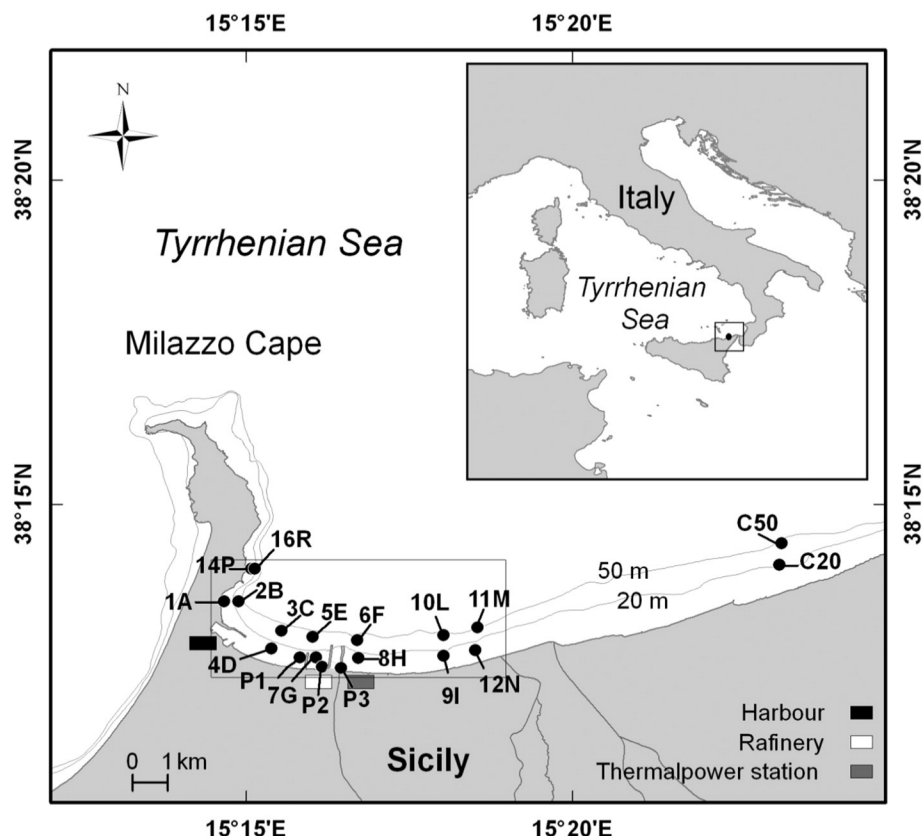


Fig. 1. Area of sampling, with sampling stations represented by black points and SIN zone by the selected area.

Table 1
Coordinate of soft and hard sites of samplings.

Latitude (°N)	Longitude (°E)	Site	Data sampling	Depth	Replicates	Sediment
38 13 500	15 14 659	1A	18/06/2010	20	3	Soft
38 13 500	15 14 884	2B	18/06/2010	50	3	Soft
38 13 050	15 15 537	3C	18/06/2010	50	3	Soft
38 12 775	15 15 387	4D	18/06/2010	20	3	Soft
38 12 953	15 16 015	5E	18/06/2010	50	3	Soft
38 12 900	15 16 700	6F	19/06/2010	50	3	Soft
38 12 638	15 16 070	7G	18/06/2010	20	3	Soft
38 12 630	15 16 720	8H	19/06/2010	20	3	Soft
38 12 663	15 18 023	9I	19/06/2010	20	3	Soft
38 12 974	15 18 023	10L	19/06/2010	50	3	Soft
38 13 095	15 18 546	11M	20/06/2010	50	3	Soft
38 12 750	15 18 512	12N	20/06/2010	20	3	Soft
38 14 001	15 15 075	14P	18/06/2010	20	3	Soft
38 14 000	15 15 130	16R	18/06/2010	50	3	Soft
38 14 065	15 23 170	W20	20/06/2010	20	3	Soft
38 14 393	15 23 200	W50	20/06/2010	50	3	Soft
38 12 388	15 15 492	Pillar1	13/09/2010	0.5, 6, 15	9	Hard
38 12 358	15 16 100	Pillar2	28/09/2010	0.5, 6, 15	9	Hard
38 12 339	15 16 254	Pillar2	14/09/2010	0.5, 6, 15	9	Hard

Chemical analyses were performed on lyophilized sediment samples. Trace elements (Al, Cd, Cr, Fe, Hg, Ni, Pb, Cu, Zn) were determined according to the United States Environmental Protection Agency (US EPA) methods (US EPA 3051A/2007; US EPA 6010C/2007; US-EPA Method 7010/2007; US-EPA Method 7473/2007). Limits of quantification of trace elements were 0.05 mg/kg dry weight (d.w.) for Al and 0.003 mg/kg d.w. for all other elements. Polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and hexachlorobenzene (HCB) were determined after extraction by the pressurized fluid extraction US-EPA 3545/A method (1996) using ASE 200 (Dionex®) instrument. Quantifications were performed as follows: i) PAHs were quantified by HPLC PDA/Fluorimeter (Waters); ii) PCBs were quantified by GC-FID/ECD (Clarus 200, PerkinElmer) according to the US-EPA method 8082A (2007); and iii) organochlorine pesticides and HCB were quantified by GC-FID/ECD (Clarus 200, PerkinElmer) according to the US-EPA method 8081B (2007). Results were expressed in mg/kg d.w. and were not recovery corrected. Limits of quantification of persistent organic pollutants were 0.001, 0.0001 and 0.0001 mg/kg d.w. for PAH single compounds, PCBs and OCPs, respectively.

Quality control and quality assurance analyses were performed using methodological blanks and standard reference materials (estuarine sediment SRM 1646a, Toronto Harbor sediment EC-4 and polychlorinated biphenyls in marine sediments HS-1). Limits of quantification were determined according to the Association of Analytical Communities (Shrivastava and Gupta, 2011) as the sum of mean concentration of the blank ($n = 10$) and 10 times the standard deviation of the blank, while the limit of blank was determined as the sum of mean of the blank ($n = 10$) and 1.645 times the standard deviation of the blank. For macrofaunal analysis, the samples were carefully washed in the laboratory and then hand-sorted under a stereomicroscope equipped with a micro-camera AxioCam Vs40 v4.8.20.

2.3. Data analysis

2.3.1. Biodiversity

The macrobenthic community was characterized by the following diversity indices for each sampling station: species richness (S), Shannon–Wiener diversity index (H') (Shannon and Weaver, 1963) and Pielou's evenness index (J). AMBI and M-AMBI indices were calculated using AMBI index software (version 4.0, available at www.azti.es). Species that are absent in the AMBI list were classified according to the literature and authors' knowledge on their ecological distribution. BOPA index and relative environmental quality were determined according to Dauvin and Ruellet's (2007) revised formula. Variations of BOPA index,

given the limits of the AMBI, to assess EcoQ were calculated according to Dauvin and Ruellet (2007).

2.4. Statistical analysis

2.4.1. Soft-bottom community

Univariate analysis by Pearson's correlation coefficient was performed to assess potential correlations between grain size and contaminant and grain size and heavy metals. For testing the most pertinent biotic indices in the Gulf of Milazzo, correlations between biotic indices AMBI, M-AMI and BOPA and trace elements and organic contaminants were also assessed.

Multivariate analyses were performed to assess potential correlations between the abundances of macrobenthic taxa and environmental variables (persistent organic pollutants (POPs) and trace elements concentration). Spatial pattern of macrobenthic community composition was assessed by using the Bray–Curtis similarity index (Bray and Curtis, 1957) on the square-root-transformed abundance matrix. Then, for the abiotic data, a similarity profile based on permutation was tested using the SIMPROF routine to group stations with similar (i.e. branch with $p > 0.05$) contaminant data. Groupings generated using the SIMPROF procedure were validated with a dissimilarity matrix based on the Euclidean distance, which was used in an agglomerative hierarchical clustering (routine CLUSTER). Differences between cluster groups were tested using permutation analysis of variance (PERMANOVA). The similarity percentage procedure (SIMPER) was used to identify the taxa that are most responsible for similarities and dissimilarities within and between clusters. A principal coordinates analysis (PCoA) was also performed to describe the contaminant data that most accounted for variation among the group identified by the SIMPROF procedure. The BIOENV procedure (BEST routine) was then applied to test the correlations between biotic and abiotic data.

Moreover, the relationships between the biodiversity indices (S, N and H') and contaminant concentrations were investigated using the Pearson correlation test.

Statistical analyses were performed using the PRIMER6 & PERMANOVA + software packages (Clarke and Warwick, 2001; Anderson et al., 2008).

2.4.2. Hard-bottom community

The biodiversity indices (S, J and H') were also calculated to describe the hard-bottom community. Moreover, PERMANOVA was performed to assess potential differences between the benthic community of the used (pillars 1 and 2) and nonused (pillar 3) refinery pillars. Abundance data were square-root transformed and analyzed on the basis of Gower distance using 4999 permutations. SIMPER test was performed to determine the contribution of each benthic category to average dissimilarity between groups.

3. Results

3.1. The abiotic environment: sediments and contaminants

Sandy silt sediments mainly characterized the study area; sands prevailed at 20 m, whilst silt prevailed at a depth of 50 m (Table 2). The highest percentages of sands were found at a depth of 20 m in the easternmost and innermost sites, with a general trend of increasing in the eastward direction. The silt fraction showed the highest percentages in the central sites at a depth of 50 m. A high percentage of pebble was locally found in site 5E at 50-m depth, outside the industrial complex. Results of chemical analysis (Table 2) showed the highest levels of PAHs in 3C and control 20. HCB showed the highest value in site 5E, while Σ PCBs in 8H. The highest DDT values were found in all stations at a depth of 20 m and in control 50. In general, trace element concentrations increased with increasing depth and decreasing grain size, except for Pb, which showed a peak in 1A. Significant positive

Table 2
Values of trace elements, organic contaminants (mg/kg d.w.) and grain size (% d.w.).

Site	Σ16PAH	ΣHCB	ΣDDT	ΣPCB	Fe	Cu	Al	Zn	Cr	Hg	Ni	Pb	Cd	Grain size %				
														Silt	Pebble	Gravel	Sand	Clay
1A	60.37	7.51	16.79	46.43	22,620.4	27.85	37,668.0	62.2	93.54	0.151	60.61	66.56	0.579	49.75	0.56	1.03	45.70	2.96
2B	70.96	1.88	7.00	69.16	26,213.5	32.95	46,070.8	73.3	92.87	0.139	59.18	18.15	0.813	52.03	4.95	4.11	36.88	2.03
3C	109.85	6.14	5.23	24.86	25,775.9	31.69	46,083.1	63.1	107.68	0.087	75.77	9.65	1.018	57.70	12.58	3.35	24.58	1.80
4D	<0.01	1.87	7.72	37.66	25,145.7	15.10	32,956.1	70.4	89.13	0.018	62.77	8.25	0.466	7.54	0.04	0.03	91.80	0.59
5E	<0.01	0.53	1.88	25.24	22,729.2	19.81	35,102.1	56.1	82.41	0.069	53.77	9.17	0.460	21.03	36.33	15.45	25.53	1.67
6F	9.59	0.92	0.75	2.73	26,575.8	38.91	50,325.7	71.6	115.72	0.048	179.77	8.75	0.533	78.47	<0.01	<0.01	18.78	2.75
7G	11.70	7.44	8.61	29.67	24,170.9	18.24	34,321.1	56.3	83.72	0.040	53.96	6.43	0.572	19.99	<0.01	<0.01	78.34	1.67
8H	14.82	3.46	18.17	93.19	23,082.0	23.21	35,044.7	52.2	81.30	0.026	62.15	7.30	0.492	35.41	<0.01	0.01	63.58	0.99
9I	30.56	4.68	1.16	18.97	22,257.9	12.23	32,191.5	48.1	72.76	0.020	49.77	6.90	0.349	25.45	<0.01	<0.01	72.09	2.46
10L	<0.01	8.35	3.62	19.95	24,828.9	31.10	42,685.2	61.9	98.50	<0.001	121.00	8.00	0.500	87.96	<0.01	<0.01	10.56	1.48
11M	66.43	3.62	2.36	16.35	25,706.8	28.60	48,710.5	65.2	97.74	0.051	63.18	9.37	0.597	82.50	<0.01	0.04	15.24	2.22
12N	74.91	3.49	9.64	17.93	21,471.5	9.14	29,339.6	44.8	61.82	0.040	38.70	6.12	0.491	10.33	0.48	0.41	87.58	1.20
14P	5.18	1.09	9.87	19.60	13,569.6	5.18	18,900.7	20.7	44.46	0.028	24.06	11.51	0.718	8.87	2.21	33.13	54.14	1.65
16R	1.05	4.64	1.89	27.43	16,326.4	12.00	26,208.6	32.9	48.52	0.055	36.08	12.46	0.437	21.84	17.61	13.61	44.67	2.27
W20	116.26	8.73	4.79	19.90	16,427.0	2.97	20,693.6	34.7	47.24	0.007	25.89	3.40	0.297	<0.01	<0.01	0.05	99.95	<0.01
W50	39.04	5.40	11.91	20.53	19,955.8	12.70	30,920.0	45.2	56.16	0.017	45.53	7.13	0.380	51.76	0.03	0.06	45.84	2.31

correlations ($p < 0.05$) were found between silt fraction and Fe, Cu, Al, Zn, Cr, Hg, Ni, Pb and Cd and between clay and Cu, Al, Hg and ΣPCBs; significant negative correlations ($p < 0.05$) were found between gravel and HCB, Fe, Al, Cr and Zn and between sand and Cu, Al, Cr, Hg, Ni and Cd. In general, trace element concentrations decreased in the following order: Al > Fe > Ni > Cr > Zn > Pb > Cu > Cd > Hg > As (Table 2).

3.2. The biotic environment

3.2.1. Soft-bottom community

A total of 2739 specimens, belonging to 246 taxa were found. The highest number of taxa (Annex 1) was recorded among class of Polychaeta (44.72%), followed by Mollusca (28.45%), Crustacea (23.58%), Echinodermata (2.03%) and Sipuncula (0.12%). The most abundant species were the molluscs *Timoclea ovata* ($N\% = 19.39$), *Lucinella divaricata* ($N\% = 8.58$), *Abra alba* ($N\% = 3.36$) and the non-indigenous polychaete *Notomastus aberans* ($N\% = 2.88$). Average values of the diversity indices for all sampling stations are reported in Table 3. Control 20 station recorded the highest value of species richness ($S = 83$) and the Shannon–Wiener diversity index ($H' = 3.0$), whilst station 2B, with 19 identified taxa, recorded the lowest corresponding values ($H' = 1.1$). The highest number of specimens was recorded in station 12N. Values of AMBI index ranged between 0.621 (2B) and 2.11 (5E). The software calculated a degree of disturbance 'Slight' for all stations, except for 12N, 3C, 2B and control 20, which showed 'Undisturbed' degree. M-AMBI ranged between 0.498 (3C) and 0.949 (control 20). The EcoQ resulted 'Good' for each station, except for control 20 and

5E, which showed 'High'. BOPA index showed the lowest value (0.0126) in station 4D, whilst the highest value (0.1040) in 8H. The calculation of EcoQ according the formula of Dauvin and Ruellet (2007) assigned a 'Good' status except for stations 2B, 14P, 16R, control 20, control 50 and 12N, where the EcoQ resulted 'High' (Table 3). (See Table 4.)

3.2.2. Hard-bottom community

A total of 670 specimens belonging to 84 taxa were found by scraping. Crustacean was the most represented ($N\% = 53.13$), followed by molluscs ($N\% = 29.55$) and polychaetes ($N\% = 17.31$). The most abundant species was the Maeridae, *Elasmopus rapax* ($N\% = 29.55$) (Annex 1). Two naturalized nonindigenous species, the Mytilidae, *Brachidontes pharaonis* ($N\% = 4.18$) and the Ostreidae, *Crassostrea gigas* ($N\% = 0.75$) were found (Annex 1). The highest values of species richness (S) and biodiversity index (H') were recorded in P2, whilst P3 recorded the highest abundance of individuals (N).

3.3. Statistical analysis

3.3.1. Soft bottom

Concerning the relationships between macrobenthic abundances and contaminants, the similarity test based on SIMPROF analysis of contaminant data revealed three distinct groups (a, b and c) ($p < 0.01$) confirmed via hierarchical cluster analysis (Fig. 2). Their characterization is shown in Table 5.

The PCO1 axis (Fig. 2) accounted for 97.6% of the total observed variation and clearly separated the three groups. PCO2 axis gathered 2.4%

Table 3
Values of biodiversity (S , N , J' and H'), biotic indices (AMBI, M-AMBI and BOPA) and corresponding disturbance/EcoQ values of soft-bottom sites.

Site	S	N	J'	H'	AMBI	Disturbance	M-AMBI	EcoQ	BOPA	EcoQ
1A	58	37	0.9	2.9	1.634	Slight	0.83773	Good	0.0963	High
2B	19	12	0.7	1.1	0.622	Undisturbed	0.64043	Good	0.0223	Good
3C	35	23	0.9	2.3	1.143	Undisturbed	0.498	Good	0.0482	High
4D	42	90	0.7	1.9	1.892	Slight	0.66255	Good	0.0126	High
5E	73	67	0.9	3.0	1.228	Slight	0.88981	High	0.0532	High
6F	56	52	0.9	2.9	2.011	Slight	0.78613	Good	0.0801	High
7G	47	83	0.8	2.4	1.168	Slight	0.528472	Good	0.0748	High
8H	32	39	0.9	2.2	1.792	Slight	0.65347	Good	0.1040	High
9I	46	66	0.9	2.5	1.738	Slight	0.73971	Good	0.0643	High
10L	56	60	0.9	2.7	1.739	Slight	0.80453	Good	0.0802	High
11M	43	44	0.9	2.3	1.844	Slight	0.72903	Good	0.0687	High
12N	53	162	0.6	2.0	0.487	Undisturbed	0.74471	Good	0.0214	High
14P	57	38	0.9	2.9	1.353	Slight	0.84761	Good	0.0461	Good
16R	30	18	0.9	2.1	1.296	Slight	0.67817	Good	0.0620	Good
Control 20	83	100	0.9	3.0	1.199	Undisturbed	0.94976	High	0.0245	Good
Control 50	29	22	0.9	2.1	1.235	Slight	0.67905	Good	0.0247	Good

Table 4
Values of biodiversity (S, N, J' and H') of hard-bottom sites.

Site	S	N	J'	H'
pillar1 (1–1)	22	25.5	0.9350	2.306
pillar1 (1–2)	18	41.0	0.8615	2.204
pillar1 (2–1)	16	12.5	0.9705	1.756
pillar1 (2–2)	7	13.0	0.9479	1.845
pillar1 (3–1)	7	4.0	0.9732	1.213
pillar1 (3–2)	18	15.0	0.9321	2.110
pillar2 (1–1)	10	22.0	0.7513	1.398
pillar2 (1–2)	14	26.0	0.8047	1.6975
pillar2 (2–1)	22	13.5	0.9734	2.3125
pillar2 (2–2)	15	11.5	0.9300	1.5258
pillar2 (3–1)	7	5.0	0.9359	0.8385
pillar2 (3–2)	23	20.0	0.9419	2.3665
pillar3 (1–1)	22	47.5	0.8634	2.248
pillar3 (1–2)	20	29.0	0.8740	2.1245
pillar3 (2–1)	13	15.5	0.7984	1.4793
pillar3 (2–2)	22	26.0	0.8511	2.049
pillar3 (3–1)	8	5.5	0.9878	1.567
pillar3 (3–2)	9	9.0	0.8166	1.2822

of the total variation. The superimposed vectors showed three sets of contaminants, each associated with one of the group.

Group 'a' included almost all sampling stations located at a depth of 50 m (2B, 3C, 6F, 10L, 11M) and characterized by the highest concentration of trace elements and Σ PAHs (Fig. 2; Table 5). This group comprised 148 macrobenthic species, 34 of which were exclusively found in the sampling stations (Fig. 3). The most characteristic species of this group were *Arabella iricolor*, *Magelona minuta*, *Lumbrineris latreilli*, *N. aberans* and *Pseudoleiocapitella fauveli*. Group 'b' comprised almost all sampling stations located at a depth of 20 m (1A, 4D, 5E, 7G, 8H, 9I and 12N) and the control at 50 m depth (control 50), and recorded the highest concentrations of Σ DDT and Σ PCB (Fig. 3; Table 5) and the highest number of identified species (169), 46 of which were exclusive of this group. In this group, the following characteristic species were found: *T. ovata*, *A. alba*, *Micronephthys stammeri*, *Aricidea (Aricidea) pseudoarticulata* and *Cossura sp.* Finally, group 'c' included only three sampling stations (14P, 16R and control 20) characterized by the lowest value of contaminant concentrations, except for HCB, which recorded the highest value

Table 5
Affinity group according to relationships between macrobenthic abundances and contaminants.

	Affinity group	a	b	c	
Trace elements	Al (mg/kg d.w.)	46,775.06	33,442.89	21,934.30	
	Fe (mg/kg d.w.)	25,820.18	22,679.18	15,441.00	
	Zn (mg/kg d.w.)	67.02	54.41	29.43	
	Cr (mg/kg d.w.)	102.50	77.61	46.74	
	Ni (mg/kg d.w.)	99.78	53.41	28.68	
	Pb (mg/kg d.w.)	10.78	14.73	9.12	
	Cu (mg/kg d.w.)	32.65	17.29	6.72	
	Cd (mg/kg d.w.)	0.69	0.47	0.48	
	Hg (mg/kg d.w.)	0.08	0.05	0.03	
	Persistent contaminants	Σ 16PAH (mg/g d.w.)	64.21	38.57	40.83
Σ HCB (mg/g d.w.)		4.18	4.30	4.82	
Σ DDT (mg/g d.w.)		3.79	9.49	5.52	
Σ PCB (mg/g d.w.)		26.61	36.20	22.31	
Sediment characteristics	Pebble (%)	3.51	4.68	6.61	
	Gravel (%)	1.50	2.12	15.60	
	Sand (%)	21.21	63.81	66.25	
	Silt (%)	71.73	27.66	10.24	
	Clay (%)	2.06	1.73	1.31	
	Median (Φ)	5.28	3.10	1.52	
	Depth (m)	50.00	27.50	30.00	
Biotic characteristics	Total species richness	148	169	122	
	N. of exclusive species	34	46	34	
	Most characteristic species (>40% similarity)	<i>Arabella iricolor</i>		<i>Timoclea ovata</i>	<i>Timoclea ovata</i>
		<i>Magelona minuta</i>		<i>Abra alba</i>	<i>Aponuphis bilineata</i>
<i>Lumbrineris latreilli</i>			<i>Micronephthys stammeri</i>	<i>Lucinella divaricata</i>	
<i>Notomastus aberans</i>			<i>Aricidea (Aricidea) pseudoarticulata</i>	<i>Goniada maculata</i>	
<i>Pseudoleiocapitella fauveli</i>		<i>Cossura sp.</i>	<i>Scoloplos (Scoloplos) armiger</i>		

and the lowest number of macrobenthic species (122). The characteristic species of the group were *T. ovata*, *Aponuphis bilineata*, *L. divaricata*, *Goniada maculata* and *Scoloplos (Scoloplos) armiger*.

The BEST analysis, implemented through the BIOENV protocol, revealed no significant correlation between contaminant concentrations and spatial distribution of macrobenthic fauna within the sampling stations. Results of Pearson test evidenced a significant negative correlation between the concentration of Σ PCBs and specific richness ($p < 0.10$). No other significant correlation was found among contaminant concentrations and biodiversity indices.

3.3.2. Hard bottom

Results of PERMANOVA evidenced no significant differences between benthic communities of used and disused pillars.

4. Discussion

Because of the link between coastal cities and seas, coastlines have often been subjected to heavy anthropogenic pressure, causing pollution of water and sediments (Estacio et al., 1997; Adami et al., 2000; Fadeeva et al., 2003). Sediments represent one of the ultimate sinks for the discharge of heavy metals into the environment (Gibbs, 1977; Luoma and Bryan, 1981; Bettinetti et al., 2003). The background concentration of hazardous substances in sediments is an important factor for evaluating the degree of contamination. Pollutants are discharged into the environment by both anthropogenic activities and natural processes, such as weathering of rocks and volcanic activities, which play a significant role in enriching the reservoirs with heavy metals (Nriagu, 1989; Veena et al., 1997). Streams are also among the main source of environmental pollutants (Alexander et al., 2000; Alam and Pathak, 2010), which is also confirmed by our data. This study shows the presence of some hotspots of metals and contaminants near mouths, testifying their unnatural role of waste receptacle (Humborg et al., 1997). Another factor responsible for contaminant accumulation is grain size of the sediment, which, in the Gulf of Milazzo, is mostly represented by fine sediment (Pepe et al., 2010) that is mainly subject to absorption of pollutants and heavy metals (Salomons and Stigliani, 2012; Farkas et al., 2007). In particular, this study shows an increase of mud fraction

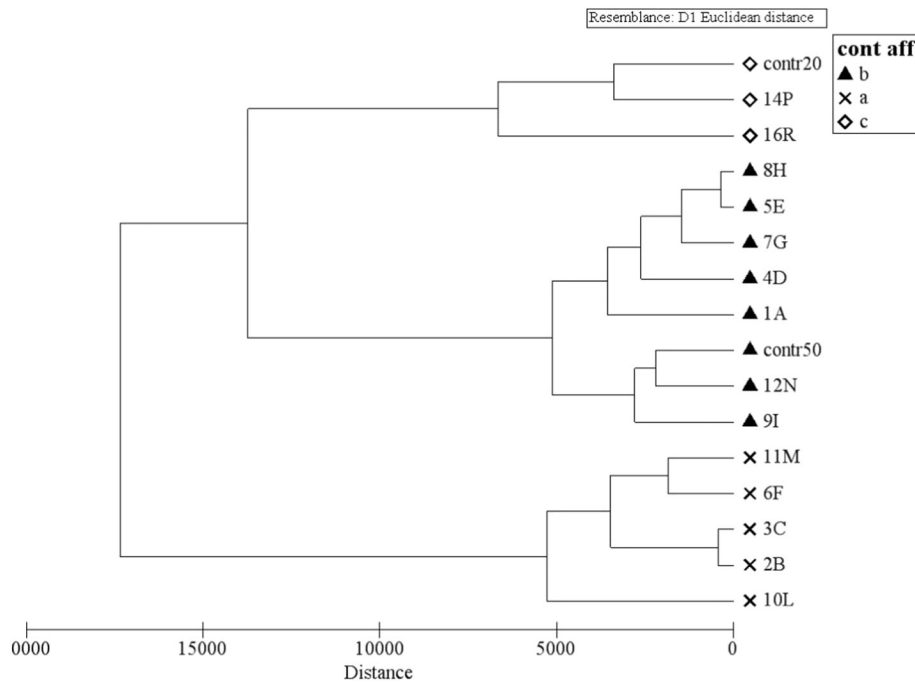


Fig. 2. Spatial distribution of three affinity groups along the study area.

westwards, reaching the maximum in front of the harbor and close to the crude oil refinery, which also represented the area in which some of the contaminant values are slightly higher than those in other sites. Nevertheless, values of trace metals and metals recorded in this study showed low levels of contamination according to regulations of reference (Annex 5 to Part IV of Legislative Decree no. 3 April 2006, n. 152; Annex A of DM 367/2003).

Human activities along the Gulf are responsible for the accumulation of pollutants in sediments and interstitial water within them. Several pollutants tend to accumulate in sediments and biota, because of their low water solubility and persistence, affecting the marine ecological health (Romeo et al., 2015). This is also confirmed by this study, which shows the highest negative effect of Σ PCBs on

species richness of the invertebrates' community. In addition, the high concentration of anthropogenic activities in this study testifies that Milazzo values of pollution are lower than other areas of the Mediterranean Sea, such as Cogoletto (Bertolotto et al., 2003), Trieste (Notar et al., 2001), Napoli (Sprovieri et al., 2007) and Bagnoli (Romano et al., 2004). In general, the values of organic contaminants and trace elements found along the area of study showed no substantial difference between the heavily anthropized area and control site. The latter, in fact, even if placed at a higher distance from the heaviest human activities, appears to be influenced by small coastal activities such as fishing and agriculture. No difference was recorded also among the values of biodiversity between used and disused pillars.

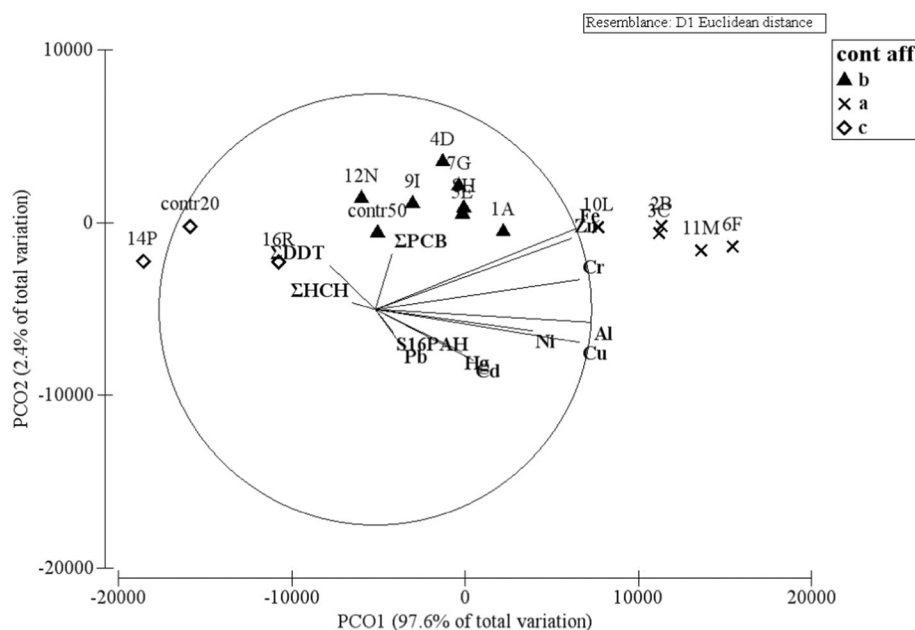


Fig. 3. Cluster analysis (A) and principal coordinates analysis (PCoA) based on the abundance community with the representation of affinity groups, superimposing the environmental factors (B) and species abundance (right-hand side of B).

The structure of macrobenthos community-recognized indicator currently used for the assessment of EcoQ (Goodnight, 1973; Borja et al., 2000; Hilty and Merenlender, 2000; Klemm et al., 2002) seems to not underline evident signs of stress. This is also confirmed by statistical analysis, which subdivided the area of study into three groups, each characterized by species with different ecological values, testifying a well-structured community (Pearson and Rosenberg, 1987; Borja et al., 2000). Most abundant species found in soft-bottom samples (*T. ovata*, *L. divaricata*) are considered by Borja et al. (2000) as 'very sensitive organism' to organic enrichment, typical of under unpolluted status. However, the presence of three nonindigenous species (*N. aberans*, *B. paraonis* and *C. gigas*) and two first records of polychaetes (*M. stammeri* and *Nicomache lumbricalis*) testify the particular environmental status of harbors. These areas, in fact, play a role as hotspots for marine species invasions, because of both a direct link with human activities (shipping and tourism) and instability of these particular environments that facilitate the advantage of opportunistic and alien species (Occhipinti-Ambrogi et al., 2011; D'Alessandro et al., 2015). Although several studies reported a close relationship between benthic communities and level of pollutants (Simboura et al., 2000; Gray and Elliott, 2009; Martins et al., 2013a,b), such a relationship was not found in this study. This may be underlined as a generalized homogeneous environmental condition without an evident site of stress.

The indices of biodiversity calculated in the Gulf of Milazzo showed values higher than other Mediterranean harbors with similar human activities, such as Genoa Voltri, Portosole, Marina degli Aregai (Moreno et al., 2008), Trieste (Afi et al., 2009) and Taranto (Maiorano et al., 2011). In this study, the applicability of three most common indices used in coastal and estuarine waters: AMBI, M-AMBI and BOPA

(Muxika et al., 2005; Dauvin and Ruellet, 2007; Borja et al., 2009) is examined. Calculation of biotic index showed elevated levels of EcoQ, which ranged between Slight–Undisturbed of AMBI and Good–High of M-AMBI and BOPA. These data confirm that the heavily anthropized marine invertebrates do not seem to be subjected to this type of pollution. By Pearson correlation between benthic indices versus trace elements and benthic indices versus organic contaminant, M-AMBI index seems to be the one with highest applicability to the environment of the Gulf of Milazzo. The correlation between pollutants and benthic community has also been affirmed by several studies that show sediments as contaminants that affect several levels of biological organization of benthos community (Luoma and Phillips, 1988; Shoults-Wilson et al., 2015; Signa et al., 2015).

5. Conclusions

The complexity of ecological systems influenced by human activities makes necessary the use of a multidisciplinary approach to accurately evaluate environmental status. Despite the presence of several human activities, our analysis testifies that the environment does not seem to show accentuate signs of stress. This study is the first of its kind to present a list of macro-invertebrate communities of the Gulf of Milazzo. This study is also recognized as the first multidisciplinary study conducted in the heavily human-stressed area of the Gulf of Milazzo. According to the results obtained, further studies are needed to reevaluate the limits of extension of the currently assigned SIN (site of national interest) zone of the Milazzo site, redimensioning the extension of the marine area, which seems to be the sector that is influenced lesser than the terrestrial and aerial environments by human activities.

Appendix Annex 1. Faunistical list of species found in hard and soft bottoms

	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3
Crustacea																			
Leptocheiliidae																			
<i>Leptocheilia savignyi</i>						3		1											
Leptocheiliidae																	4	1	2
Eriphiidae																			
<i>Eualus pusiolus</i>																			1
<i>Eualus occultus</i>																		3	
<i>Eualus cranchii</i>																	7	1	4
Stenothoidae																			
<i>Stenothoe elachista</i>																	16	10	15
<i>Stenothoe eduardi</i>																		1	
<i>Stenothoe gallensis</i>																	2	2	3
<i>Stenothoe tergestina</i>																	1		
<i>Stenothoe valida</i>																	3		4
<i>Stenothoe sp</i>																			
Maeridae																			
<i>Elasmopus affinis</i>																			2
<i>Elasmopus brasiliensis</i>																		1	
<i>Elasmopus pectenicrus</i>																		2	
<i>Elasmopus rapax</i>																	29	8	33
<i>Maerella tenuimana</i>																		1	
Ischyroceridae																			
<i>Erichthonius punctatus</i>																	2	3	16
<i>Jassa marmorata</i>																		1	
Apseudidae																			
<i>Apseudes spinosus</i>	3			1								1		1					
<i>Apseudes latreillei</i>				9		2								1					
Xantidae																			

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(continued)

	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3
<i>Paractaea monodi</i>																	2		1
Paguridae																			
<i>Pagurus bernhardus</i>																	5	3	5
Galatheidae																			
<i>Galathea strigosa</i>																	1	1	
Anthuridae																			
<i>Anthura gracilis</i>	4			1										3		7			1
<i>Isopoda sp.</i>																		1	
Pilumnidae																			
<i>Pilumnus hirtellus</i>																	10	3	26
<i>Pilumnus villosissimus</i>																	1		
Epialtidae																			
<i>Acanthonyx lunulatus</i>																	6		1
Grapsidae																			
<i>Pachygrapsus marmoratus</i>																	1		
Gnathiidae																			
<i>Gnathia phallonajopsis</i>									4		3								
<i>Gnathia dentata</i>											1					11			
Cirolanidae																			
<i>Cirolanidae</i>							1												
<i>Eurydice pulchra</i>					1														
Mysidacea																			
<i>Mysidacea</i>														2				1	
Dyastilidae																			
<i>Diastylis</i>		4																	
<i>Diastylis rugosa</i>		1																	
<i>Dyastilidae</i>				3															
Bodotridae																			
<i>Bodotridae</i>		1																	
<i>Iphinoe sp</i>	3	2				1					1				2				
Cumacea			1						1		3				7				
Corophiidae																			
<i>Corophium aculeatum</i>									2					3					
<i>Apocorophium acutum</i>																			3
<i>Ampithoe ramondi</i>																			1
<i>Corophium rotundirostre</i>											1				2				
Atylidae																			
<i>Nototropis guttatus</i>																		1	1
Gammaridae																			
<i>Cheirocratus sundevalli</i>							1												
Leucothoidae																			
<i>Leucothoe serraticarpa</i>	1										1	1		1		1			
<i>Leucothoe spinicarpa</i>																		1	1
<i>Leucothoe venetiara</i>																		1	6
<i>Leucothoe oboa</i>			1												1				
Ampeliscidae																			
<i>Ampelisca typica</i>																			1
<i>Ampelisca multispinosa</i>		1																	
<i>Ampelisca tenuicornis</i>	1								1		2			2	5				
<i>Ampelisca pseudosarsi</i>													2	1					
<i>Ampelisca sarsi</i>											2			2					
<i>Ampelisca brevicornis</i>														4					
<i>Ampelisca sp</i>	1																		
Aoridae																			
<i>Leptocheirus pectinatus</i>	1						3				5				1				
<i>Leptocheirus mariae</i>			1			1						1			4	1			
<i>Aora gracilis</i>								1											
<i>Lembos spiniventris</i>				1		2								2					
Lysianassidae																			
<i>Pardia punctata</i>							1	1						1					
<i>Lepidocream longicorne</i>														1					
<i>Hippomedon massiliensis</i>			1											2					
Phoxocephalidae																			
<i>Phoxocephalidae</i>			2		2	1			10		12				5				
Oedicerotidae																			
<i>Perioculodes longimanus</i>			1								6			14					
<i>Synchelidium haplocheles</i>							1												
Haustoriidae																			
<i>Urothoe pulchella</i>															7				
<i>Urothoe grimaldii</i>															2				
Pontoporeiidae																			
<i>Bathyporeia phaiophthalma</i>															3				
Caprellidae																			
<i>Phtisica marina</i>															1				

(continued)

	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3	
<i>Pseudoprotella phasma</i>																	1		1	
Caprellidae																		1	1	
Photidae																				
<i>Photis longicauda</i>	3											1		22	1					
<i>Photis longipes</i>				2										8						
Crangonidae																				
<i>Philocheras trispinosus</i>														1						
Processidae																				
<i>Processa edulis</i>						1					1	1	1				1			
Penaeidae																				
<i>Parapenaeus longirostris</i>			1																	
Alpheidae																				
<i>Alpheus glaber</i>									1		1						15		12	
<i>Alpheus macrocheles</i>																	2	6	6	
<i>Athanas nitescens</i>																	2	5	3	
<i>Synalpheus gambarelloides</i>																	2		2	
<i>Eriphia verrucosa</i>																	2	1	4	
Goneplacidae																				
<i>Goneplax rhomboides</i>			1	12							3				1					
Polybiidae																				
<i>Liocarcinus maculatus</i>																			1	
Inachidae																				
<i>Achaeus gracilis</i>						1														
Callianassidae																				
<i>Callinassa sp.</i>																			1	
<i>Callinassa truncata</i>				12															2	
Upogebiidae																				
<i>Upogebia tipica</i>									2											
Paguridea																				
<i>Anapagurus petiti</i>		1					2								1	1				
Diogenidae																				
<i>Clibanarius erythropus</i>							2	2												
Stomatopoda																				
Copepoda									1											
Ostracoda																			2	
Amphiuridae																				
Echinodermata																				
<i>Amphiura chiajei</i>	1	2			2	1		1	2				1		1	1				
<i>Amphiura filiformis</i>		2	1						2		1	3	3	1	1					
Ophiuridae																				
<i>Ophiura ophiura</i>						1		1								1				
Astropectinidae																				
<i>Astropecten irregularis</i>						1											1			
Synaptidae																				
<i>Labidoplax digitata</i>			1								6			1		1				
Caecidae																				
<i>Caecum trachea</i>	1																			
Chamidae																				
<i>Chama gryphoides</i>																		2	3	5
Haminoeidae																				
<i>Atys rochii</i>	1																			
Calyptraeidae																				
<i>Calyptraea chinensis</i>		1																		
Iravadiidae																				
<i>Hyalia vitrea</i>																1				
Naticidae																				
<i>Tectonatica sagraiana</i>		1								1		1								
<i>Euspira pulchella</i>	1							1												
Eulimidae																				
<i>Nanobalcis nana</i>						1														
Mangeliidae																				
<i>Bela nebulosa</i>	1																			
Skeneinae																				
<i>Dikoleps nitens</i>																			1	
Nassariidae																				
<i>Nassarius mutabilis</i>																			1	
Rissoidae																				
<i>Peringiella elegans</i>											6	1								
Haminoeidae																				
<i>Weinkauffia turgidula</i>								1											1	
Pyramidellidae																				
<i>Odostomia conoidea</i>																			1	
<i>Eulimella turris</i>	2															3				
<i>Chrysallida flexuosa</i>								1												

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(continued)

	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3
<i>Turbonilla acuta</i>	1																		
Retusidae																			
<i>Retusa mammillata</i>				1							1	1				1			
<i>Cylichna nitidula</i>																1			
<i>Retusa leptoeilema</i>	3																		
Cylichnidae																			
<i>Cylichna crossei</i>														1					
Ringiculidae																			
<i>Ringicula conformis</i>																	1		
Philinidae																			
<i>Philina scabra</i>											1								
<i>Philina aperta</i>	7	2										2		1		1			
Spondyliidae																			
<i>Spondylus gaederopus</i>																	2	1	
Cerithiidae																			
<i>Bittium latreillii</i>																		9	11
Littorinidae																			
<i>Littoraria intermedia</i>																		1	
Patellidae																			
<i>Patella caerulea</i>																		7	
Ostreidae																			
<i>Crassostrea gigas</i>																	5		
<i>Ostrea edulis</i>																	9	1	2
Noetiidae																			
<i>Striarca lactea</i>																	4	2	3
Mytilidae																			
<i>Brachidontes pharaonis</i>																	13	8	1
<i>Gregariella semigranata</i>																	1		
<i>Modiolus barbatus</i>																	1		
<i>Musculus costulatus</i>																	2	10	7
<i>Mytilaster solidus</i>																		17	
<i>Mytilus galloprovincialis</i>																		1	
Arcidae																			
<i>Arca noae</i>																	11	1	5
<i>Arca tetragona</i>																		4	1
<i>Barbatia barbata</i>																			1
Gastrochaenidae																			
<i>Roccellaria dubia</i>																			1
Nuculidae																			
<i>Nucula nitidosa</i>	1									1			1	2					
<i>Nucula sulcata</i>			1																
<i>Nucula sp.</i>									1										
<i>Ennucula aegeensis</i>														1					
<i>Nuculana fragilis</i>													1						
<i>Nuculana pella</i>		3		1	2				1							6			
Anomiidae																			
<i>Anomia ephippium</i>															1		3	1	1
Lucinidae																			
<i>Anodontia fragilis</i>						1		1				1							1
<i>Ctena decussata</i>								2											
<i>Lucinella divaricata</i>	44				13	6	1	5		2		21	4	1	3	135			
Thyasiridae																			
<i>Thyasira biplicata</i>			1																
<i>Thyasira alleni</i>	1		1	1						1	6	14	5		13	2			
<i>Leptaxinus subovatus</i>									1										
Carditidae																			
<i>Glande aculeata</i>								1											
Pharidae																			
<i>Sinupharus africanus</i>		1						2						1					
Montacutidae																			
<i>Epilepton clarkiae</i>				1															
<i>Kutiella bidentata</i>												2		3	1	2			
Astartidae																			
<i>Astarte sulcata</i>							1												
<i>Digitaria digitaria</i>														2					
Cardiidae																			
<i>Acanthocardia paucicostata</i>			1									1		4					
<i>Laevicardium crassum</i>						1													
<i>Parvicardium minimum</i>		1																	
<i>Plagiocardium papillosum</i>		2																	1
Mactridae																			
<i>Spisula subtruncata</i>	24	1		3										5		2			
Lottiidae																			
<i>Tectura virginea</i>																			1
Tellinidae																			

(continued)

	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3
<i>Tellina distorta</i>	21			6									10	17		7			
Limidae																			
<i>Limatula Gwyni</i>							1												
Psammobiidae																			
<i>Gari fervensin</i>	1			1									3			4			
Semelidae																			
<i>Scrobicularia cottardi</i>				37															
<i>Abra alba</i>	12	2		40		1		2	2	2	1	11		3	6	10			
<i>Abra nitida</i>				1							3		1						
<i>Abra prismatica</i>				1								3		3	2				
<i>Azorinus chamasolen</i>																			1
Veneridae																			
<i>Cardites antiquata</i>								1											
<i>Gouldia minima</i>	2					2		1											
<i>Dosinia lupinus</i>	2																		2
<i>Petricola lajonkairii</i>	2																		
<i>Pitar rudis</i>														3					
<i>Callista chione</i>														2					
<i>Timoclea ovata</i>	31	53	19	77		9	16	7	16		2	28	17	48	10	198			
<i>Chamelae gallina</i>								1											
Corbulidae																			
<i>Corbula gibba</i>	7	2		2					2					2	1	10			
Hiatellidae																			
<i>Hiatella rugosa</i>						1													
<i>Hiatella arctica</i>																			6
Thraciidae																			
<i>Thracia papyracea</i>	4	1											3						
Cuspidariidae																			
<i>Cuspidaria rostrata</i>											1								
<i>Cardiomya costellata</i>		2																	
Ungulinidae																			
<i>Diplodonta intermedia</i>		1																	
Polychaeta																			
Ampharetidae																			
<i>Ampharete grubei</i>		2						2						1	1	2	1		1
<i>Melinna palmata</i>								1											
Capitellidae																			
<i>Capitellidae sp.</i>														2					
<i>Capitella capitata</i>		1						3											
<i>Capitomastus minimus</i>	3	1																	
<i>Dasybranchus gajolae</i>					1														1
<i>Heteromastus filiformis</i>	2	1							4		1				6				
<i>Mastobranthus trinchessii</i>							1	1											
<i>Notomastus aberans</i>	8	2	1	4		2	1			26	2	15	1	1		16			
<i>Notomastus latericeus</i>		2				1				1						2			
<i>Notomastus sp.</i>								1											1
<i>Notomastus sp.</i>										5		5		4		4			1
<i>Peresiella sp.</i>						1													
<i>Pseudoleiocyprina fauveli</i>		2	1			6	1	1	3	1	2			1	1				
Cossuridae																			
<i>Cossura</i>		4	3		1	1	1	1	3		2	7	1		1				
Aphroditidae																			
<i>Aphroditidae sp.</i>																			1
Ophelidae																			
<i>Polyopthalmus pictus</i>																			1
Phyllodoceidae																			
<i>Phyllodoce lineata</i>																	0	1	2
Chrysopetalidae																			
<i>Chrysopetalum debile</i>								1											
Polinoe																			
<i>Harmothoe fraserthomsoni</i>																			1
Polinoidea																			1
Dorvilleidae																			
<i>Dorvilleidae</i>											1								
Eunicidae																			
<i>Eunicidae sp.</i>						2	2												
<i>Eunice vittata</i>	2	3				3	1					1				1			
<i>Marphysa bellii</i>	3			2	1		1	1		1		2				4			
<i>Palola siciliensis</i>																		3	1
<i>Nematonereis unicornis</i>			1		1	3		2						1					
Flabelligeridae																			
<i>Armandia cirrhosa</i>		4	2						2		3								
<i>Flabelligera sp.</i>		3		1		1									1				
<i>Pherusa plumosa</i>						1													

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	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3
Glyceridae																			
<i>Glycera alba</i>		3		1	2	1			9		1	1			4				
<i>Glycera capitata</i>		1									2								
<i>Glycera fallax</i>		3	3																
<i>Glycera lapidum</i>		1				1	2						2		1				
<i>Glycera tessellata</i>													1						
<i>Glycera tridactyla</i>		1		2				2	3				1		3		1		1
<i>Glycera unicornis</i>		1				1			1	3			1		1				
Goniadidae																			
<i>Goniada maculata</i>	3				1					12	1		1	3		7			
<i>Goniadidae sp.</i>								1				1				2			
Hesionidae																			
<i>Kefersteinia cirrata</i>		1						3	1										
Lumbrineridae																			
<i>Abyssoninoe bidentate</i>						1							2		1				
<i>Abyssoninoe hibernica</i>	3	1				2			1	6	2	2	2			2			
<i>Gallardoneris iberica</i>		1		4		2		6	3	1		1		4	1	4			
<i>Lumbrineris funchanlensis</i>						2								2					
<i>Lumbrineris geldiaiyi</i>		4		2	1			5	3	2	13	3	4						
<i>Lumbrineris latreilli</i>		2	2	8		4	1	4	1	8	8								
<i>Lumbrineris luciliae</i>		2		1				1			1								
<i>Lumbrineris lusitanica</i>		1		3						3						2			
<i>Lumbrineriopsis paradoxa</i>								1											
<i>Lumbrineris impatiens</i>														7					
<i>Scoletoma emandibulata mabiti</i>	1																		
Magelonidae																			
<i>Magelona minuta</i>		2	4		1	3		3	12	4	15	1		1		2			
<i>Praxillella gracilis</i>					1				3		1								
Maldanidae																			
<i>Maldane sarsi</i>					1	2			1		1								
<i>Maldanidae sp.</i>	1	1	1			1	2							1					
<i>Nicomache lumbricalis</i>			1			1		4	2	1				1		1			
Nereididae																			
<i>Nereididae sp.</i>		1						1		1			1			1	1	1	
Nereididae																			
<i>Ceratonereis (Composetia) costae</i>																	8	3	4
<i>Pseudonereis sp.</i>								1											
<i>Websterinereis glauca</i>										1									
Nephtyidae																			
<i>Micronephthys stammeri</i>	3		1	4		3	3	8	1			7		9		6			
<i>Nephtys hombergii</i>				2		1				2		1		1					
<i>Nephtys sp.</i>						1							1						
Oeonidae																			
<i>Arabella iricolor</i>		6	4	1		7			2	7	4	2		3	1				
Onuphidae																			
<i>Aponuphis bilineata</i>	3	1		3			1	2	2	2		3	3	4		6			
<i>Hyalinoecia brementi</i>			2		1			1					1			1			
<i>Hyalinoecia fauveli</i>						3			2										
<i>Hyalinoecia sp.</i>		2																	
<i>Onuphidae spp.</i>					1			1											
Orbiniidae																			
<i>Scoloplos (Scoloplos) armiger</i>	4			6		1				8	2	3	1	16		2			
<i>Schroederella laubieri</i>								8						2		1			
Paralacydoniidae																			
<i>Paralacydonia paradoxa</i>		4	4						1		1	1	2						
Paraonidae																			
<i>Aricidea capensis bansei</i>		1		1					1										
<i>Aricidea (Aricidea) pseudoarticulata</i>	2			3		2	1		3	8	1	4	2	1	9	9			
<i>Aricidea cerrutii</i>														6					
<i>Cirrophorus branchiatus</i>	1		1						1	1									
<i>Cirrophorus furcatus</i>									1										
<i>Levinsenia gracilis</i>														1					
<i>Levinsenia oculata</i>		3		1		4				2		11		1		4			
<i>Paraonis fulgens</i>	1	1				1					2			1					
<i>Paraonis lyra</i>								3	2										
<i>Paraonis paucibranchiata</i>		2																	
<i>Paraonis sp.</i>							1		1										
Pilargidae																			
<i>Ancystrosyllis</i>								1											
<i>Pilargidae sp.</i>																	2		
Poecilochaetidae																			
<i>Poecilochaetidae sp.</i>									1										
<i>Poecilochaetus serpens</i>						1		2	1			1							
Phyllococidae																			
<i>Eteone</i>		3	1						1										

(continued)

	7G	5E	3C	4D	2B	1A	16R	14P	6F	8H	10L	9I	c50	c20	11M	12N	P1	P2	P3
<i>Phyllodoce lineata</i>						1	1		3			1							
Polynoidae																			
<i>Lepidonotus clava</i>										1									
<i>Lepidonotus squamatus</i>			1																
<i>Harmothoe</i>														1					
<i>Harmothoe fraserthomsoni</i>																1			
<i>Polynoë</i>		3							1		2								
Sabellinae																			
<i>Amphiglena mediterranea</i>														1					
<i>Branchiomma</i>								1						3					1
<i>Bispira fabricii</i>		2						1							1				
<i>Sabella</i> sp.		1						1	1										
Serpulidae																			
<i>Serpulidae</i>											3						6		10
<i>Filograna</i>																	4	1	2
<i>Hydroides pseudouncinatus</i>																			1
<i>Vermiliopsis striaticeps</i>																		1	6
Sigalionidae																			
<i>Sigalion</i>								1						1					
<i>Sigalion mathildae</i>							1				1			6			1	1	
Spionidae																			
<i>Laonice cirrata</i>		1					1												
<i>Laonice</i> sp.										1									
<i>Malacoceros fuliginosus</i>		2																	
<i>Microspio mecznikowianus</i>	2			3		1		1	1		1	3	2	2					
<i>Polydora</i> sp.																			
<i>Prionospio fallax</i>		1	1				1		8										
<i>Prionospio ehlersi</i>			1						1										
<i>Prionospio malmgreni</i>		4	1			1			14		15	2		3	9				
<i>Prionospio</i> sp.	1		1			1			3										
<i>Prionospio steenstrupi</i>											5				6				
<i>Spio</i> sp.		2				2													
<i>Spio filicornis</i>				3							5			6					2
<i>Spionidae</i> sp.		1	1	2				1	1	1									
Sternaspidae																			
<i>Sternaspis scutata</i>											1								
Syllidae																			
<i>Syllis gracilis</i>		1			1	1	1	1			1								
<i>Syllis hyalina</i>																		7	10
<i>Syllis</i> sp.																		13	7
Terebellidae																			
<i>Amphitritides gracilis</i>						1													2
<i>Terebellidae</i>		1																	1
Sipunculidae																			
Phascolosomatidae																			
<i>Onchesoma steenstrupii</i>		19							2		9		1	1	4	1			
<i>Phascolion (Phascolion) strombus</i>																			1
<i>Sipuncula</i> sp.		1			2														2

References

- Adami, G., Barbieri, P., Piselli, S., Predonzani, S., Reisenhofer, E., 2000. Detecting and characterizing sources of persistent organic pollutants (PAHs and PCBs) in surface sediments of an industrialized area (harbour of Trieste, northern Adriatic Sea). *J. Environ. Monit.* 2 (3), 261–265.
- Afli, A., Boufahja, F., Sadraoui, S., Ben Mustapha, K., Aissa, P., Mrabet, R., 2009. Functional organization of the benthic macrofauna in the Bizerte lagoon (SW Mediterranean Sea), semi-enclosed area subject to strong environmental/anthropogenic variations. *Cah. Biol. Mar.* 50 (2), 105.
- Alam, M., Pathak, J.K., 2010. Rapid assessment of water quality index of Ramganga river, western Uttar Pradesh (India) using a computer programme. *Nat. Sci.* 8 (11), 1–8.
- Alexander, R.B., Smith, R.A., Schwarz, G.E., 2000. Effect of stream channel size on the delivery of nitrogen to the Gulf of Mexico. *Nature* 403 (6771), 758–761.
- Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA+ For PRIMER: Guide to Software and Statistical Methods. PRIMER-E, Plymouth.
- Bax, N., Williamson, A., Aguero, M., Gonzalez, E., Geeves, W., 2003. Marine invasive alien species: a threat to global biodiversity. *Mar. Policy* 27 (4), 313–323.
- Beaumont, N.J., Austen, M.C., Mangi, S.C., Townsend, M., 2008. Economic valuation for the conservation of marine biodiversity. *Mar. Pollut. Bull.* 56 (3), 386–396.
- Bertolotto, R.M., Tortarolo, B., Frignani, M., Bellucci, L.G., Albanese, S., Cuneo, C., 2003. May. Heavy metals in coastal sediments of the Ligurian sea off Vado Ligure. *Journal de Physique IV (Proceedings)* vol. 107. EDP Sciences, pp. 159–162.
- Bettinetti, R., Giare, C., Provini, A., 2003. Chemical analysis and sediment toxicity bioassays to assess the contamination of the River Lambro (Northern Italy). *Arch. Environ. Contam. Toxicol.* 45 (1), 0072–0078.
- Borja, A., Franco, J., Pérez, V., 2000. A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Mar. Pollut. Bull.* 40 (12), 1100–1114.
- Borja, A., Muxika, I., Rodríguez, J.G., 2009. Risposte paradigmatiche delle comunità bentoniche marine a diverse pressioni antropiche, con M-AMBI, nell'ambito della direttiva quadro europea sull'acqua. *Ecol. Mar.* 30 (2), 214–227.
- Bray, R.J., Curtis, J.T., 1957. An ordination of the upland forest communities of southern Wisconsin. *Ecol. Monogr.* 27, 325–349.
- Buchanan, J.B., Kain, J.M., 1971. Measurement of the Physical and Chemical Environment. Methods for the Study of Marine Benthos. p. 16.
- Clarke, K.R., Warwick, R.M., 2001. An approach to statistical analysis and interpretation. *Chang. Mar. Communities* 2.
- Costanza, R., d'Arge, R., De Groot, R., Farber, S., Grasso, M., Hannon, B., ... Van Den Belt, M., 1998. The value of the world's ecosystem services and natural capital. *Ecol. Econ.* 1 (25), 3–15.
- Cruz-Motta, J.J., Collins, J., 2004. Impacts of dredged material disposal on a tropical soft-bottom benthic assemblage. *Mar. Pollut. Bull.* 48 (3), 270–280.
- Currie, D.R., Isaacs, L.R., 2005. Impact of exploratory offshore drilling on benthic communities in the Minerva gas field, Port Campbell, Australia. *Mar. Environ. Res.* 59 (3), 217–233.
- D'Alessandro, M., Castriota, L., Consoli, P., Romeo, T., Andaloro, F., 2015. *Pseudonereis anomala* (Polychaeta, Nereididae) expands its range westward: first Italian record in Augusta and Siracusa harbours. *Mar. Biodivers.* 1–5.
- Daskalakis, K.D., O'Connor, T.P., 1995. Distribution of chemical concentrations in US coastal and estuarine sediment. *Mar. Environ. Res.* 40 (4), 381–398.
- Dauvin, J.C., Ruellet, T., 2007. Polychaete/amphipod ratio revisited. *Mar. Pollut. Bull.* 55 (1), 215–224.

- De Groot, R.S., Wilson, M.A., Boumans, R.M., 2002. A typology for the classification, description and valuation of ecosystem functions, goods and services. *Ecol. Econ.* 41 (3), 393–408.
- Estadio, F.J., García-Adiego, E.M., Fa, D.A., García-Gómez, J.C., Daza, J.L., Hortas, F., Gómez-Ariza, J.L., 1997. Ecological analysis in a polluted area of Algeciras Bay (Southern Spain): external 'versus' internal outfalls and environmental implications. *Mar. Pollut. Bull.* 34 (10), 780–793.
- Fadeeva, N.P., Bezverbnaja, I.P., Tazaki, K., Watanabe, H., Fadeev, V.I., 2003. Composition and structure of marine benthic community regarding conditions of chronic harbour pollution. *Ocean Polar Res.* 25 (1), 21–30.
- Farkas, A., Erratico, C., Vigano, L., 2007. Assessment of the environmental significance of heavy metal pollution in surficial sediments of the River Po. *Chemosphere* 68 (4), 761–768.
- Gibbs, R.J., 1977. Transport phases of transition metals in the Amazon and Yukon Rivers. *Geol. Soc. Am. Bull.* 88 (6), 829–843.
- Goodnight, C.J., 1973. The use of aquatic macroinvertebrates as indicators of stream pollution. *Trans. Am. Microsc. Soc.* 1–13.
- Gray, J.S., Elliott, M., 2009. *Ecology of Marine Sediments: From Science to Management*. Oxford University Press.
- Grigg, R.W., 1994. Community structure, succession and development of coral reefs in Hawaii. A Natural History of the Hawaiian Islands: Selected Readings II, p. 196.
- Hilty, J., Merenlender, A., 2000. Faunal indicator taxa selection for monitoring ecosystem health. *Biol. Conserv.* 92 (2), 185–197.
- Humborg, C., Ittekkot, V., Cociasu, A., Bodungen, B.V., 1997. Effect of Danube River dam on Black Sea biogeochemistry and ecosystem structure. *Nature* 386 (6623), 385–388.
- ISPRA, 2009. Piano di caratterizzazione ambientale dell'area marina costiera prospiciente il sito di bonifica di interesse nazionale area industriale di Milazzo. CII-Pr-SI-MI-01.23.
- Klemm, D.J., Blockson, K.A., Thoeny, W.T., Fulk, F.A., Herlihy, A.T., Kaufmann, P.R., Cormier, S.M., 2002. Methods development and use of macroinvertebrates as indicators of ecological conditions for streams in the Mid-Atlantic Highlands region. *Environ. Monit. Assess.* 78 (2), 169–212.
- Lindegarth, M., Hoskin, M., 2001. Patterns of distribution of macro-fauna in different types of estuarine, soft sediment habitats adjacent to urban and non-urban areas. *Estuar. Coast. Shelf Sci.* 52 (2), 237–247.
- Lotze, H.K., Reise, K., Worm, B., van Beusekom, J., Busch, M., Ehlers, A., ... Wolff, W.J., 2005. Human transformations of the Wadden Sea ecosystem through time: a synthesis. *Helgol. Mar. Res.* 59 (1), 84–95.
- Luoma, S.N., Bryan, G.W., 1981. A statistical assessment of the form of trace metals in oxidized estuarine sediments employing chemical extractants. *Sci. Total Environ.* 17 (2), 165–196.
- Luoma, S.N., Phillips, D.J.H., 1988. Distribution, variability, and impacts of trace elements in San Francisco Bay. *Mar. Pollut. Bull.* 19 (9), 413–425.
- Maiorano, P., Mastrototaro, F., Beqiraj, S., Costantino, G., Kashta, L., Gherardi, M., ... Tursi, A., 2011. Bioecological study of the benthic communities on the soft bottom of the Vlora Gulf (Albania). *J. Coast. Res.* 95–105.
- Martins, R., Quintino, V., Rodrigues, A.M., 2013a. Diversity and spatial distribution patterns of the soft-bottom macrofauna communities on the Portuguese continental shelf. *J. Sea Res.* 83, 173–186.
- Martins, R., Sampaio, L., Quintino, V., Rodrigues, A.M., 2013b. Soft-bottom Portuguese continental shelf polychaetes: diversity and distribution. *J. Mar. Syst.* 123–124, 41–54.
- Mason, C.F., 2002. *Biology of Freshwater Pollution*. Pearson Education.
- Matthiessen, P., Law, R.J., 2002. Contaminants and their effects on estuarine and coastal organisms in the United Kingdom in the late twentieth century. *Environ. Pollut.* 120, 739–757.
- McCauley, D.J., Pinsky, M.L., Palumbi, S.R., Estes, J.A., Joyce, F.H., Warner, R.R., 2015. Marine defaunation: animal loss in the global ocean. *Science* 347 (6219), 1255641.
- Moreno, M., Vezzulli, L., Marin, V., Laconi, P., Albertelli, G., Fabiano, M., 2008. The use of meiofauna diversity as an indicator of pollution in harbours. *ICES J. Mar. Sci.: J. Conseil.* 65 (8), 1428–1435.
- Muxika, I., Borja, A., Bonne, W., 2005. The suitability of the marine biotic index (AMBI) to new impact sources along European coasts. *Ecol. Indic.* 5 (1), 19–31.
- Notar, M., Leskovsek, H., Faganeli, J., 2001. Composition, distribution and sources of Polycyclic aromatic hydrocarbons in sediments of the Gulf of Trieste, northern Adriatic Sea. *Mar. Pollut. Bull.* 42, 36–44.
- Nriagu, J.O., 1989. A global assessment of natural sources of atmospheric trace metals. *Nature* 338 (6210), 47–49.
- Occhipinti-Ambrogi, A., 2007. Global change and marine communities: alien species and climate change. *Mar. Pollut. Bull.* 55 (7), 342–352.
- Occhipinti-Ambrogi, A., Marchini, A., Cantone, G., Castelli, A., Chimenz, C., Cormaci, M., ... Piraino, S., 2011. Alien species along the Italian coasts: an overview. *Biol. Invasions* 13 (1), 215–237.
- Ojaveer, H., Galil, B.S., Minchin, D., Olenin, S., Amorim, A., Canning-Clode, J., ... Zenetos, A., 2014. Ten recommendations for advancing the assessment and management of non-indigenous species in marine ecosystems. *Mar. Policy* 44, 160–165.
- Otway, N.M., 1995. Assessing impacts of deepwater sewage disposal: a case study from New South Wales, Australia. *Mar. Pollut. Bull.* 31 (4), 347–354.
- Pearson, T.H., Rosenberg, R., 1978. Feast and famine: structuring factors in marine benthic communities. Symposium of the British Ecological Society.
- Pepe, F., Scopelliti, G., Di Leonardo, R., Ferruzza, G., 2010. Granulometry, mineralogy and trace elements of marine sediments from the Gulf of Milazzo (NE Sicily): evaluation of anthropogenic impact. *Ital. J. Geosci.* 129, 385–394.
- Romano, E., Ausili, A., Zharova, N., Magno, M.C., Pavoni, B., Gabellini, M., 2004. Marine sediment contamination of an industrial site at Port of Bagnoli, Gulf of Naples, Southern Italy. *Mar. Pollut. Bull.* 49 (5–6), 487–495.
- Romeo, T., D'Alessandro, M., Esposito, V., Scotti, G., Berto, D., Formalewicz, M., Noventa, S., Giuliani, S., Macchia, S., Sartori, D., Mazzola, A., Andaloro, F., Giacobbe, S., Deidun, A., Renzi, M., 2015. Environmental quality assessment of Grand Harbour (Valletta, Maltese Islands): a case study of a busy harbour in the Central Mediterranean Sea. *Environ. Monit. Assess.* <http://dx.doi.org/10.1007/s10661-015-4950-3>.
- Salomons, W., Stigliani, W. (Eds.), 2012. *Biogeochemistry of Pollutants in Soils and Sediments: Risk Assessment of Delayed and Non-linear Responses*. Springer Science & Business Media.
- Shannon, C. E., & Weaver, W. (1963). University of Illinois Press. Urbana, IL.
- Shoultz-Wilson, W.A., Elsayed, N., Leckrone, K., Urnine, J., 2015. Zebra mussels (*Dreissena polymorpha*) as a biomonitor of trace elements along the southern shoreline of Lake Michigan. *Environ. Toxicol. Chem.* 34 (2), 412–419.
- Shrivastava, A., Gupta, V.B., 2011. Methods for the determination of limit of detection and limit of quantitation of the analytical methods. *Chronicles Young Sci.* 2 (1), 21–25.
- Signa, G., Mazzola, A., Costa, V., Vizzini, S., 2015. Bottom-up control of macrobenthic communities in a eutrophic coastal system. *PLoS One* 10 (2), e0117544.
- Simboura, N., Nicolaidou, A., Thessalou-Legaki, M., 2000. Polychaete communities of Greece: an ecological overview. *Mar. Ecol. Prog. Ser.* 21 (2), 129–144.
- Sitran, R., Bergamasco, A., Decembrini, F., Guglielmo, L., 2009. Microzooplankton (tintinnid ciliates) diversity: coastal community structure and driving mechanisms in the southern Tyrrhenian Sea (Western Mediterranean). *J. Plankton Res.* 31 (2), 153–170.
- Sprovieri, M., Feo, M.L., Prevedello, L., Salvagio, Manta D., Sammartino, S., Tamburrino, S., Marsella, E., 2007. Heavy metals, polycyclic aromatic hydrocarbons and polychlorinated biphenyls in surface sediments of the Naples harbour (Southern Italy). *Chemosphere* 67, 998–1009.
- US EPA, 2007a. Method 3051A. Microwave Assisted Acid Digestion of Sediments, Sludges, Soils, and Oils (Washington, DC).
- US EPA, 2007b. Method 6010C. Inductively Coupled Plasma-Atomic Emission Spectrometry (Washington, DC).
- US EPA, 2007c. Method 7010. Graphite Furnace Atomic Absorption Spectrophotometry (Washington, DC).
- US EPA, 2007d. Method 7473 "Mercury in Solids and Solutions by Thermal Decomposition, Amalgamation, and Atomic Absorption Spectrophotometry" (Washington, DC).
- US EPA method 3545 revision B, 1996. Pressurized Fluid Extraction (PFE). Office of Water, Washington, D.C., USA.
- US EPA method 8081B revision 2, 2007a. Organochlorine Pesticides by Gas Chromatography. Office of Water, Washington, D.C., USA.
- US EPA method 8082A revision 1, 2007b. Polychlorinated Biphenyls (PCBs) by Gas Chromatography. Office of Water, Washington, D.C., USA.
- Veena, K.B., Radhakrishnan, C.K., Chacko, J., 1997. Heavy metal induced biochemical effects in an estuarine teleost. *Indian J. Mar. Sci.* 26 (1), 74–78.
- Vitousek, P.M., D'Antonio, C.M., Loope, L.L., Westbrooks, R., 1996. Biological invasions as global environmental change. *Am. Sci.* 84 (5), 468–478.
- Wentworth, C.K., 1922. A scale of grade and class terms for clastic sediments. *J. Geol.* 377–392.
- Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., ... Watson, R., 2006. Impacts of biodiversity loss on ocean ecosystem services. *Science* 314 (5800), 787–790.