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Environmental quality assessment of Grand Harbour (Valletta, Maltese Islands): a case study of a busy harbour in the Central Mediterranean Sea

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Abstract Contamination levels by plastic debris, trace elements and persistent organic pollutants were assessed and related to macrobenthic diversity within soft bottoms of Grand Harbour (Malta, Central Mediterranean).

Sediment toxicity was evaluated by ecotoxicological method, deploying Bacteria (*Vibrio fischeri*), Echinodermata (*Paracentrotus lividus*) and Crustacea (*Corophium orientale*). Univariate analysis (Pearson's test) was used to test relationships between biodiversity indices, pollutants and grain size. A multivariate approach (PERMANOVA) was applied to investigate for any significant differences among sampling stations concerning plastic abundances and to test the relationship between infaunal abundances and pollutant concentrations (the BIOENV test). Significant differences in the plastic abundances were found between sampling stations. The lowest value for Shannon-Wiener biodiversity index was associated to the highest sediment pollution level. Multivariate analyses suggest that MBT and TBT were factors that most influenced macrozoobenthic abundance and biodiversity. The bivalve *Corbula gibba* and the introduced polychaete *Monticellina dorsobranchialis* were the most abundant found species

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Introduction

Harbours represent hotspots of human-mediated environmental pressures which may significantly

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affect the general quality of surrounding coastal and marine waters, as well as the composition of autochthonous species assemblages through the spread of non-indigenous species they promote (ballast water, fouling, passive spread through canals). Consequently, the environmental impact footprint of the harbours extends far beyond their confines and could impinge on the conservation status of vital biocenoses, located outside the same harbours (Bellan et al. 2010). In spite of this assumption, harbours are neither included in Water Framework Directive nor in Marine Framework Strategy Directive provisions, with the consequence that some MFSD descriptors defining marine ecosystem health (i.e. D2 non-indigenous species and D10 marine litter) are not included in harbour monitoring schemes, even if they could significantly affect local species assemblages. Regular monitoring of the sediment quality within harbour environments is an important pursuant to assessing the possible influence of anthropogenic activities on ecosystem quality. In fact, human activities centred around the harbour sampled in this study are fuelling the accumulation of chemical pollutants in sediments and in interstitial water within the same harbour. In view of their low water solubility and persistence, several chemical pollutants tend to accumulate in sediments and biota (Kang et al. 2000; Wiberg and Harris 2002). This is the case of polychlorinated biphenyls (PCBs), extensively used in past as industrial coolants and in electrical transformers (Sprovieri et al. 2007) or of organotin compounds (as TBT, MBT, DBT), extensively used in the past as biocides in antifouling paints along the hulls of recreational vessels, large ships and docks (De Mora 1996; Yebra et al. 2004). All these pollutants deposited within harbour sediments can be released into the overlying water column through sediment re-suspension via natural or anthropogenic processes, affecting marine ecosystem health in the area (Dickinson et al. 1996; Poulton et al. 1996; Fatoki and Mathabatha 2001; McCready et al. 2006).

The global shipping-mediated transport of goods also indirectly designates harbours as hotspots for introduction of non-indigenous marine species (NIS) which, in most cases, due to the instability of the same harbour environments, fail to establish viable populations (Occhipinti-Ambrogi 2007). However, several studies also suggest that floating litter can greatly assist the transport of species beyond their natural boundaries and their introduction to new environments (Winston 1982; Winston et al. 1997; Barnes 2002; Barnes and Milner 2005). Barnes (2002) estimated that human litter more than doubles the rafting opportunities for biota, assisting the dispersal of NIS. Recent assessments suggest (Zenetos et al. 2012; Katsanevakis et al. 2013; Galil et al. 2014) that more than 80 % of the known NIS in the Mediterranean might have been introduced by colonizing marine litter or could potentially use litter for expanding their range further. Large amounts of litter enter in the Mediterranean through the Suez Canal, further supporting rafting on marine litter as a viable introduction pathway and vector for Lessepsian species into the Mediterranean (Galil et al. 1995).

The Grand Harbour of Valletta, strategically located within the Sicily Channel, represents a key shipping trade waterway, straddled between Europe, North Africa and Asia. Shipping traffic within the same harbour adds up to 10,000 vessels/year (Transport Malta, local authorities' data), ranging from container vessels, bulk carriers, motor tankers, Ro-Ro vessels, fuel tankers, fishing vessels and even cruise liners. Shipping activities within the harbour directly and indirectly impinge on the marine biota through ship repair, shipbuilding, grit blasting and ship hull painting. Anthropogenic pressures within the same harbour also arise from recreational boat moorings (yacht marinas), the national fisheries landing station and an electrical power station. The biotic and abiotic components of bottom sediments of such a highly impacted and stressed Mediterranean harbour were investigated throughout a multivariate analysis of macrozoobenthic communities and an assessment of the contamination degree by trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn), persistent organic pollutants (organotin compounds, polychlorinated biphenyls and hydrocarbons) and plastic debris (MSFD D10 marine litter). The aims of this study are to evaluate the abundance of plastic debris, chemical and ecotoxicological parameters, trend of the biodiversity indices and the presence of non-indigenous species in an harbour subject to high level of anthropogenic stress. The major aim of this investigation is to conduct a marine ecosystem health assessment of the harbour in Valletta by exploring the relationship between multiple stressors on infaunal assemblages.

Material and methods

The study area encompassed a large swathe of the Grand Harbour (Fig. 1). This historic and heavily fortified harbour is one of the largest natural harbours in the world, representing an example of a heavily-indented coastline which was etched through the partial drowning of valleys.

Surface sediment samples (about 30 cm of thickness) were collected by means of a 0.1-m^2 van Veen grab at eight sampling stations, six of which were located in the more anthropized part of the harbour and two were located in an area which is not directly subjected to human activities (control stations MP_BN7 and MP_BN9) (Table 1).The water depths for the different sediment sampling stations range between 4 and 22 m; the possible influence on infaunal assemblages of bathymetric differences was statistically tested by including bathymetry as a discrete factor. Three replicates were collected at each sampling station for benthic infauna analyses ($n = 24$). Sediment samples were washed on-board through a 0.5-mm-mesh sieve and rapidly stored in 90 % ethanol. An undisturbed sediment sample was additionally collected at each sampling station for subsequent physical (grain size), chemical (trace elements, persistent organic pollutants) and ecotoxicological analyses and for the determination of plastic debris contamination. Sediment aliquots for physical and ecotoxicological analyses were preserved at + 4 °C; aliquots for chemical analysis were stored at −20 °C.
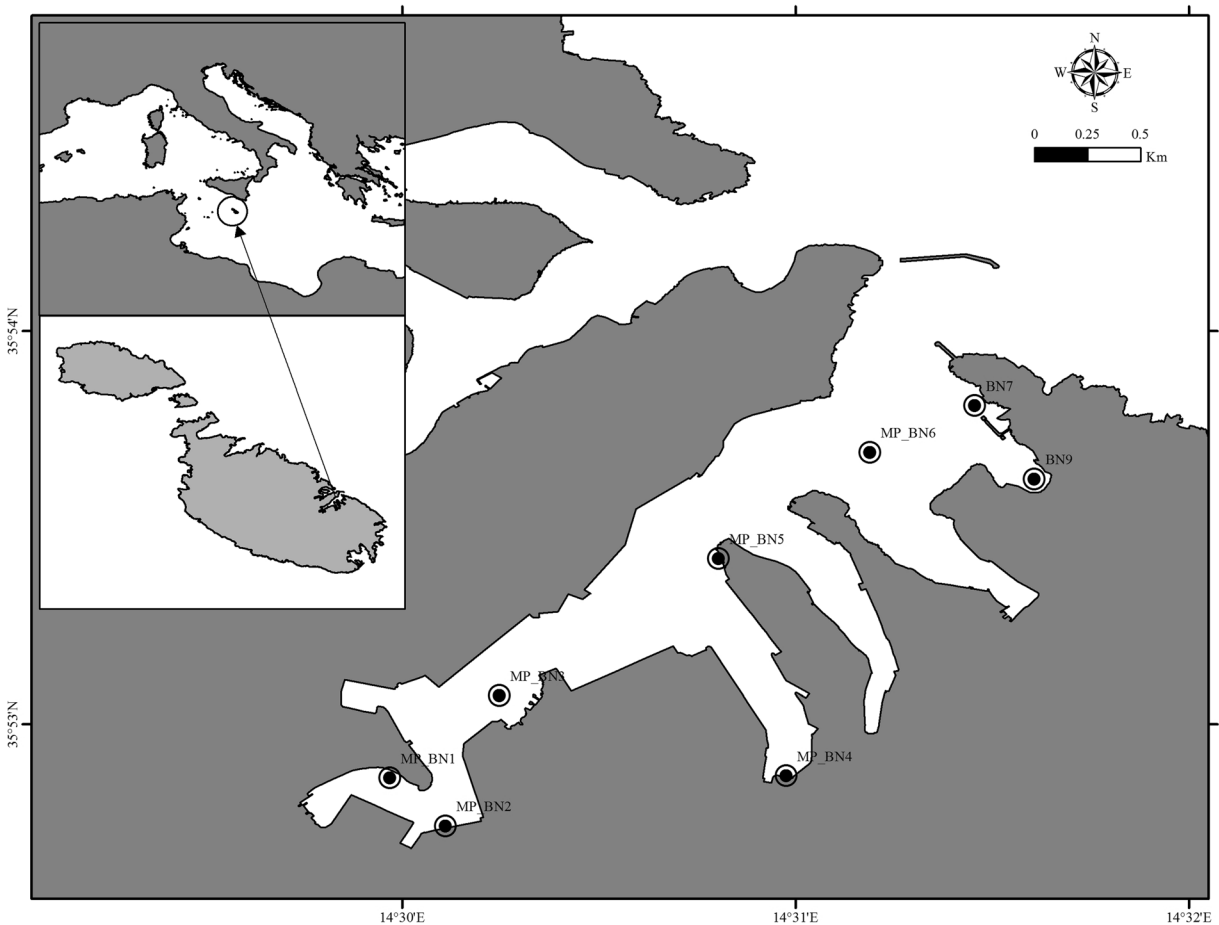

Fig. 1 Map of the study area reporting the sampling stations

Table 1 Percentages of granulometric classes in each of the sampling stations

Code	Depth (m)	Human Activities	Latitude	Longitude	%plebbe	%gravel	%sand	%silt	%clay
MP_BN1	4	Drainage pipe	35°52.863′N	14°29.968′E	5.23	6.34	40.72	42.60	5.11
MP_BN2	10	Power station	35°52.730′N	14°30.112′E	0.02	0.13	7.98	87.58	4.29
MP_BN3	14	Wharves	35°53.072′N	14°30.247′E	0.00	0.59	15.55	80.00	3.86
MP_BN4	10	Grit blasting	35°52.868′N	14°30.976′E	0.43	0.62	21.87	72.43	4.65
MP_BN5	7	Wharves	35°53.421′N	14°30.803′E	2.78	2.30	16.23	74.45	4.24
MP_BN6	22.5	Port access	35°53.691′N	14°31.188′E	0.72	0.61	15.12	80.32	3.22
MP_BN7	6	No activity	35°53.810′N	14°31.455′E	0.40	1.46	85.94	11.66	0.54
MP_BN9	12	No activity	35° 53.623′N	15°31.606′E	3.11	5.22	88.65	1.66	1.36

Grain size of sediment

The sediment grain-size analysis was carried out according to Buchanan and Kain (1971), using a sieve series with intervals of 1 Φ for grains greater than 0.063 mm (4 Φ) and subsequently applying the Wentworth scale to classify the sediment. The silt (4.0–8.0 Φ) and clay (8.0–10.0 Φ) fractions were quantified through the column-dispersion method.

Plastic debris

Plastic debris was classified in four size classes, with some modifications from the hierarchy described in Barnes (2002): microplastics (<5 mm), macroplastics (5–10 mm), megaplastics (10–20 mm) and plastics (>20 mm). Microplastics were extracted according to the methodology given in Claessens et al. (2011). In brief, 3 L of a concentrated saline solution were added to 1 kg of wet sediment and stirred for 2 min. Sediment sample was then allowed to settle for 1 h, before the supernatant was poured through a 500-μm-mesh sieve. For each sediment sample, this extraction was performed three times and collected particles (sieve) were examined using a binocular stereomicroscope (Zeiss, Discovery V 8) equipped with micro-camera (Axio-Cam vs40 v4.8.20); larger plastic categories were extracted through hand-sorting under a stereomicroscope. Plastic particles were visually classified on the basis of their colour, size and shape; the three shape categories adopted were fibrous, rounded and irregular. All the extracted plastic debris were counted and weighed.

Chemical parameters

Trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn) were determined after mineralization of 0.5 g of dried sediment employing a mixture of H_2O_2–HNO_3 (4:1 v/v), according to the US-EPA 6020A (2015a) method, through inductively coupled plasma-mass spectrometry (ICP-MS; mod. Agilent Technologies). Results were expressed as microgrammes/kilogramme dry weight (d.w.); the lowest sensitivity thresholds were lower than 0.002 mg/kg d.w. for all the analysed trace elements.

Persistent organic pollutants were determined through extraction from about 5–10 g of dried sediment, according to the methodologies described in ICRAM (2003); quantifications of PCBs (congeners 28, 52, 77, 81, 101, 118, 126, 128, 138, 153, 156, 169, 180) were conducted according to the US-EPA 8082A/2007 (2015b) standard; quantifications of 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, indenopyrene, naphthalene, phenanthrene, pyrene, perylene, acenaphthene) were determined as per the US-EPA Method 8270D (2015c). Results were expressed as microgrammes/kilogramme dry weight (d.w.). The lowest sensitivity threshold for the persistent organic pollutant analyses was that of 0.0001 mg/kg d.w.

The chemical analyses of butyltins (BT) for surface sediments was conducted according to a modified method from Binato et al. (1998) and Morabito et al. (1995). Tributyltin (TBT), dibutyltin (DBT), monobutyltin (MBT) and total butyltins (BT = TBT + DBT + MBT) were determined through a four-step analysis consisting

of: (I) extraction with a methanol solution of tropolone (0.05 %) in acid environment through a sonication bath, followed by liquid–liquid separation with dichloromethane and hexane solvent exchange; (II) BT derivatization with Grignard reagents and elimination of the excess with NH_4Cl (20 %); (III) clean-up through a solid phase extraction (SPE) column packed with silica gel and anhydrous sodium sulphate and (IV) quantification through GC-MS/MS analysis. A gas chromatograph (mod. Trace GC, Thermo Finnigan, USA), equipped with an automatic injector (mod. AS2000, Thermo Finnigan, USA), was fitted with a capillary column Rtx-5MS (i.d. 0.25 mm, length 30 m, film thickness 0.25 μm, Altech, USA). The column temperature was initially held at 50 °C for 2 min, increased at a rate of 9 °C per minute up to a maximum to 164 °C and then at a rate of 50 °C per minute to reach a final temperature of 300 °C. The splitless injection port and the transfer line were kept at 250 and 240 °C, respectively. Helium was employed as a carrier gas, with a constant flow of 1.5 mL per minute, and the injected hexane extract had a volume of 1.5 μL. The fragmentation was obtained through an ion trap mass spectrometer (mod. Polaris Q, Thermo Finnigan, USA), setting the electron impact (EI) source at 250 °C, the electron energy at 70 eV and the accelerating voltage at 1.1 kV. Concerning mass-to-mass method (MS/MS), the fragmentation ions chosen were 193, 137 and 120 m/z for TBT, 151, 135 and 120 m/z for DBT and 165, 150 and 135 m/z for MBT. Concentrations were expressed as nanogrammes Sn/gramme (d.w.). The lowest sensitivity thresholds were 2 ng Sn/g for TBT and 4 ng Sn/g for DBT and MBT.

Quality assurance and quality control protocols were performed in order to guarantee the quality of the chemical analyses performed in this study. Laboratories complied with ISO 9001:2000 and ISO 14001 standards. Chemicals and reagents were of analytical grade, and glassware was carefully washed to avoid sample cross-contamination. Data quality assurance and quality control protocols included spiked samples, laboratory blanks and continuous calibration verification. The accuracy and precision of the procedures were tested by analysing laboratory-induced spiked samples in statistical replicates ($n = 3$) and standard reference materials to calculate the average and standard deviation (SD) of recoveries and deviations from certified values.

Concerning trace elements (As, Cd, Cr, Cu, Hg, Ni, Pb, Zn), the precision and accuracy of the analytical method were evaluated by analysing the certified reference material MESS-3 (Hg) LGC 6137 (all the other trace elements). The recovery levels ($n = 3$) were within 95–103 % for all considered elements. As regards persistent organic pollutants, PCB and PAH recoveries ($n = 5$) were tested by standard reference materials purchased from the National Institute of Standards and Technologies (NIST-Estuarine Sediment SRM1646a), the UK Department of Trade and Industry, as part of the UK's National Measurement System (SRM-Harbour sediment). Results obtained by replicates were highly consistent with the certified values (mean errors 4 % for PCBs and 6 % for PAHs). Due to the high percentages of obtained recoveries (over than 90 % for each PCB and within 70–80 % for PAHs), analytical concentrations were not recovery corrected to avoid the introduction of any additional mathematical error. Concerning TBTs, the precision and accuracy of the analytical method were evaluated by analysing the certified reference material BCR-CRM 462 (EC-JRC Institute for Reference Materials and Measurements) along with the samples. The recovery levels ($n = 9$) for TBT, DBT and MBT were 87 ± 7, 89 ± 13 and 90 ± 15 %, respectively.

Ecotoxicological analyses

Ecotoxicological tests, according to ISPRA Guidelines (ISPRA 2011), must be performed at different trophic levels: Bacteria (*Vibrio fischeri*), Echinodermata (*Paracentrotus lividus*), Crustacea and Amphipoda (*Corophium orientale*). Sediment elutriates were prepared according to the US-EPA method (US-EPA 2001). Ecotoxicological tests on *V. fischeri* are widely used for toxicity evaluations (Isenberg 1993; ISO 1998) in soil (Vanhala and Ahtiainen 1994), sediment and elutriate samples (Brouwer et al. 1990; Heida and van der Oost 1996; Pedersen et al. 1998; Salizzato et al. 1998). The lyophilized NRRL-B-11177 strain stored within the American Type Culture Collection (12301 Parkland Drive, Rockville, Maryland, USA) was acquired and tested following the Microtox® method.

The embryo toxicity test with *P. lividus* was performed according to Gaion et al. (2013). A minimum of three adult *P. lividus* individuals per sex were used to obtain gametes by injecting 1 mL of 0.5 M KCl solution into the coelom through the peristome. Sperm was then

diluted into filtered seawater in order to reach a sperm: eggs ratio of 15,000:1 (Lera and Pellegrini 2006); fertilization was executed by gently mixing the sperm and egg suspension. A few minutes after the fertilization, an aliquot of embryos was observed under the inverted microscope to verify the presence of the fertilization membrane. After most of the eggs (approx. 95 %) were fertilized, volumes of the suspensions containing around 1000 fertilized eggs were transferred into the 10-mL-well plate containing elutriates in order to evaluate the proper embryonic development against a control. Controls were conducted as untreated negative controls (0.22 μm FSW), and positive controls with an exposure to $CuNO_3*3H_2O$ (Sigma Aldrich, USA) (FSW 0.22 μm) until the P4 stage was reached (pluteus). After the incubation period, larval development was blocked by adding one drop of 40 % paraformaldehyde. The percentage of normal developed plutei in each treatment was determined by making observations on 100 larvae.

The *C. orientale* test was conducted as described in the ISO protocol (ISO 2005) and in Bigongiari et al. (2001). At the end of the test (10 days), the survivors were counted; apparently, dead individuals were considered as living if movements were exhibited after gentle stimulation; missing amphipods were assumed to be dead. The acceptability of the test results was fixed at a mortality rate in the negative control ranging from 0 to 10 %, and LC_{50} values were included in the acceptable range defined by the control chart. The sensitivity of the population was checked by performing the 96-h LC_{50} test with atomic absorption standard cadmium solution $Cd(NO_3)_2*4H_2O$ (1000 mg/L) as reference toxicants.

Biodiversity

For macrofaunal analysis, the samples were hand-sorted under a stereomicroscope equipped with micro-camera Axio-Cam vs40 v4.8.20 and under an optical microscope equipped with Optika Vision Lite 2.1 software for examination of the smallest diagnostic characters. Molluscs and crustaceans were determined, when possible, at species level, as well as Cirratulidae since this represents the most abundant and diversified family of polychaetes.

The macrobenthic community was characterized by calculating the following diversity indexes for each sampling station: specific richness (S), expressed as the total number of species recorded at each sampling;

the Shannon-Wiener diversity index (H') (Shannon and Weaver 1963), calculated as $H' = -i = 1SPi*lnPi$ where P_i is the proportion of specimens in the ith species and the Pielou's evenness index (J) expressed as $J = H'/ln S$ where H' is the Shannon-Wiener diversity index value and S is the specific richness. J index values range between 0 and 1, with values close to 1 being recorded when individuals are homogeneously distributed between different species, while when the community is dominated by few species only, J tends to approach 0.

Statistical analysis

Statistical analyses were performed using the PRIMER6 & PERMANOVA+ software packages (Clarke and Warwick 2001; Anderson et al. 2008). To evaluate potential variations in the abundance of the four different plastic sizes in relation to the factor "sampling stations" in the anthropized area, a non-parametric multivariate analysis of variance (PERMANOVA) was performed. Data were transformed to square root and analysed on the basis of Bray-Curtis similarity (4999 permutations). Pairwise comparisons were computed when significant differences ($p < 0.05$) among factor levels were detected. A principal coordinate (PCO) analysis was also performed to describe the plastic size categories that most accounted for variation among the sampling stations considered.

Multivariate analyses were also performed to assess potential correlations between the abundances of macrobenthic *taxa* and environmental variables (plastic category abundances, POPs and trace elements concentration). Spatial pattern of macrobenthic community composition was assessed using a Bray-Curtis similarity index (Bray and Curtis 1957) on the square root-transformed abundance matrix. Then, for the normalized 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 Euclidean distance that was used in an agglomerative hierarchical clustering (routine CLUSTER). A PCO analysis 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. The relationships between the abundance of each plastic size class, contaminant concentrations, biodiversity indices and abundance of alien species were investigated using the Pearson correlation test. Moreover, the same test was used to evaluate potential correlations between the following pairs of variables: grain size vs. biodiversity indices, grain size vs. PAHs, grain size vs. TBT, grain size vs. trace elements and biodiversity indices vs. persistent organic pollutants (TBT, PAH).

Results

Grain size of sediment

Sediment samples were mainly characterized by silt (Table 1). Only in two stations, MP_BN7 and MP_BN8, sand represented the predominant fraction (85.94 and 88.65 %, respectively). MP_BN2 showed the highest percentage of silt (82.6 %). A coarser texture was recorded at MP_BN1 (40.72 % of sand).

Plastic debris

A total of 59 fragments of plastic debris (mean 2 ± 2.725 particles/kg dry sediment) have been recorded within all the sampling stations. The highest mean density per site (mean 7 ± 3.66 particles/kg dry sediment) occurred at MP_BN1 (Table 2). Microplastics ($n = 35$ particles/kg dry sediment) represented the most abundant plastic category (Table 2), followed by macroplastics ($n = 11$ particles/kg dry sediment), megaplastics ($n = 8$ particles/kg dry sediment) and plastics ($n = 5$ particles/kg dry sediment). MP_BN1 was the station with the highest abundance of microplastics ($n = 12$ particles/kg dry sediment), megaplastics ($n = 3$ particles/kg dry sediment) and plastics ($n = 2$ particles/kg dry sediment). The highest number of macroplastic debris was recorded in MP_BN4 ($n = 5$ particles/kg dry sediment). Ninety-three percent of all plastic debris presented an irregular shape; the most abundant and frequently recorded category (F% = 91.30) having an irregular shape was that of microplastics (Table 2).

Chemical parameters

Trace elements showed the highest measured concentrations at MP_BN4, with the exception of Hg which prevailed at MP_BN5. In general, trace element concentrations respected the following decreasing order: Cu > Zn > Pb > Cr > As > Ni > Hg > Cd (Table 3).

All persistent organic pollutants showed the lowest values in MP_BN6 and the highest in MP_BN4. The concentration hierarchy for the same nine dominant PAH congeners is as follows: fluoranthene > benzo(b)fluoranthene > benzo(a)pyrene > benzo(k)fluoranthene > anthracene > indeno(1,2,3-cd)pyrene > benzo(g,h,i)perylene > naphthalene. At all the sampling stations, all PCB congeners and the total amount of PCBs amounted to less than 5 μg/kg d.w.

As regards TBT, DBT and MBT, the highest values were recorded at MP_BN4 while the lowest values were recorded at the control station MP_BN9 (Table 2). The

Table 2 Biotic indices (S, H and J) and plastic abundances (per size and shape) in each sampling stations

Code	Biodiversity			Plastic size				Plastic shape		
	S	H'	J	Micro	Macro	Mega	Plastic	Rounded	Filamentous	Irregular
MP_BN1	16.03	2.02	0.08	12	4	4	3	–	1	22
MP_BN2	13.03	1.09	0.07	4	1	–	–	–	1	4
MP_BN3	10.07	1.08	0.08	2	–	–	–	–	–	2
MP_BN4	0.03	0	0	4	4	3	1	–	–	12
MP_BN5	13.00	1.08	0.07	9	1	–	–	–	–	1–
MP_BN6	13.07	1.08	0.08	4	1	1	1	–	1	6
MP_BN7	14.05	2.04	0.09	–	–	–	–	–	–	–
MP_BN9	9.00	1.05	0.07	–	–	–	–	–	–	–

Table 3 Chemical compound concentrations measured in each of the sampling station. Trace elements expressed in mg/kg d.w; TBT, DBT, MBT in (ng Sn/g d.w.), organic compounds in mg/kg d.w

	Benzo(a)pyrene	Ben(b)fluoranthen	Ben(k)fluoranthene	Benzo(g,h,i)perylene	Indenopyrene	Anthracene	Fluoranthene	Naftalene	Cd	Ni
BN1	3077	4276	1770	316	453	935	5615	67	<0.3	21.8
BN2	844	1028	302	141	322	113	604	34	<0.3	7.4
BN3	438	418	195	111	256	72	335	<10	<0.3	6.8
BN4	536	725	226	324	428	53	389	11	0.8	31.3
BN5	893	1060	367	38	301	122	827	7	<0.3	10.5
BN6	311	254	124	112	238	43	270	12	0.3	6.7
BN7	1797	2490	603	652	666	364	3025	<10	<0.3	5.9
BN9	1390	1938	459	516	510	322	2998	<10	<0.3	4.4

	Pb	As	Totalcrome	Hg	Cu	Zn	TBT	DBT	MBT
BN1	136.1	7.7	25.1	0.65	194.6	205.2	156.0	92.0	62.0
BN2	56.3	5.4	14.9	0.39	61.6	76.8	421.0	224.0	117.0
BN3	113.0	8.6	17.6	1.05	59.0	65.3	109.0	116.0	82.0
BN4	366.0	33.0	89.4	3.01	1026.5	933.3	5654.0	929.0	489.0
BN5	276.7	18.3	44.4	3.93	125.2	342.6	144.0	99.0	63.0
BN6	148.4	12.8	20.4	3.12	79.2	98.9	12.0	9.0	7.0
BN7	357.2	26.4	12.4	4.0	51.8	87.2	23.0	13.0	12.0
BN9	80.9	13.7	12	1.9	29.6	38.3	8	5	4

average contribution of TBT to total BT was 50 %, with the highest percentage in the MP_BN4 station (80 %).

Ecotoxicological responses

The three tested organisms evidenced different ecotoxicological responses. For instance, concerning *V. fischeri*, EC_{20} levels ranged between 11.53 and 13.39 % for all harbour stations, with the exclusion of MP_BN3 and MP_BN4 for which no degree of such responses were observed. All tested harbour sediments are able to significantly affect early life stages of *P. lividus* ($EC_{50} < 4 0 \%$), with the exclusion of the control station MP_BN7 and MP_BN9. As regards *C. orientale*, the direct exposure to sediment produced a considerable mortality rate only for MP_BN1 (88 %) and MP_BN4 (90 %) sediments.

Biodiversity

A total of 1567 individuals belonging to 67 species under 53 families of Polychaeta, Crustacea, Bivalvia and Gastropoda were identified (Table 4). Macrobenthic individual abundance varied between 10 (MP_BN4) and 2204 individuals/m^2 (MP_BN5). Polychaeta accounted for half of the total abundance (53.7 %) and included 16 families. Cirratulidae was the most abundant family, dominated by the introduced *Monticellina dorsobranchialis* (12.2 % of total abundance). The highest abundance of this species was recorded at MP_BN2 (80 individuals), while no individuals of this species were recorded at MP_BN4 and at control stations. Mollusca (39.8 % of total abundance) was the second largest component of the harbour infauna. Corbulidae was the most abundant family, solely represented by *Corbula gibba*, with the highest abundance recorded at MP_BN2. No individuals of this species were recorded at MP_BN4 and only one individual was recorded at the control stations. Crustacea represented 6.5 % of the total macrobenthic abundance, with *Apseudopsis latreilli* and *Alpheus glaber* being the most abundant species (Table 4).

The average values of the diversity indices for each of the sampling stations are reported in Table 2. The highest value for the number of species (*S*) was recorded at MP_BN1 (16.07), whilst MP_BN7 (control station) showed the highest values for Shannon diversity (*H′*) and for Pielou eveness (*J*) (2.04 and 0.90, respectively). The lowest values for biological diversity indices were

recorded at MP_BN4 ($S = 0.03$; $H′ = 0$; $J = 0$), with only one specimen of Eunicidae (Polychaeta) being reported for this sampling station.

Statistical analyses

PERMANOVA analysis revealed significant differences ($p < 0.01$) between the abundance of the four different plastic size categories among the sampling stations in the anthropized area. Results of the pairwise comparisons are reported in Table 5.

The PCO1 axis (Fig. 2) accounted for 64.7 % of the total observed variation and clearly separated the MP_BN1 and MP_BN3 from all the other sampling stations. PCO2 axis gathered 24.7 % of the total variation and separated MP_BN5 from the other sampling stations. The superimposed vectors showed that all the plastic categories were mainly associated with the MP_BN1 station; moreover, microplastic resulted associated also with MP_BN5 and macroplastics, megaplastics and plastics with MP_BN4. No plastic category resulted associated with MP_BN3.

No significant correlations were found between plastic fragment abundance and BT concentrations nor between the fragment abundance and sediment grain-size parameters and trace element concentrations. A positive relationship between TBT and DBT ($r = 0.985$; $p < 0.01$) as well as DBT and MBT were found ($r = 0.999$; $p < 0.01$) in all stations, which may indicate that the contamination of sediments originate from the same sources, such as antifouling paints. Significant positive correlations were found between silt content vs. acenaphthene, anthracene, fluoranthene, benzo(a)anthracene, benzo(k)fluoranthene and benzo(a)pyrene ($r > 0.91$; $p < 0.05$). Indeno(1,2,3-cd)pyrene and dibenzo(a,h)anthracene concentrations were also positively correlated with clay content ($r > 0.86$; $p < 0.05$). Furthermore, significant positive correlations were found between microplastic abundances and benzo(b)fluoranthene, benzo(a)pyrene and fluoranthene concentrations ($r > 0.820$; $p < 0.05$) and between all the other plastic categories and benzo(g,h,i)perylene concentrations ($r > 0.93$; $p < 0.05$). As regards trace elements, significant positive correlations were found between Ni concentrations and macroplastic and megaplastic abundances ($r = 0.927$ and 0.837, respectively; $p < 0.05$).

Concerning the relationships between macrobenthic abundances vs. contaminants, the similarity test based

Table 4 Number of individuals of the identified families in each of the sampling station

No. of individuals	MP_BN1	MP_BN2	MP_BN3	MP_BN4	MP_BN5	MP_BN6	MP_BN7	MP_BN9	%N TOT
Crustacea									6.5
Aoridae	1	–	–	–	–	–	–	–	0.1
Corophiidae	4	–	6	–	4	–	–	–	0.9
Dexaminidae	–	–	–	–	–	1	–	–	0.1
Lysianassidae	–	–	–	–	–	1	–	–	0.1
Maeridae	3	–	–	–	–	–	–	–	0.2
Oedicerotidae	–	–	2	–	–	2	–	1	0.3
Mysidae	–	–	–	–	2	1	–	–	0.2
Bodotriidae	–	–	–	–	–	1	–	–	0.1
Diastylidae	–	–	–	–	–	1	–	–	0.1
Anthuridae	–	–	–	–	–	–	5	–	0.3
Apseudopsis latreilli	–	–	–	–	–	–	*15.0*	*33.0*	*3.1*
Alpheus glaber	–	*2*	*1*	–	–	–	–	–	*0.2*
Crangonidae	–	–	1	–	–	–	–	–	0.1
Processidae	–	–	1	–	–	–	–	–	0.1
Varunidae	1	–	–	–	–	–	–	–	0.1
unid. Brachiura	–	1	–	–	–	–	–	–	0.1
Diogenidae	–	–	–	–	–	1	–	–	0.1
Paguridae	–	–	–	–	6	1	–	–	0.4
Callianassidae	1	–	1	–	1	–	–	–	0.2
Ostracoda	–	–	–	–	–	1	–	–	0.1
Mollusca									39.8
Cardiidae	8	18	1	–	4	1	4	2	2.4
Corbula gibba	*48*	*157*	*20*	–	*82*	*47*	*4*	–	*22.8*
Lasaeidae	–	–	–	–	3	–	1	–	0.3
Lucinidae	1	–	–	–	–	–	–	2	0.2
Montacutidae	3	–	–	–	–	–	–	–	0.2
Nuculidae	–	–	1	–	1	–	–	–	0.1
Semelidae	19	99	2	–	–	–	–	–	7.7
Tellinidae	1	–	–	–	–	8	9	2	1.3
Mactridae	–	–	–	–	–	–	–	3	0.2
Veneridae	2	–	–	–	4	13	16	15	3.2
unid. Bivalvia	–	–	–	–	–	2	14	–	1.0
Retusidae	–	–	–	–	–	–	1	–	0.1
unid. Mollusca	–	–	–	–	5	–	–	–	0.3
Polychaeta									53.7
Ampharetidae	–	1	–	–	1	6	4	–	0.8
Capitellidae	1	1	26	–	18	64	4	–	7.3
Monticellina dorsobranchialis	*4*	*80*	*38*	–	*63*	*6*	–	–	*12.2*
Other Cirratulidae	43	114	13	–	22	2	1	2	12.6
Cossuridae	–	4	–	–	–	14	–	–	1.1
Eunicidae	–	–	–	1	–	4	–	–	0.3
Glyceridae	–	–	2	–	3	4	1	–	0.6
Lumbrineridae	–	–	–	–	–	13	1	–	0.9

Table 4 (continued)

No. of individuals	MP_BN1	MP_BN2	MP_BN3	MP_BN4	MP_BN5	MP_BN6	MP_BN7	MP_BN9	%N TOT
Maldanidae	–	–	–	–	–	10	–	1	0.7
Nereidae	8	1	11	–	14	2	–	–	2.3
Oneonidae	–	–	1	–	–	–	–	–	0.1
Onuphidae	–	–	–	–	–	9	–	–	0.6
Orbinidae	–	1	7	–	–	6	3	3	1.3
Paraonidae	40	1	1	–	6	1	4	–	3.4
Poecilochaetidae	–	5	2	–	–	3	–	–	0.6
Spionidae	20	26	51	–	20	9	–	–	8.0
Sabellidae	–	–	–	–	–	–	8	2	0.6
Syllidae	–	–	–	–	–	–	2	–	0.1
Terebellidae	–	–	–	–	1	–	2	–	0.2

Dominant species are evidenced in italic. Total percent abundance (%N) in respect to the whole sampling was also reported

on SIMPROF analysis of contaminant data revealed three distinct groups (A, B, C) ($p < 0.01$) confirmed via hierarchical cluster analysis (Fig. 3a). Their characterization is shown in Table 6.

The PCO1 axis (Fig. 3b) accounted for 53.7 % of the total observed variation and clearly separated the B and C groups. PCO2 axis gathered 27.3 % of the total variation and separated A from the other two groups. The superimposed vectors showed two sets of

Table 5 Pairwise comparisons between sampling stations in the anthropized area of Grand Harbour

Groups	t	P
MP_BN1 vs MP_BN2	4.1829	0.006
MP_BN1 vs MP_BN3	5.0826	0.004
MP_BN1 vs MP_BN4	1.6982	n.s.
MP_BN1 vs MP_BN5	2.5512	0.025
MP_BN1 vs MP_BN6	1.7034	n.s.
MP_BN2 vs MP_BN3	3.1163	0.015
MP_BN2 vs MP_BN4	3.4164	0.008
MP_BN2 vs MP_BN5	1.4685	n.s.
MP_BN2 vs MP_BN6	0.58323	n.s.
MP_BN3 vs MP_BN4	4.6592	0.004
MP_BN3 vs MP_BN5	2.2855	0.043
MP_BN3 vs MP_BN6	1.8835	n.s.
MP_BN4 vs MP_BN5	2.7874	0.024
MP_BN4 vs MP_BN6	1.2722	n.s.
MP_BN5 vs MP_BN6	0.99735	n.s.

n.s. not significant

contaminants, one associated with the group A and one related with group B; no contaminants appeared to be associated with group C.

Group A included the sampling station MP_BN1, located close to a sewage outfall and characterized by the highest concentrations of PAHs (benzo(a)pyrene and anthracene) and the highest plastic fragment abundance values (Fig. 2; Table 6). This group comprised 18 families of macrobenthic species, eight of which, identified as opportunistic, constituted the highest average abundance values within this group. Group B comprised MP_BN4 located near a grit-blasting activity and characterized by the highest concentrations of trace elements and organotin compounds (MBT, DBT and TBT) and by the presence of only one macrobenthic family (Fig. 3; Table 6). Group C was composed by the remaining sampling stations (6) that showed lower concentrations of all contaminants and the highest total number of macrobenthic families (45) with respect to the other groups (Table 6).

The BEST analysis, implemented through the BIOENV protocol, showed that TBT and MBT were the environmental variables which were best related with the spatial distribution of macrobenthic fauna within the studied sites (rho = 0.808; $p < 0.05$). In particular, the Bubble plot option, chosen to visualize the MDS graph with superimposed circles of increasing size with increasing macrobenthic abundance or contaminant concentrations, showed that highest concentrations of TBT and MBT correspond to lowest macrobenthic abundances (Fig. 4).

Fig. 2 Principal coordinate (PCO) analysis biplot representing the spatial distribution of the sampling stations on the basis of abundance of the plastic size categories identified (*superimposed vectors*)
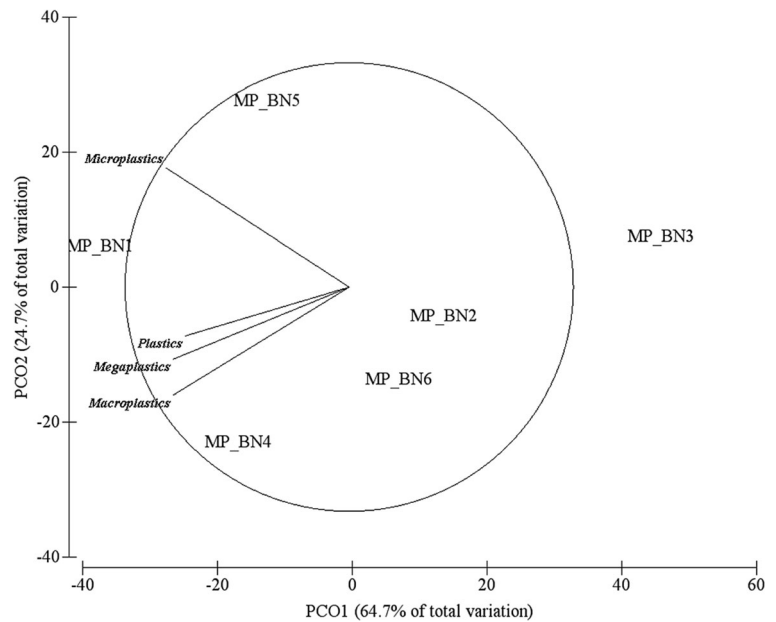
As regards relationship between diversity indices vs. trace element concentrations and plastic abundances, Pearson's test showed significant positive correlations between the Shannon diversity index and all the plastic categories, except for microplastic abundances ($p < 0.05$). Significant negative correlations were found

Table 6 Simprof affinity groups characterization

		A	B	C
Heavy metals	Ni (mg/kg d.w.)	21.8	31.3	41.7
	Pb (mg/kg d.w.)	136.1	366.0	1032.5
	As (mg/kg d.w.)	7.7	33.0	85.2
	Cr tot (mg/kg d.w.)	25.1	89.4	121.7
	Hg (mg/kg d.w.)	0.65	3.01	14.39
	Cu (mg/kg d.w.)	194.6	1026.5	406.4
	Zn (mg/kg d.w.)	205.2	933.3	709.1
Organic contaminants	Benzo(a)pyrene (mg/kg d.w.)	3077	536	5673
	Anthracene (mg/kg d.w.)	935	53	1036
	TBT (ng Sn/g d.w.)	156.0	5654.0	717
	DBT (ng Sn/g d.w.)	92.0	929.0	466
	MBT (ng Sn/g d.w.)	62.0	489.0	285
Plastic debris	Microplastic (n/kg)	12	4	19
	Macroplastic (n/kg)	4	4	3
	Megaplastic (n/kg)	4	3	1
	Plastic (n/kg)	3	1	1
Biotic characteristics	Total number of families	18	1	45
	No. of opportunistic families	8	0	9

between species richness "S" and Zn ($r = −0.767$; $p < 0.05$), Cu ($r = −0.820$; $p < 0.05$) and Cr ($r = −0.762$; $p < 0.05$) concentrations.

The Pearson's test conducted on comparisons between diversity indices values and contaminant concentrations showed significant negative correlations among infaunal species richness and BT concentrations ($p < 0.05$). Moreover, H' was negatively correlated with the sand fraction percentage and positively correlated with all the other sediment grain-size classes.

Discussion

Harbours, as a major interface between coastal cities and the sea, are often under heavy anthropogenic pressure and increasingly bear the brunt of pressures linked to poor water and sediment quality (Estacio et al. 1997).

Our study confirms the correlation between particulate and PAH concentration that, thanks to their highly hydrophobic nature, have a strong affinity for fine sediments (Li et al. 1998). The abundance of plastic fragments recorded from the Grand harbour supports the increasing detection of waste in the marine environment, fuelled in turn by an increase in accidental and indiscriminate release of the same waste (Wright et al. 2013). Microplastics were found in all the sediments collected within the harbour, and significant concentration differences were recorded with respect to the control stations. In general, the concentrations of microplastic (<5 mm) debris reported in this study were higher than those found in other similar studies conducted in the UK (Thompson et al. 2009) and Singapore (Ng and Obbard 2006), where three and eight plastic debris fragments per kilogram of dried sediments were recorded, respectively. In contrast, concentrations of plastic (>20 mm) found in the Maltese harbour were less than the values reported for harbours in Belgium (Claessens et al. 2011) and India (Reddy et al. 2006). The highest abundance of debris reported at MP_BN1 station could be due to a high terrestrial input via the sewage pipe. The absence of debris in control stations suggests that plastic concentrations measured in this Maltese harbour could be mainly related to human activities within the same harbour, rather than to transport dynamics shunting plastic pollution from contiguous areas. A combination of physical, biological and chemical processes that reduce the structural integrity of plastic (Cole et al. 2011) may be active in producing the high

densities of debris of small size as nanoplastic and microplastic. However, some of micro and nanoplastic used in consumer products are introduced directly into the oceans via runoff (Maynard 2006). Since microplastics occupy the same size fraction as sediments, they are potentially bioavailable to a wide range of organisms. Nanoplastics and microplastics can be ingested by low trophic suspension, filter and deposit feeders, detritivores and planktivores (Thompson et al. 2004, Graham and Thompson 2009; Murray and Cowie 2011). Therefore, they may accumulate within organisms, resulting in physical harm, such as internal abrasions and blockages. In addition, as confirmed by correlations found in our study between microplastic abundances and benzo(b)-fluoranthene, benzo(a)pyrene, fluoranthene benzo(g,h,i)perylene and Ni, these debris are liable to concentrate environmental contaminants, which have a greater affinity for the hydrophobic surface of plastic compared to seawater (Rochman et al. 2013).

The positive correlation between Shannon diversity index values and plastic category densities may be in accordance with the use of plastic debris as a substrate by many benthic species (Winston 1982; Barnes 2002; Derraik 2002). Our results seems to suggest that pattern of distribution of plastic debris are unlinked to grain size of sediment.

Moreover, the current study shows a clear separation between the environmental quality of human-stressed and control areas within the Grand Harbour.

Statistical analysis showed two sites, MP_BN1 and MP_BN4, with environmental features different from the other stations that are in correspondence of drainage pipe and grit-blasting activities. These stations, characterized by highest levels of contaminants and lowest levels of biodiversity, testified the negative effects of these compounds on communities (Apeti et al. 2009).

Different factors could affect the bioavailability and the final toxicity of pollutants measured in sediments as well as redox conditions, pH, microbiological activity, water salinity and hydrodynamics (Borghini 1999; Renzi et al. 2012) which can affect their chemical form, and for this reason, the observed levels could not be significant of attended effects on biota (Bigongiari et al. 2015). Mobility rather than levels should be properly considered for trace elements within environmental studies (Tessier et al. 1979). Furthermore, literature evidenced as trace element levels are also linked to local geomorphology (Renzi et al. 2011; Andaloro et al.

2012). In fact, fine-grained and organic-enriched sediments tend to have relatively high metal contents (Sternbeck and Östlund 2001; Zhang et al. 2007) and silt fractions are rich in feldspars and natural trace elements (Borghini 1999); consequently, the concentrations of trace elements measured in sediments indicate the enrichment caused by human activities and levels that are due to local natural geomorphologic structures. Due to the contribute of both human-due inputs and natural sources to trace element levels in sediments, absolute discussions of measured levels are of low significance but local thresholds should be determined to detect outliers (Renzi et al. 2015). Even if natural thresholds in local non-polluted sediments represents a key point to evaluate harbour pollution, at the best of our knowledge, a solid statistically structured study to define these types of data is missing in Malta Island and comparison with local geological backgrounds are not possible.

Levels of Cd recorded in Malta harbour resulted lower than those of other Mediterranean harbours, as Iskenderun Bay, Ligurian sea (Bertolotto et al. 2005), but other heavy metals showed concentrations higher than Iskenderun Bay (Yilmaz 2003), Gulf of Trieste (Covelli et al. 2001) and Ligurian Sea (Bertolotto et al. 2005). Some values are comparable with Ancona harbour (Beolchini et al. 2009), except for Pb that in Malta harbour showed higher value. The concentrations measured in Malta harbour results higher also respect to the Gulf of Leone, except for Ni that is lower (Roussiez et al. 2005).

Ecotoxicological tests developed and applied to improve classical environmental monitoring approaches based only on chemistry, promote the quali-quantitative evaluation of biological effects produced by the bioavailable fractions of environmental pollutants, including persistent organic pollutants and trace elements (Volpi-Ghirardini et al. 2001).

Ecotoxicological responses conducted within this study highlighted the fact that harbour areas are able to significantly affect the development of more sensitive species or life stages. Tests performed with sensitive life stages of *P. lividus* reveals toxicity levels for sediment originating from all the sampling stations. The high sensitivity of this species is also confirmed by other studies on sediments and is difficult to explain conclusively (Bigongiari et al. 2015). *V. fischeri* is more sensitive to trace elements pollution than to organic pollutants (Salizzato et al. 1998), so the lower toxicity levels

observed at MP_BN3 and MP_BN4 could be pointing towards a lower solubility of trace elements than organic pollutants within sediments. On the contrary, results obtained through the direct exposure tests performed with *C. orientale* confirms a significant biotic mortality for MP_BN4 sediments that could be reliably associated with the direct toxicity of sediment (Onorati et al. 1999; ISPRA 2011); this hypothesis is also corroborated by the values of the diversity indices.

The spatially homogeneous values of polychlorinated biphenyls (PCBs) recorded in this Maltese harbour (all lower than 5 µg/kg d.w.), suggest that this type of pollution is not a particularly important stressor for the harbour. A similar scenario is reported for Mar Piccolo in Taranto (Cardellicchio et al. 2007), Venice Lagoon (Frignani et al. 2001), Cretan Sea (Gogou et al. 2000) and Trieste Harbour (Adami et al. 2000).

The majority of the PAH congeners attained their maximum values at MP_BN1, and this may be related to the presence of a sewage outfall and the consequent waste effluents accumulating near the same station (Law 1994; Savinov et al. 2000; Kannan et al. 2005).

The high concentrations of BT reported in the present study may be related to the high density of maritime activity experienced within the Grand Harbour and to an extensive past use of these pollutants as biocides in antifouling paints for recreational vessels, large ships and docks (De Mora 1996; Yebra et al., 004). Our data underscores the persistence and toxicity of these chemicals when released in the marine environment. In fact, the percentage of TBT towards the ΣBT showed a past contamination of butyltins in almost all stations. Differently, in the MP_BN4 site, the elevated amount of TBT (5654 ng Sn/g, representing 80 % of ΣBT) might suggest a recent and illegal use of TBT-based antifouling paints (Turner and Glegg 2014) or an accumulation of TBT-containing paint particles, particularly resistant to biodegradation (Page et al. 1996).

It cannot be excluded that the minor part of the content of TBT and MBT could be related to their leaching from PVC and related materials, considering their extensive use as stabilizer compounds in the
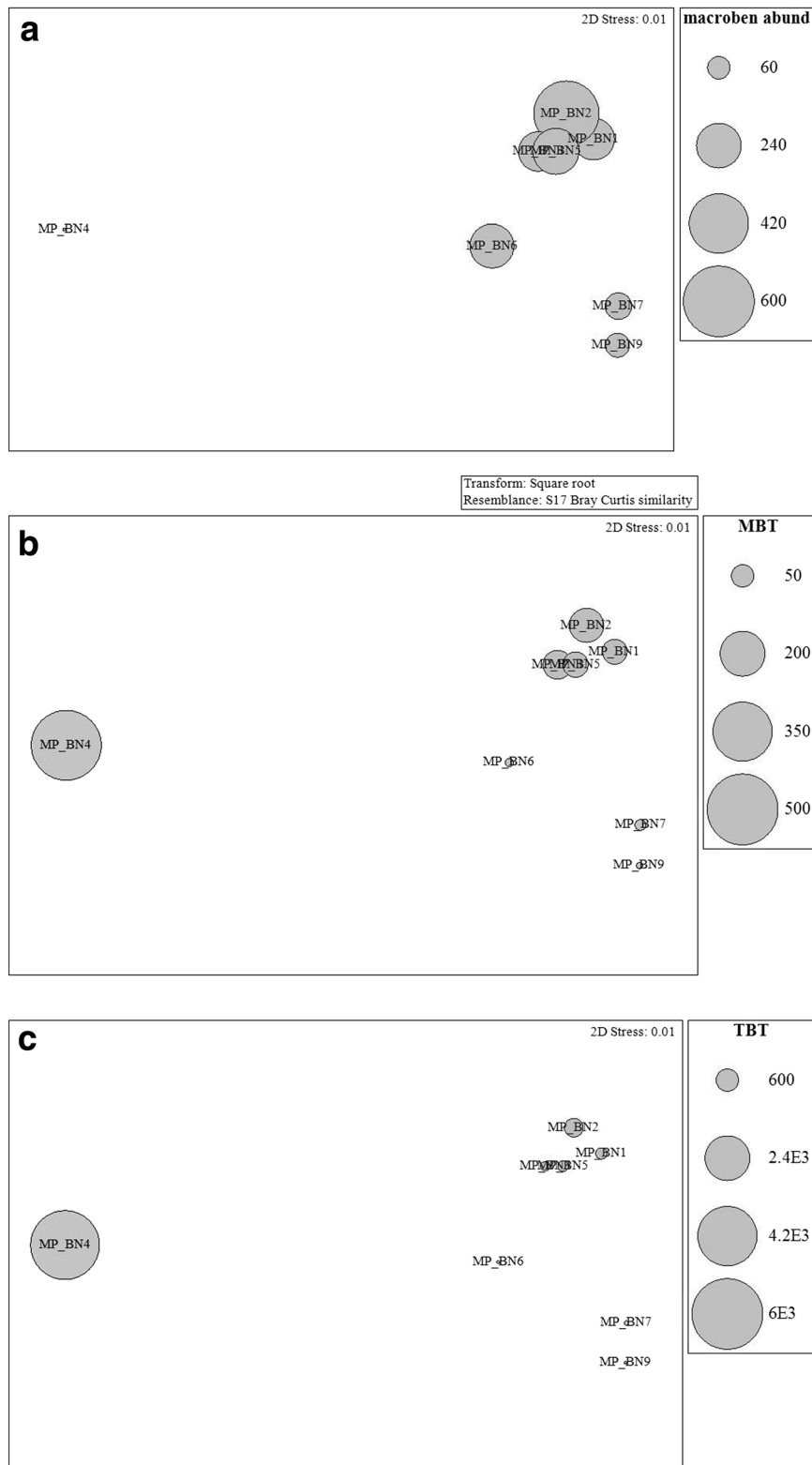
a

b
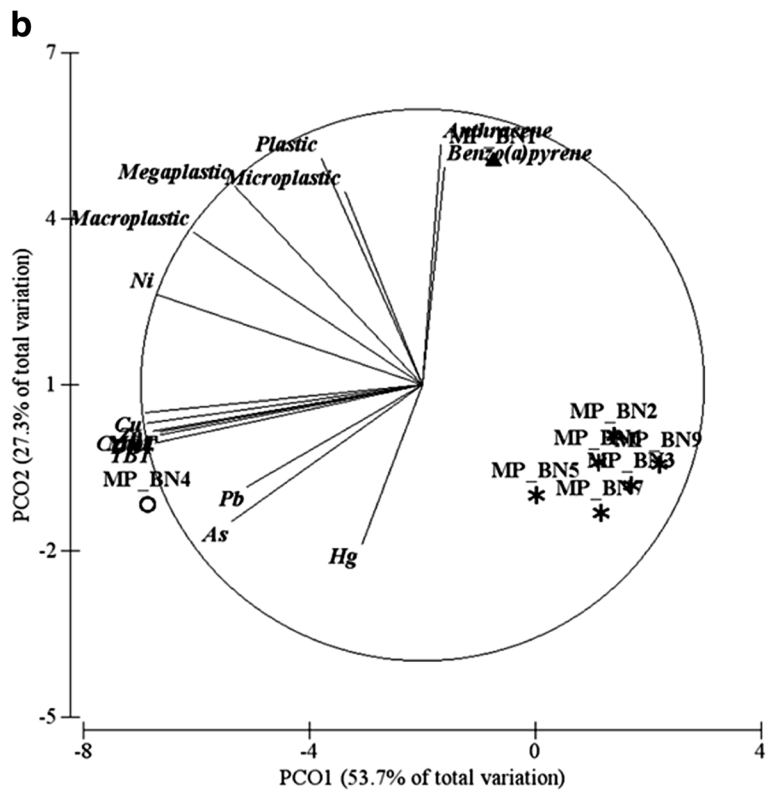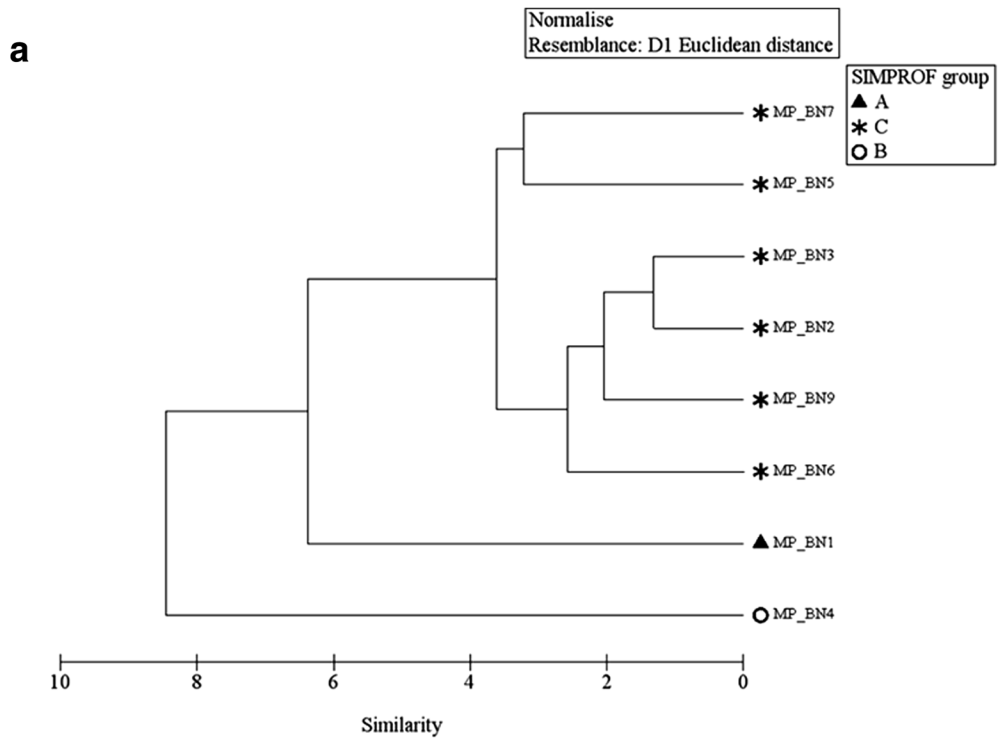
Fig. 4 Bubble plot of macrobenthic abundance (**a**), MBT (**b**) and TBT (**c**) concentrations, recorded at each sampling stations

manufacture of polyvinylchloride (Blunden et al. 1984) and within cooling pipes of thermoelectric power (Bacci and Gacci 1989; Bressa et al. 1999).

Pattern of invertebrate distribution in La Valletta harbour confirms the importance of zoobenthos as bioindicators within marine monitoring (Pearson and Rosenberg 1978; Borja et al. 2000). For instance, high abundance of second-order opportunistic species, typical of environmentally unbalanced situations, such as *C. gibba* and *M. dorsobranchialis*, and of first-order opportunistic family which proliferate in reduced sediments, typical of pronounced environmentally unbalanced situations, such as members of the Capitellidae polychaete family, represent a critical environmental status (Borja et al. 2000). Such conditions are also normally typified by low biodiversity values. The presence of non-indigenous species, which are favoured by environmental unbalance (Occhipinti-Ambrogi 2007), underscores the strategic position of the study area as a major shipping transit route within the Central Mediterranean Sea, that designate the Maltese Islands as a stepping stone for already established NIS to expand their range from the western half to the eastern half of the Mediterranean Basin and vice-versa (Sciberras and Schembri 2007). The present study, by confirming the depletion effects of contaminants on benthic macroinvertebrates (Apeti et al. 2009; Horng et al. 2009; Guerra-Garcia et al. 2010), puts in evidence a significant negative correlation between macrobenthic diversity and copper concentration that suggests a possible cause-effect link, tied to the toxicity of this heavy metal (Brage 1985). Following the ban of TBT, copper is the biocide of choice for antifouling paints (Finnie 2006). These products are also the main source of copper loading in to the marine environment and might cause local concentration peaks of metal in water and overcoming of environmental quality criteria (Srinivasan and Swain 2007).

Moreover, the strong relation between low specific richness values for the macrobenthic community and high sediment concentrations of organotin pollutants confirm the role of these xenobiotics as an important driver of biodiversity loss (Boulajfene et al. 2014). In this study, no significant correlations were found between indices of biodiversity and trace elements; this, as suggested by Tweedley et al. (2015), may be due to different degrees of sensitivity of each species to each metal. Indeed, having found in the same samples both metals and species in different combinations, it is difficult to generalize regarding cause and effect relationships.

Conclusions

In order to unravel the complexity of ecological systems influenced by human activities, workers need to include different aspects of the problem that are frequently not considered due to economy reasons. This is the case of plastic debris and other chemicals which have only recently made the limelight. MBT and TBT have a major influence on macrozoobenthic abundance and biodiversity in harbours. Results of this study suggest that the concentration within sediments of organotin compounds could be one of the major discriminating factors shaping the macrobenthic biodiversity of the same sediment, synergistically making the same community more susceptible to a niche shift through its colonization by non-indigenous species.

The present paper, the first multidisciplinary study carried out to assess the marine health of the Grand Harbour la Valletta, provides evidence that the physical-chemical characterization of sediments represents only a small tassel within the complex web-like ecotoxicological and ecological interactions witnessed within marine ecosystems.

Ecotoxicological responses obtained in this study might evidence differences in the bioavailability of key sediment pollutants, with salient implications for biotic assessment. Harbours are not frequently characterized from a biotic point of view because they are, a priori, considered to be severely stressed ecosystems characterized by low biodiversity. On the contrary, as evidenced by this research, NIS species (i.e. *M. dorsobranchialis*) could take advantage of the relative lack of competition, present within human-stressed ecosystems, exploiting harbours as refuges and strongholds from where to colonize contiguous areas. Gaps in the biotic assessment of harbour ecosystems could lead to a missed opportunity in modelling and controlling colonization processes of coastal marine areas by non-indigenous species.

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). *Journal of Environmental Monitoring*, 2(3), 261–265.

Andaloro, F., Romeo, T., Renzi, M., Guerranti, C., Perra, G., Consoli, P., Perzia, P., & Focardi, S. E. (2012). Alteration of trace element levels in sediments and biota from the Aeolian archipelago (Italy) following an episode of intense volcanic activity. *Environmental Monitoring and Assessment*, 184(7), 4035–4047.

Anderson, M. J., Gorley, R. N., & Clarke, K. R. (2008). *PERMANOVA+ for PRIMER: guide to software and statistical methods*. Plymouth: PRIMER-E.

Apeti, D. A., Lauenstein, G. G., & Riedel, G. F. (2009). Cadmium distribution in coastal sediments and mollusks of the US. *Marine Pollution Bulletin*, 58(7), 1016–1024.

Bacci, E., & Gacci, C. (1989). Organotins compounds in harbours and marina waters from the Northern Tyrrhenian Sea. *Marine Pollution Bulletin*, 206(6), 290–292.

Barnes, D. K. A. (2002). Invasions by marine life on plastic debris. *Nature*, 416, 808–809.

Barnes, D. K. A., & Milner, P. (2005). Drifting plastic and its consequences for sessile organism dispersal in the Atlantic Ocean. *Marine Biology*, 146, 815–825.

Bellan, G., Dauvin, J. C., & Bellan-Santini, D. (2010). Knowledge, protection, conservation and management in the marine domain: the example of the mainland French coast. *Open Conservation Biology Journal*, 4, 9–18.

Beolchini, F., Dell'Anno, A., De Propris, L., Ubaldini, S., Cerrone, F., & Danovaro, R. (2009). Auto-and heterotrophic acidophilic bacteria enhance the bioremediation efficiency of sediments contaminated by heavy metals. *Chemosphere*, 74(10), 1321–1326.

Bertolotto, R. M., Tortarolo, B., Frignani, M., Bellucci, L. G., Albanese, S., Cuneo, C., & Gollo, E. (2005). Heavy metals in superficial coastal sediments of the Ligurian Sea. *Marine Pollution Bulletin*, 50(3), 348–356.

Blunden, S. J., Hobbs, L. A., & Smith, P. J. (1984). *The environmental chemistry of organotin compounds*. The Royal Society of Chemistry, London: Environmental Chemistry.

Bigongiari, N., Braida, T., & Pasteris, A. (2001). Saggio biologico con l'anfipode *Corophium orientale*: metodiche ed esempi di applicazione ai sedimenti marini. *Biologia Marina Mediterranea*, 8(2), 60–71.

Bigongiari, N., Cipriani, L.E., Pranzini, E., Renzi, M., & Vitale, G. (2015). Assessing shelf aggregate environmental compatibility and suitability for beach nourishment: A case study for Tuscany (Italy). Marine Pollution Bulletin, http://dx.doi.org/10.1016/j.marpolbul.

Binato, G., Biancotto, G., Piro, R., & Angeletti, R. (1998). Atomic adsorption spectrometric screening and gas chromatographic-mass spectrometric determination of organotin compounds in marine mussels: an application in samples from the Venetian Lagoon. *Fresenius Journal of Analytical Chemistry*, 361, 333–337.

Borghini, F. (1999). Gli elementi in tracce negli ecosistemi costieri dell'Argentario. Atti Società Toscana Scienze Natutali, Mem. Serie A, 105, 9–16.

Borja, A., Franco, J., & Perez, V. (2000). A marine biotic index to establish the ecological quality of soft-bottom benthos within European estuarine and coastal environments. *Marine Pollution Bulletin*, 40(12), 1100–1114.

Boulajfene, W., Boukhicha, J., Berto, D., Romeo, T., Ben Hassine, P. K., & Tlig-Zouari, S. (2014). Biomonitoring of the environmental contamination by organotins in the Gulf of Tunis: occurrence of imposex in *Stramonita hemastoma* (Linnaeus, 1767). *Marine and Freshwater Research, MF*, 133301.

Brage, R. (1985). Effect of sediment copper on benthic fauna. *Marine Ecology Progress Series*, 25, 83–89.

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs*, 27, 325–349.

Bressa, G., Caravello, G. U., Da Villa, G., & Sisti, E. (1999). Organotins in mussels and sediments from the lagoon of Venice. *Fresenius Environment Bulletin*, 8, 107–115.

Brouwer, H., Murphy, T., & McArdle, L. (1990). A sediment-contact bioassay with *Photobacterium phosphoreum*. *Environmental Toxicology Chemistry*, 9, 1353–1358.

Buchanan, J. B., & Kain, J. H. (1971). Measurement of the physical and chemical environment. In N. A. Holme, & A. D. McIntyre (Eds.), *Methods for the study of marine benthos* (pp. 30–53). Oxford: Blackwell Scientific Publications.

Cardellicchio, N., Buccolieri, A., Giandomenico, S., Lopez, L., Pizzulli, F., & Spada, L. (2007). Organic pollutants (PAHs, PCBs) in sediments from the Mar Piccolo in Taranto (Ionian Sea, Southern Italy). *Marine Pollution Bulletin*, 55(10), 451–458.

Claessens, M., Meester, S. D., Landuyt, L. V., Clerck, K. D., & Janssen, C. R. (2011). Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin*, 62(10), 2199–2204.

Clarke, K. R., & Warwick, R. M. (2001). *Change in marine communities: an approach to statistical analysis and interpretation* (2nd ed.,). Plymouth: PRIMER-E.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. *Marine Pollution Bulletin*, 62(12), 2588–2597.

Covelli, S., Faganeli, J., Horvat, M., & Brambati, A. (2001). Mercury contamination of coastal sediments as the result of long-term cinnabar mining activity (Gulf of Trieste, northern Adriatic sea). *Applied Geochemistry*, 16(5), 541–558.

De Mora, S. J. (1996). The Tributyltin debate: ocean transportation versus seafood harvesting. In S. J. de Mora (Ed.), *Tributyltin: case study of an environmental contaminant* (pp. 1–15). Cambridge: Cambridge University Press.

Derraik, J. G. B. (2002). The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin*, 44, 842–852.

Dickinson, W. W., Dunbar, G. B., & McLeod, H. (1996). Heavy metal history from cores in Wellington Harbour New Zealand. *Environmental Geology*, 27, 59–69.

Estacio, F. J., García-Adieg, 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. *Marine Pollution Bulletin*, *34*, 780–793.

Fatoki, O. S., & Mathabatha, S. (2001). An assessment of heavy metal pollution in East London and Port Elisabeth Harbour. *Water SA*, *27*, 233–240.

Finnie, A. A. (2006). Improved estimates of environmental copper release rates from antifouling products. *Biofouling*, *22*(5), 279–291.

Frignani, M., Bellucci, L. G., Carraro, C., & Raccanelli, S. (2001). Polychlorinated biphenyls in sediments of the Venice Lagoon. *Chemosphere*, *43*, 567–575.

Gaion, A., Scuderi, A., Pellegrini, D., & Sartori, D. (2013). Arsenic exposure affects embryo development of sea urchin, *Paracentrotus lividus* Lamarck, 1816). *Bulletin of Environmental Contamination and Toxicology*, *91*(5), 565–570.

Galil, B., Golik, A., & Turkay, M. (1995). Litter at the bottom of the sea: a sea bed survey in the Eastern Mediterranean. *Marine Pollution Bulletin*, *30*(1), 22–24.

Galil, B. S., Marchini, A., Occhipinti-Ambrogi, A., Minchin, D., Narščius, A., Ojaveer, H., & Olenin, S. (2014). International arrivals: widespread bioinvasions in European seas. *Ethology Ecology and Evolution*, *26*(2–3), 152–171.

Gogou, A., Bouloubassi, I., & Stephanou, E. G. (2000). Marine organic geochemistry of the Eastern Mediterranean: aliphatic and polyaromatic hydrocarbons in Cretan Sea superficial sediments. *Marine Chemistry*, *68*, 265–282.

Graham, E. R., & Thompson, J. T. (2009). Deposit- and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. *Journal of Experimental Marine Biology and Ecology*, *368*(1), 22–29.

Guerra-Garcia, J. M., Ruiz-Tabares, A., Baeza-Rojano, E., Pilar Cabezas, M., Diaz-Pavon, J. J., Pacios, I., Maestre, M., Gonzalez, A. R., Espinosa, F., & Garcia-Gomez, J. C. (2010). Trace metals in *Caprella* (Crustacea: Amphipoda). A new tool for monitoring pollution in coastal areas? *Ecological Indicators*, *10*, 734–743.

Heida, H., & van der Oost, R. (1996). Sediment pore water toxicity testing. *Water Science Technology*, *34*, 109–116.

Horng, C. Y., Wang, S. L., & Cheng, I. J. (2009). Effects of sediment-bound Cd, Pb, and Ni on the growth, feeding, and survival of *Capitella* sp. *Journal of Experimental Marine Biology and Ecology*, *371*, 68–76.

ICRAM (2003). "Metodologie analitiche di riferimento" edito dal Ministero dell'Ambiente e della tutela del territorio – Servizio Difesa Mare. Scheda n. 7 Sedimenti mediante Cromatografia Liquida ad Alta Prestazione HPLC.

Isenberg, D. L. (1993). The Microtox® toxicity test—a developer's commentary. In M. Richardson (Ed.), *Ecotoxicology monitoring* (pp. 3–15). VCH Verlagsgesellschaft MBH, Weinheim. Germany.

ISO (1998). *Water quality—determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (luminescent bacteria test) ISO/DIS 11348*. Geneva, Switzerland: International Organization for standardization.

ISO (2005). Water quality-Determination of acute toxicity of marine or estuarine sediment to amphipods. ISO 16712–05 E.

ISPRA (2011). *Manuali e linee guida 67/2011, Batterie di saggi ecotossicologici per sedimenti di acque salate e salmastre*. Guideline in Italian language.

Kang, Y., Sheng, G., Fu, J., Mai, B., Zhang, G., Lin, Z., & Min, Y. (2000). Polychlorinated biphenyls in surface sediments from Pearl River Delta and Macau. *Marine Pollution Bulletin*, *40*, 794–797.

Kannan, K., Johnson, B.-R., Yohn, S. S., Giesy, J. P., & Long, D. T. (2005). Spatial and temporal distribution of polycyclic aromatic hydrocarbons in sediments from inland lakes in Michigan. *Environmental Science and Technology*, *39*, 4700–4706.

Katsanevakis, S., Zenetos, A., Belchior, C., & Cardoso, A. C. (2013). Invading European seas: assessing pathways of introduction of marine aliens. *Ocean Coastal Management*, *76*, 64–74.

Law, R. J. (1994). Polycyclic aromatic hydrocarbons (PAH) - problems and progress in sampling, analysis and interpretation. *Marine Pollution Bulletin*, *29*, 235–241.

Lera, S., & Pellegrini, D. (2006). Evaluation of the fertilization capability of *Paracentrotus lividus* sea urchin storaged gametes by the exposure to different aqueous matrices. *Environmental Monitoring and Assessment*, *119*, 1–13.

Li, A., Ab Razak, I. A., Ni, F., Gin, M. F., & Christensen, E. R. (1998). Polycyclic aromatic hydrocarbons in the sediments of the Milwaukee Harbor estuary, Wisconsin, USA. *Water, Air, and Soil Pollution*, *101*(1–4), 417–434.

Maynard, A. (2006). Nanotechnology: a research strategy for addressing risk. Woodrow Wilson International Center for Scholars Project on Emerging Nanotechnologies. http://www.nanotechproject.org/file_download/files/PEN3_Risk.pdf.

McCready, S., Birch, G. F., & Long, E. R. (2006). Metallic and organic contaminants in sediments of Sidney Harbour, Australia and vicinity—a chemical dataset for evaluating sediment quality guidelines. *Environmental International*, *32*, 455–465.

Morabito, R., Chiavarini, S., & Cremisini, C. (1995). *Speciation of organotin compounds in environmental samples by GC-MS, In: P. Quevauviller, E. A. Maier, & B. Griepink (eds). Quality Assurance for Environmental Analysis (cap. 17, pp. 435–464)*. Amsterdam: Elsevier.

Murray, F., & Cowie, P. R. (2011). Plastic contamination in the decapod crustacean Nephrops norvegicus (Linnaeus, 1758). *Marine Pollution Bulletin*, *62*(6), 1207–1217.

NG, K. L., & Obbard, J. P. (2006). Prevalence of microplastic in Singapore's coastal marine Environment. *Marine Pollution Bulletin*, *52*, 791–767.

Occhipinti-Ambrogi, A. (2007). Global change and marine communities: alien species and climate change. *Marine Pollution Bulletin*, *55*(7), 342–352.

Onorati, F., Bigongiari, N., Pellegrini, D., & Giuliani, S. (1999). The suitability of Corophium *orientale* (Crustacea, Amphipoda) in harbour sediment toxicity bioassessment. *Aquatic Ecosystem Health & Management*, *2*(4), 465–473.

Page, D. S., Ozbal, C. C., & Lanphear, M. E. (1996). Concentration of butyltin species in sediments associated with shipyard activities. *Environmental Pollution*, *2*, 237–243.

Pearson, T. H., & Rosenberg, R. (1978). Macrobenthic succession in relation to organic enrichment and pollution of the marine

environment. *Oceanography Marine Biology Annual Review, 16*, 229–311.

Pedersen, F., Bjørnestad, E., Andersen, H. V., Kjølholt, J., & Poll, C. (1998). Characterization of sediments from Copenhagen harbour by use of biotests. *Water Science Technology, 37*, 233–240.

Poulton, D. J., Morris, W. A., & Coakley, J. P. (1996). Zonation of contaminated bottom sediments in Hamilton harbor as defined by statistical classification techniques. *Water Quality Research Journal Canada, 31*, 505–528.

Reddy, S. M., Basha, S., Adimurthy, S., & Ramachandraiah, G. (2006). Description of small plastics fragments in marine sediments along the Alang-Sosiya ship-breaking yard, India. *Estuarine Coastal and Shelf Science, 68*, 656–660.

Renzi, M., Specchiulli, A., Baroni, D., Scirocco, T., Cilenti, L., Focardi, S., Breber, P., & Focardi, S. (2012). Trace elements in sediments and bioaccumulation in European silver eels (*Anguilla anguilla* L.) from a Mediterranean lagoon (SE Italy). *International Journal of Environmental Analytical Chemistry, 92*(6), 676–697.

Renzi, M., Romeo, T., Guerranti, C., Perra, G., Italiano, F., Focardi, S. E., Esposito, V., & Andaloro, F. (2011). Temporal trends and matrix-dependent behaviours of trace elements closed to a geothermal hot-spot source (Aeolian Archipelago, Italy). *Procedia Earth and Planetary Science, 4*, 10–28.

Renzi, M., Bigongiari, N., & Focardi, S. E. (2015). Baseline levels of trace elements in coastal sediments from the central Mediterranean (Tuscany, Italy). *Chemistry and Ecology, 31*(1), 34–46.

Sternbeck, J., & Östlund, P. (2001). Metals in sediments from the Stockholm region: geographical pollution patterns and time trends. *Water, Air, & Soil Pollution: Focus, 1*, 151–165.

Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers contaminants to fish and induces hepatic stress. *Natural Scientific Report, 3*, 3263. doi:10.1038/srep03263.

Roussiez, V., Ludwig, W., Probst, J. L., & Monaco, A. (2005). Background levels of heavy metals in surficial sediments of the Gulf of Lions (NW Mediterranean): an approach based on 133 Cs normalization and lead isotope measurements. *Environmental Pollution, 138*(1), 167–177.

Salizzato, M., Pavoni, B., Volpi Ghirardini, A., & Ghetti, P. F. (1998). Sediment toxicity measured using *Vibrio fischeri* as related to the concentrations of organic (PCBs, PAHs) and inorganic (metals, sulphur) pollutants. *Chemosphere, 36*, 2949–2968.

Savinov, V. M., Savinova, T. N., Carroll, J., Matishov, G. G., Dahle, S., & Naes, K. (2000). Polycyclic aromatic-hydrocarbons (PAHs) in sediments of the White-Sea, Russia. *Marine Pollution Bulletin, 40*, 807–818.

Sciberras, M., & Schembri, P. J. (2007). A critical review of records of alien marine species from the Maltese Islands and surrounding waters (Central Mediterranean). *Mediterranean Marine Science, 8*(1), 41–66.

Shannon, C. E., & Weaver, W. (1963). *The mathematical theory of communication*. University of Illinois Press, Urbana.

Sprovieri, M., Feo, M. L., Prevedello, L., Manta, D. S., 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*(5), 998–1009.

Srinivasan, M., & Swain, G. W. (2007). Managing the use of copper-based antifouling paints. *Environmental Management, 39*(3), 423–441.

Tessier, A., Campbell, P. G. C., & Bisson, M. (1979). Sequential extraction procedure for the speciation of particulate trace metals. *Analytical Chemistry, 51*(7), 844–851.

Thompson, R. C., Olsen, Y., Mitchell, R. P., Davis, A., Rowland, S. J., John, A. W. G., McGonigle, D., & Russel, A. E. (2004). Lost at sea: where is all the plastic? *Science, 304*, 838.

Thompson, R. C., Moore, C. J., Vom Saal, F. S., & Swan, S. H. (2009). Plastics, the environment and human health: current consensus and future trends. *Philosophical Transactions of the Royal Society of London B: Biological Science, 364*(1526), 2153–2166.

Turner, A., & Glegg, G. (2014). TBT-based antifouling paints remain on sale. *Marine Pollution Bulletin, 88*, 398–400.

Tweedley, J. R., Warwick, R. M., & Potter, I. C. (2015). Can biotic indicators distinguish between natural and anthropogenic environmental stress in estuaries? *Journal of Sea Research, 102*, 10–21.

US-EPA 6020A/2007. Inductively coupled plasma-mass spectrometry. http://www.epa.gov/solidwaste/hazard/testmethods/sw846/pdfs. Last access February 16th, 2015a

US-EPA 8082A/2007. Polychlorinated Biphenyls (PCBs) by Gas Chromatography. http://www.epa.gov/solidwaste/hazard/testmethods/sw846/pdfs. Last access February 16th, 2015b.

US-EPA 8270D. Semivolatile organic compounds by gas chromatography/mass spectrometry (GC/MS). http://www.epa.gov/solidwaste/hazard/testmethods/sw846/pdfs/. Last access February 16th, 2015c.

US-EPA (2001). Methods for Collection, Storage and Manipulation of Sediments for Chemical and Toxicological Analyses - Contaminated Sediments in Water. Technical Manual. EPA-823-F-01-023.

Vanhala, P. T., & Ahtiainen, J. H. (1994). Soil respiration ATP-content and *Photobacterium* toxicity test as indicators of metal pollution in soil. *Environmental Toxicology Water Quality, 9*, 115–121.

Volpi-Ghirardini, A., Arizzi Novelli, A., Borsetto, E., Delaney, B., & Tagliapietra, D. (2001). Sediment toxicity assessment in the Venetian Lagoon using *Paracentrotus lividus* (Echinodermata: Echinoidea). *Biologia Marina Mediterranea, 8*(1), 489–496.

Wiberg, P. L., & Harris, C. K. (2002). Desorption of p,p0-DDE from sediment during resuspension events on the Palos Verdes shelf, California: a modelling approach. *Continental Shelf Research, 22*, 1005–1023.

Winston, J. E. (1982). Drift plastic—an expanding niche for a marine invertebrate? *Marine Pollution Bulletin, 13*, 348–357.

Winston, J. E., Gregory, M. R., & Stevens, L. M. (1997). Encrusters, epibionts, and other biota associated with pelagic plastics: a review of biogeographical, environmental, and conservation issues. In J. M. Coe, & D. B. Rogers (Eds.), *Marine debris: sources, impacts, and solution* (pp. 81–97). New York: Springer.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. *Environmental Pollution, 178*, 483–492.

Yebra, D. M., Kiil, S., & Dam-Johansen, K. (2004). Antifouling technology—past, present and future steps towards efficient and environmentally friendly antifouling coatings. *Progress in Organic Coatings*, *50*, 75–104.

Yilmaz A. B. (2003). Levels of heavy metals (Fe, Cu, Ni, Cr, Pb and Zn) in tissue of *Mugil cephalus* and *Trachurus mediterraneus* from Iskenderun Bay, Turkey. *Environmental Research, 92*, 277–281.

Zenetos, A., Gofas, S., Morri, C., Rosso, A., Violanti, D., García Raso, J. E., et al. (2012). Alien species in the Mediterranean Sea by 2012. A contribution to the application of European Union's Marine Strategy Framework Directive (MSFD). part 2. Introduction trends and pathways. *Mediterranean Marine Science*, *13*, 328–352.

Zhang, L., Ye, X., Feng, H., Jing, Y., Ouyang, T., Yu, X., Liang, R., Gao, C., & Chen, W. (2007). Heavy metal contamination in western Xiamen Bay sediments and its vicinity, China. *Marine Pollution Bulletin*, *54*(7), 974–982.