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Article

Ecological Risk Assessment of Heavy Metals and Environmental Quality of Tunisian Harbours

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Abstract: Harbours are one of the most disturbed coastal ecosystems due to intensive anthropogenic pressures. This study aimed for the first time to compare anthropogenic impacts in three harbours from the central coast of Tunisia (Mediterranean Sea) employing analysis of heavy metal contamination and ecological quality status (EcoQS). Sampling was carried out in spring 2019 in the fishing harbour of Teboulba, the marina of Monastir, and the commercial harbour of Sousse. The high levels of concentration in heavy metals and organic matter were closely related to the fine-grained fraction of the sediment in the fishing and commercial harbours. A total of 94 macrobenthic species, including five nonindigenous species, were identified belonging to six zoological groups. Multivariate analyses highlighted a strong influence of the harbour activity on the diversity of macrozoobenthic communities. Three benthic assemblages were identified according to their environmental characteristics such as sediment type, organic matter content, and heavy metal contamination. Benthic and biotic indices (H′, J′, AMBI, and BO2A) showed that the EcoQS varied from poor (commercial harbour) to good (marina), and was significantly influenced by harbour activity, organic matter, and heavy metal contamination of the sediment. The present work could be considered as providing important baseline data for the implementation of national environmental policies and management plans in the future.

Keywords: benthic macrofauna; harbour activity; heavy metals; biotic indices; ecological quality status; central Mediterranean

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1. Introduction

Harbours are known as confined ecosystems that are considered as among the most affected coastal areas faced with a wide range of environmental problems including the discharge of sewage waste and ballast water, petroleum and its derivatives, and antifouling paints, together with dredging activities, which may affect both the dredged and the disposal sites [1–5]. Moreover, harbour habitats are considered as major pathways for nonindigenous species (NIS) [6–9]. These anthropogenic activities increase the environmental pressures in harbour habitats and change the structure and functioning of the biological communities [5,10]. Therefore, the question of how biological communities respond to environmental perturbations in these marine ecosystems is becoming increasingly relevant for environmental managers and ecologists [11]. Macrofaunal benthic invertebrates are recommended as well-established targets for the evaluation of quality status in coastal ecosystems over time [3,5,12] and for the assessment of heavy metal pollution in enclosed environments including harbours [2,13]. To evaluate benthic environmental quality, different indices are available, such as the widely used AZTI marine biotic index (AMBI) [14] and BO2A [15,16].

The sheltered and semisheltered coastal ecosystems of Tunisia have been under increasing anthropogenic pressures for several years, due to the influence of massive quantities of phosphogypsum (PG) discharged as a byproduct generated during the production of phosphate fertilizers, along with urban wastes and shipping activities which damage the environment and its marine biodiversity [9,17–20]. Along the 1600 km of Tunisian coastline, there are eight commercial and industrial harbours open to international marine traffic, eight marinas with a total capacity of nearly 3266 moorings, and 43 fishing harbours with a landing capacity of 150,000 tonnes of fishing products annually.

Harbours are specifically characterized by their limited water exchange with the open sea, and are known as sinks for the accumulation of pollutants, especially, heavy metals, whose concentrations can reach five times the values found in seawater. As a result, many harbours are classified as harmfully polluted environments, due to the persistence of high contents of contaminants in the sediments and their toxicity and ability to be absorbed in the trophic chain [21,22]. Several studies had focused on the assessment of heavy metal contamination in the sediments and seawater along the Tunisian coast. In the Gulf of Tunis, Ennouri et al. [23] showed diverse spatial distribution of metals due to anthropogenic activities. Furthermore, Helali et al. [24] pointed out that iron, cadmium, lead, and zinc were the most frequently encountered trace metals in the offshore areas of the Mejerda catchment. On the southern part of the Tunisian coast, several studies showed that high concentrations of many heavy metals (e.g., Pb, Cr, Cd, Cu, Zn, and Fe) in surface sediments around the Gulf of Gabès [9,19,20,25]. These high concentrations were clearly related mainly to phosphogypsum waste discharges. Other studies had considered the effects of heavy metal and hydrocarbon pollution of sediments and benthic communities, indicating that the Gulf of Gabès is one of the most polluted areas in Tunisian waters [20,26,27]. Previous studies of the spatial distribution and contamination with heavy metals in the Gulf of Gabès [28] showed that the marine sediments were devoid of pollution by As, Ni, and Pb; moderately polluted by Cr and Cu; and moderately to strongly polluted by P, Y, and Zn, while all sites were extremely polluted by Cd. Recently, Mosbahi et al. [9] showed that the Gulf of Gabès harbours were characterized by high levels of heavy metal contamination correlated with the surrounding heavy industrialization and the intense harbour activities.

Despite the large number of harbours and the importance of the coastal ecosystems, only few biological and ecological studies have been carried out in Tunisia that specifically address the benthic macrofauna of the harbours. The earliest of these studies was carried out by Aloui-Bejaoui and Afli [29] in the Kerkennah islands, followed by [5,30,31], who worked on the benthic communities of Mediterranean harbours and identified the NIS from these enclosed environments. More recently, [9] focused on the assessment of the ecological quality status (EcoQS) and the impacts of NIS in the Gulf of Gabès harbours. Moreover, to our knowledge, no studies have yet targeted the effects of heavy metals on the structure and functional responses of benthic macrobenthic assemblages and the EcoQS of harbour ecosystems on the eastern coast of Tunisia (central Mediterranean Sea).

The present study aims to improve our knowledge of the heavy metal distribution, the structure and diversity of macrobenthic communities, and the EcoQS in three harbours on the central part of the Tunisian coast. The correlations between the benthic ecological quality and heavy metal contamination are evaluated. The following question arises: How do harbour marine activities influence the contamination and EcoQS of such harbour ecosystems?

2. Materials and Methods

2.1. Study Area

The three harbours are located on the northeastern coast of Tunisia (Figure 1). In the north, the Sousse commercial harbour occupies a surface area of 21 ha and has a maximum depth of 10.5 m. This harbour was constructed in 1899 and is currently visited by more than 550 vessels per year transporting various goods, leading to the reception of 65,410 containers. The harbour of Teboulba is the major fishing port in the Monastir region, constructed in 1970 and covering an area of approximately 8.5 ha with a maximum depth of 4.5 m. It has a very large number of shipyards and hosts more than 900 fishing boats (trawlers, tuna, and sardine vessels), contributing 14% of the Tunisian national fish production. Farther south, the marina of Monastir, also constructed in 1970, is an attractive destination for coastal holiday tourism, hosting 400 international recreational boats; its maximum depth is only 4 m [31].

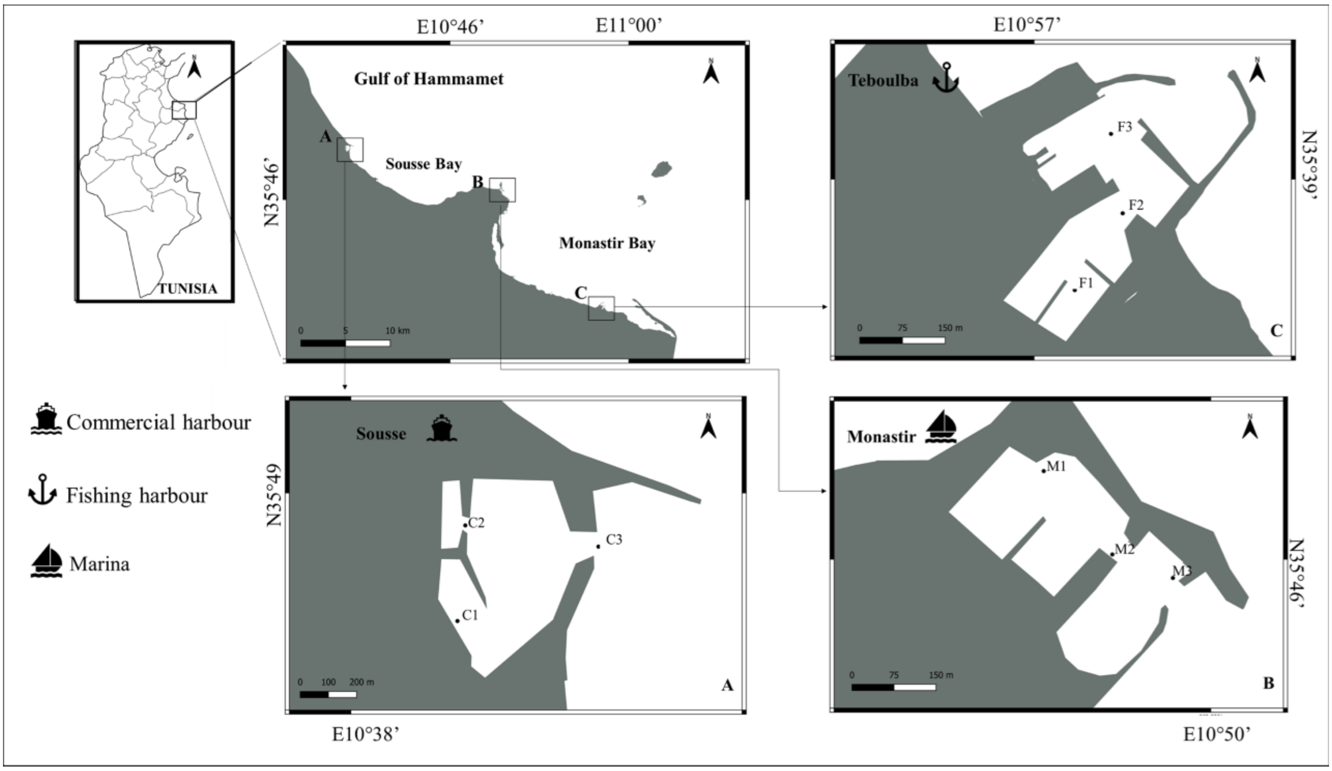

Figure 1. Map of the study areas showing location of sampling stations. (**A**): commercial harbour of Sousse, (**B**): Monastir marina, (**C**): fishing harbour of Teboulba.

2.2. Sampling Design and Laboratory Procedures

Macrobenthic Fauna Sampling

Sampling was carried out from 18 to 20 April 2019. For each harbour, sampling was carried out using a motorized boat to access all the stations. Three stations were sampled to cover the entire harbour basin (Figure 1). At each station, eight replicate samples were taken with a Van Veen grab covering a unit surface of 0.05 m^2: i.e., four samples for biological analyses representing a total surface of 0.2 m^2, and four replicates for sediment analyses (sediment type, organic matter content, and chemical contamination analyses). Seawater samples were taken at ~0.1 m depth using PVC Van Dorn bottles (1.5 L volume) deployed horizontally.

Benthic samples were sieved on a 1 mm circular mesh and the residue fixed in 70% alcohol [19,29,32]. A portable GPS (WGS84) and a depth meter were available on board to record the exact position and depth at each station (Table 1). In addition to temperature and salinity, other hydrological parameters were measured in situ close to the seabed using a WTW 3420 multimeter, dissolved oxygen with an oximeter (WTW3B30-010), pH with a pH meter (WTW 3110), and transparency with a Secchi disk.

The chlorophyll a concentration (Chl a: $\mu g \cdot L^{-1}$) was estimated in the laboratory from a 1 L water sample collected in situ and transported in the dark and at low temperature to the laboratory and then filtered on GFC filters (0.2 µm pore size) and extracted using 100% acetone [20]. The absorbance was measured with a spectrophotometer at 630, 647, 664, and 750 nm, and the concentration was estimated according to [33].

Table 1. Main characteristics of sampling stations and environmental factors measured during spring 2019. H. type: harbour type; Code: station code; Lat N: Latitude; Log E: Longitude; D: depth (m); Tr: transparency (m); Sal: salinity; Temp: surface temperature (°C); Chl a: chlorophyll (μg/g); DO: dissolved oxygen (mg.L^{-1}); OM: organic matter (%); Folk classif: Folk classification; Sand (63–2000 μm); Mud (<63 μm) (%); *Po*: *Posidonia oceanica*; *Cn*: *Cymodocea nodosa.*

Zone	H Type	Code	Lat N	Log E	Dep	Tr	Sal	Temp	pH	Chl a	DO	Sand	Mud	Folk Classif	OM	Macrophytes
Teboulba	Fishing	F1	10.9561°	35.6582°	2.3	1.0	37.3	21.3	7.88	8.02	7.22	76.40 ± 2.1	24.60 ± 1.2	Fine sand	20.40	*Ulva* spp.
		F2	10.9569°	35.6606°	2.5	1.5	37.8	21.3	7.82	9.24	7.42	80.20 ± 1.2	19.80 ± 0.2	Fine sand	18.20	*Ulva* spp.
		F3	10.9571°	35.6594°	2.4	1.2	38.2	22.01	7.90	7.81	8.01	84.41 ± 0.1	15.59 ± 0.1	Fine sand	16.40	*Ulva* spp.
Monastir	Marina	M1	10.8346°	35.7791°	4.2	3.0	40.5	19.8	8.01	10.16	8.50	96.2 ± 0.2	3.8 ± 2.1	Medium sand	10.04	*Po*
		M2	10.8358°	35.7787°	4.4	3.2	39.6	20.1	8.22	12.42	8.42	89.0 ± 5.3	11.0 ± 1.8	Medium sand	8.30	*Po*
		M3	10.8333°	35.7805°	4.5	3.5	40.1	20.2	8.02	12.20	9.02	97.7 ± 0.1	2.3 ± 1.1	Medium sand	7.88	*Po*; *Cn*
Sousse	Commercial	C1	10.6425°	35.8228°	6.0	1.4	39.1	18.7	6.13	3.98	4.66	3.3 ± 0.2	96.7 ± 0.1	Mud	28.80	*Ulva* spp.
		C2	10.6429°	35.8259°	7.5	0.9	38.5	19.2	7.50	5.21	6.52	1.3 ± 2.1	98.7 ± 0.1	Mud	36.42	*Ulva* spp.
		C3	10.6481°	35.8251°	9.0	1.3	38.6	18.5	7.38	5.31	6.77	5.3 ± 1.5	94.7 ± 0.1	Mud	32.18	*Ulva* spp.

The benthic macrofauna was carefully sorted, identified, when possible, to species level under a stereomicroscope, and then counted. The nomenclature of macrobenthic species was updated according to the World Register of Marine Species (WoRMS, last accessed on 8 October 2022). The vegetation type found at each station was also identified (Table 1).

2.3. Sediment Analysis

For sediment analysis, the topmost 5 cm layer was sampled in each replicate (12 sediment samples for each harbour). The particle-size distribution was determined after sieving the dry sediment through a series of sieves with decreasing mesh size (1000, 500, 250, 125, and 63 µm) according to the standard recommended by AFNOR (French Association for Standardization). Sediment samples (200 g) were then dried at 80 °C to constant weight and ground to a fine powder [9,34,35]; the median grain size was then estimated to allow classification of the type of sediment. The organic matter (OM) content was determined on the powder samples (100 g) by loss on ignition at 500 °C for 4 h [20].

The concentrations of heavy metals (copper, chromium, cadmium, magnesium, mercury, lead, iron, aluminium, zinc), fluorine, phosphorus, and nitrogen were determined after digesting the powder sample (5 g) in aqua regia (HCl, HNO_3, H_2O) at 95 °C, and analysed by inductively coupled plasma atomic emission spectrometry (ICP–AES) and mass spectrometry (ICP–MS) [36]. During heavy metal analysis, calibration verification standards were regularly used to evaluate the calibration curve. The minimum correlation coefficient of the calibration curve accepted was 0.999 [27,28]. The assessment of heavy metal pollution in the three harbours is based on regional and international standard guidelines applicable for heavy metals in marine sediments [28,37,38].

2.4. Data Analysis

2.4.1. Taxonomic Diversity

Taxonomic richness (S; the number of taxa per 0.05 m^2) and the abundance (N: number of individuals per 0.05 m^2 calculated from the four replicated samples) and the indices of diversity (H′: Shannon–Weaver diversity index) in log_2 and evenness (J′: Pielou's evenness) were evaluated for the nine stations from the three harbours.

A taxa-by-station abundance matrix and a correlation matrix of environmental variables were constructed and imported into the PRIMER®-v6 for statistical analysis [39].

The trophic organization of the macrozoobenthic communities was characterized using the feeding guilds proposed by several marine ecologists [9,19,20,32].

One-factor analysis of variance (ANOVA) was carried out to test the differences in environmental variables, taxonomic richness, abundance, H′, J′, trophic groups, and biotic indices (AMBI and BO2A) between the stations and the harbours. A post hoc Tukey test ($p < 0.05$) was used for a posteriori multiple comparisons. Prior to the ANOVAs, analyses were performed to test the normality (Kolmogorov-Smirnov) and check the homogeneity of variances. These statistical analyses were performed using the R software vegan package (R version 2.12.0).

2.4.2. Ecological Indicators

We applied two currently available biotic indices commonly used to evaluate the EcoQS of harbour ecosystems, namely AMBI [14] and BO2A [15,16]. AMBI qualifies the ECoQS within a five-class scale of pollution, which considers five ecological groups ranging from sensitive species (EGI) to first-order opportunistic species (EGV); we used the species list published by the AZTI web site on 30 June 2019. The BO2A (benthic opportunistic polychaete amphipod index) index was calculated as log10 of the ratio of frequencies for opportunistic annelids and amphipods: i.e., the total number of opportunistic annelids and total number of amphipods +1 divided by the average abundance counted in replicate samples [15,16].

2.4.3. Multivariate Analysis

Cluster analysis and n-MDS based on the Bray–Curtis similarity index were applied to visually assess differences in macrofaunal assemblages among stations of the harbours.

The similarity percentages (SIMPER) routine was used to establish which species contribute most to the observed differences in the determined data. The BIO-ENV procedure was employed to analyse the correlations between the macrobenthic community and environmental variables [9]. Principal component analysis (PCA) was performed to identify the main environmental parameters and anthropogenic pressures determining the benthic community distribution. The Spearman rank correlation coefficient (ρs) was used to find the best matches of environmental factors that affect the benthic and biotic indices [9]. The significance of the 'maritime activity' factor in determining the observed similarity pattern was tested by nested permutation analysis of variance (PERMANOVA) (for more details see Tempesti et al. [39]).

3. Results

3.1. Environmental Factors

Water Characteristics

Water-column temperature (ANOVA; F = 49.52; $p > 0.05$) and salinity (ANOVA; F = 23.05; $p > 0.05$) were insignificant differences between the harbours. Conversely, pH, transparency, chlorophyll, and dissolved oxygen concentrations illustrated statistically significant differences between the three harbours. The pH varied between 6.13 (at C1; near shipyard) and 8.22 (at F2) (ANOVA; F = 3.14; $p < 0.05$), the transparency between 0.9 m (C2) and 3.5 m (M3) (ANOVA; F = 4.24; $p < 0.05$), the chlorophyll a concentration between 3.98 mg L^{-1} (C1) and 12.42 (M2) (ANOVA; F = 6.18; $p < 0.05$) and dissolved oxygen between 4.66 mg L^{-1} (C1) and 9.02 mg L^{-1} (M3) (ANOVA; F = 10.22; $p < 0.05$) (Table 1).

3.2. Sediment Features

Sediment type and organic matter content (Table 1) were significantly different between the harbours. The cluster analysis of the 'grain size × 9 stations' (four replicates for each station) matrix reveals three different groups that can be distinguished according to the percentage of each sediment grain-size fraction (ANOVA; F = 24.16; $p < 0.05$). These groups correspond to three sediment types (Figure 2). The first group (GA) comprises the medium sand stations located in the marina of Monastir. The second group (GB) corresponds to fine sand stations located in the fishing harbour of Teboulba, while the third group (GC) corresponds to mud stations located in the commercial harbour of Sousse. The organic matter content of the sediment ranged from 7.88% (M3) to 36.42% (C2), exhibiting significant differences between sediment types for each harbour (ANOVA; F = 1.67; $p < 0.05$). The highest percentages of OM were recorded for mud and fine sediments of fishing and commercial harbours, while the lowest values were found in the medium sands of the Monastir marina.

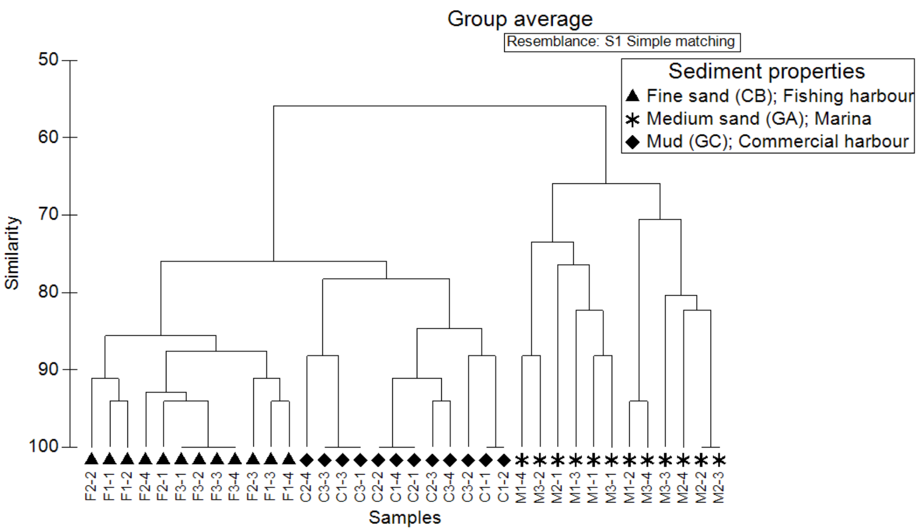

Figure 2. Cluster dendrogram showing three distinct subgroups of stations (GA, GB, and GC) according to the sediment characteristics of 36 samples (12 replicates for each harbour).

3.3. Sediment Contamination

Heavy metal concentrations were found to exhibit significant variability between stations depending on sediment type and harbour activity (Appendix A). The highest values were recorded for mud sediments from the commercial harbour of Sousse, where Pb ranged from 606.99 to 846.80 µg/g, Hg from 421.80 to 682,30 µg/g, Zn varied from 298.46 to 305.80 µg/g and Cd from 4.20 to 5.42 µg/g (Table 2). Conversely, the Monastir marina, which is characterized by medium sand, shows the lowest concentrations of chemical pollutants except for Fe (85.06 µg/g), which represents the highest value compared to levels found in the fishing or commercial harbours. A huge concentration of Hg is found in the fishing harbour (927.00 µg/g in F3) compared to the other sampled stations, while Cu shows the same levels as in the commercial port, along with the highest values for chemical elements such as N (36.45 to 66.65 µg/g) and F (12.40 to 49.60 µg/g) (Table 2). The metal pollutants were significantly different between the three harbours (ANOVA; for all chemical elements; $p < 0.05$), the highest value being recorded for the mud and fine sediments (commercial and fishing harbour) (see Table A2; Appendix B).

Table 2. Pollutant concentrations in surface sediments of the stations sampled in the three harbours in spring 2019.

	Fishing Harbour of Teboulba			Marina of Monastir			Commercial Harbour of Sousse		
	F1	F2	F3	M1	M2	M3	C1	C2	C3
F (µg/g)	12.40	28.50	49.60	2.10	4.70	3.20	1.20	7.70	18.00
P (µg/g)	10.88	8.65	11.02	9.88	5.85	7.02	29.88	10.63	12.40
N (µg/g)	36.45	40.48	66.65	17.30	13.18	10.24	18.40	12.28	9.62
Cu (µg/g)	198.50	179.80	172.60	62.30	34.10	63.80	198.80	178.40	172.40
Cr (µg/g)	62.70	44.90	43.70	14.60	22.80	37.30	28.64	46.40	23.85
Cd (µg/g)	1.86	2.12	2.88	0.98	0.84	0.76	5.42	4.20	4.88
Mg (µg/g)	344.20	371.90	328.50	231.40	104.50	99.64	546.20	361.90	438.70
Hg (µg/g)	281. 80	379. 20	927.00	102.80	98.46	63.20	421.80	579.20	682.70
Pb (µg/g)	143.90	88.46	298.20	13.20	24.50	18.00	728.30	846.80	606.99
Fe (µg/g)	23. 79	12.35	22.12	85.06	69.58	51.78	54.31	67.86	48.36
Al (µg/g)	64.510	101.66	38.58	13.94	13.35	19.42	20.24	15.13	19.72
Zn (µg/g)	171.82	124.40	198.80	64.70	164.30	98.12	305.80	298.46	303.20

3.4. Macrobenthic Community Composition

The abundance ranges between 1025 ind.m^{-2} (at C2: commercial harbour) and 4224 ind.m^{-2} (at M2), showing a significant spatial variation between the three different harbours (F = 101.03; $p = 0.011$). The diversity indices indicate a significant difference between the three harbours. The number of taxa varies between 8 (at F1: fishing harbour) and 30 (at M3: marina), and differs significantly between the three harbours (F = 32.79; $p = 0.001$). Equally, the Shannon–Weaver diversity index (H′) ranges between 1.06 (at F1) and 3.42 (at M3), and Pielou's evenness varies between 0.48 (at F1) and 0.82 (at M1 and M3), with the two ecological indices showing a significant variation between the three harbours (F = 20.27; $p = 0.002$ (for H′) and F = 3.903; $p = 0.022$ (for J′)) (Figure 3).

A total of 3510 individuals from 94 taxa were identified belonging to six zoological groups in the three different harbours (Appendix B). Among them, the most diverse group are molluscs (46% of total number of species), followed by polychaetes (27%), crustaceans (23%), and other taxa (echinoderms, bryozoans, and cnidarians) (4%). The molluscs (44% of the total abundance) and polychaetes (38%) represent the most abundant groups in terms of individuals for each sampling site (Figure 4A). A high number of macrobenthic taxa are recorded for Monastir marina (46 taxa), following by the fishing harbour of Monastir (37 taxa), and the commercial harbour of Sousse (28 taxa). A total of five NIS are recorded: the gastropod *Cerithium scabridum*, the bivalve *Ruditapes philippinarum*, *Pinctada imbricata radiata*, *Fulvia fragilis*, and the decapod *Pilumnus minutus*, with high abundances in the marina and the commercial harbour, while the nonindigenous gastropod *Cerithium scabridum* is particularly well represented in the fishing harbour.

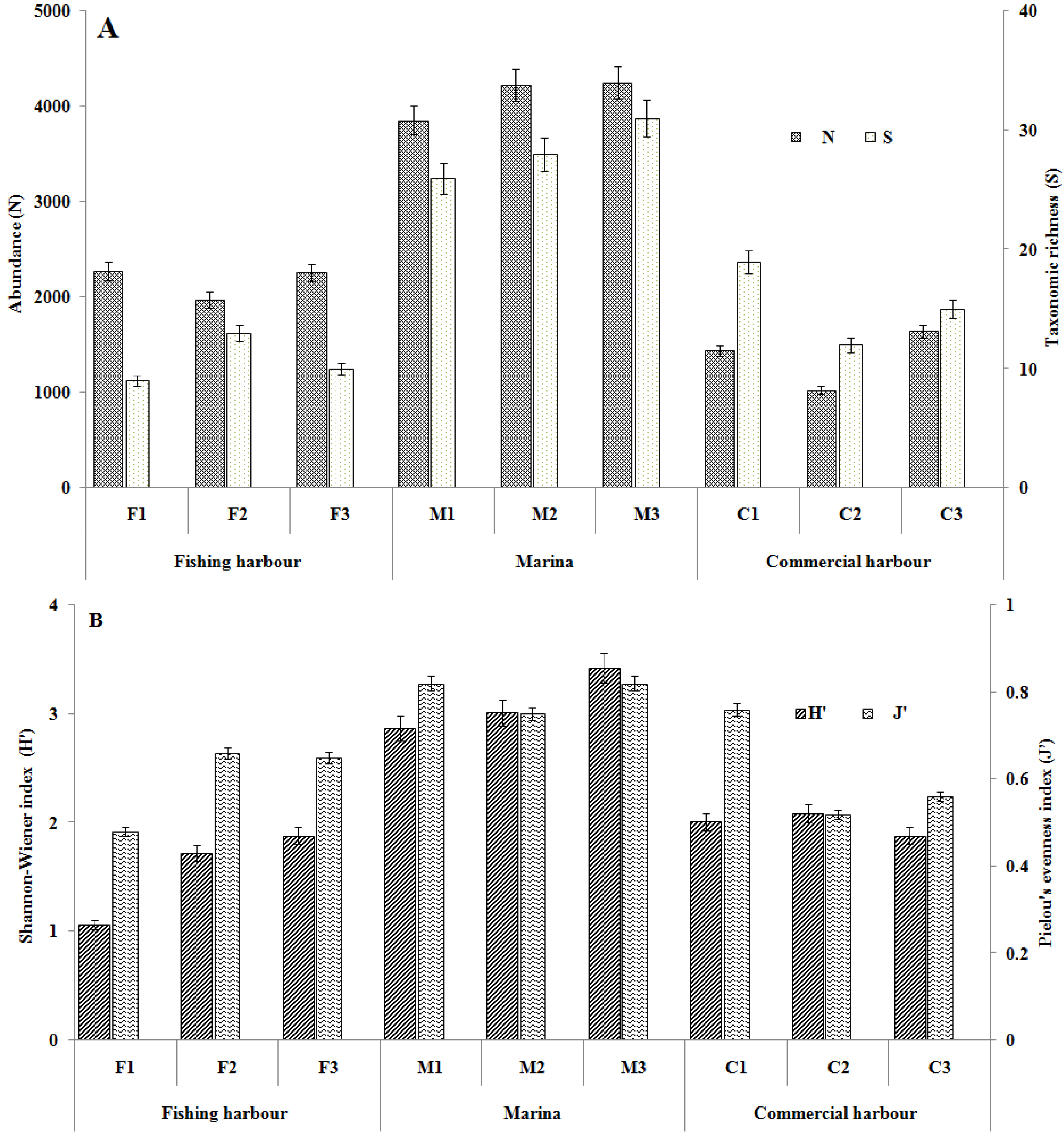

Figure 3. Diversity indices (mean of four replicates ± SD) calculated for each sampled harbour. (**A**): abundance (N; ind·m^{-2}) and species richness (S); (**B**): Shannon index (H′; bits·ind^{-1}) and Pielou's index (J′).

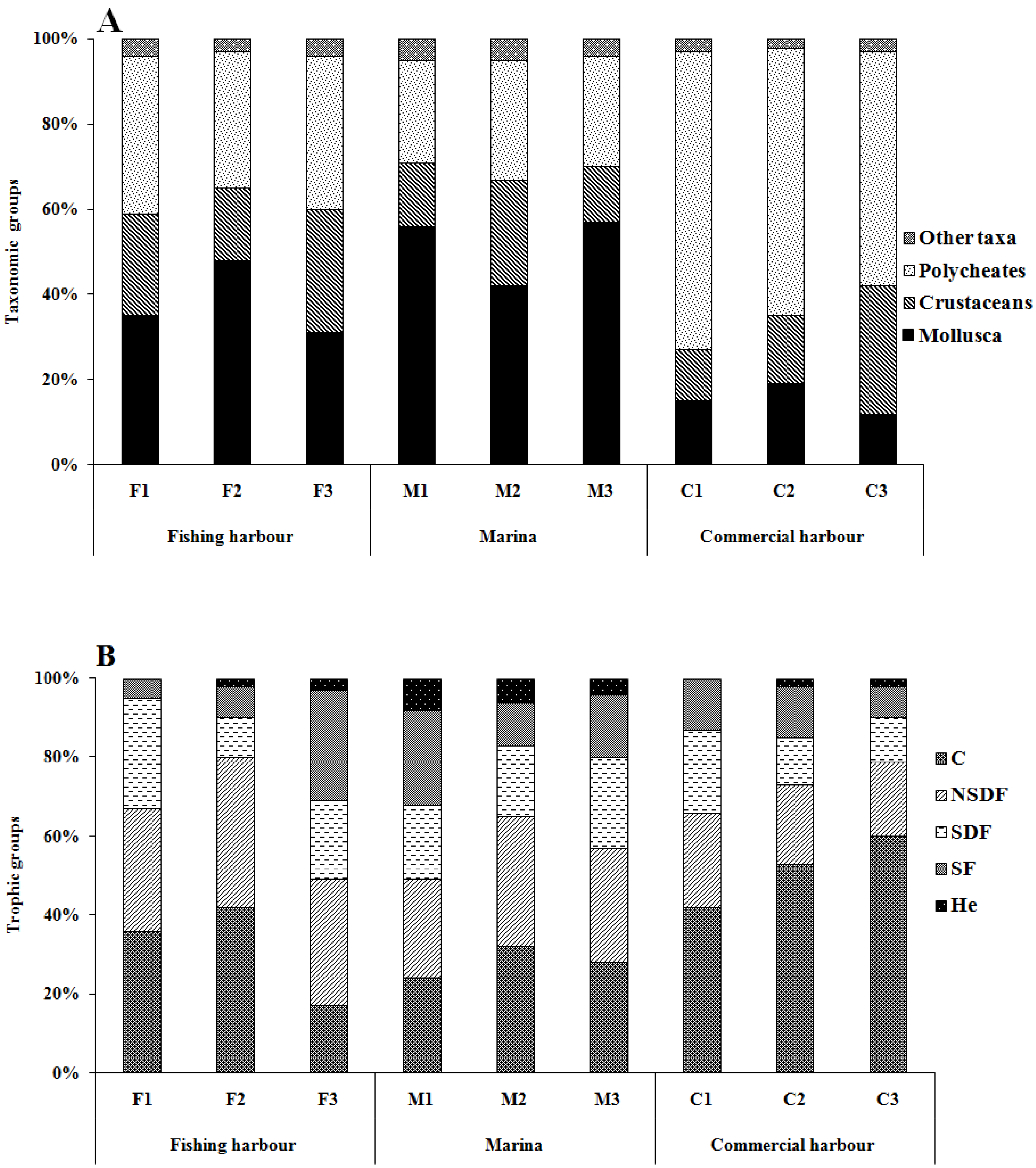

Figure 4. Proportion of taxonomic (**A**) and trophic groups (**B**) of the macrobenthic communities sampled in each harbour. C: carnivores, He: micrograzers, SF: suspension feeders, SDF: selective deposit feeders, NSDF: non selective deposit feeders.

In the three harbours, the dominant species are the polychaetes Cirratulus cirratus, Perinereis cultrifera, and Marphysa sanguinea; the bivalve Pinctada imbricata radiata; the gastropod Cerithium scabridum; and the amphipods Gammarus insensibilis, Dexamine spinosa, and Leucothoe incisa.

Trophic structure analysis reveals a significantly different distribution of trophic groups between the harbours (ANOVA; F = 112.3; p = 0.05). In the fishing harbour of Teboulba, the trophic groups show a spatial variation (ANOVA; F = 81.2 p = 0.022); F1 and F2 are dominated by carnivores and non selective deposit feeders, but F3 is dominated by suspension feeders and non selective deposit feeders. The commercial harbour exhibits a predominance of carnivores followed by non selective and selective deposit feeders, while the marina shows equivalent proportions of trophic groups (Figure 3B).

3.5. Spatial Patterns of the Benthic Assemblages

The spatial ordination of samples obtained by n-MDS highlights the distinction between three main groups corresponding to the commercial harbour (C1, C2, and C3), fishing harbour (F1, F2, and F3), and marina (M1, M2, and M3); the within-group similarity (measured by cluster analysis) exceeds 65% (Figure 5). According to PERMANOVA analyses, the separation of samples into three main groups, which correspond to the three types of harbour area activity, is highly significant. This is confirmed by pairwise comparisons between the different harbour area activities (Table 3). SIMPER analysis shows that the percentage of similarity within the same maritime activity varies from 19.25% for the fishing harbour to 25.04% for the marina (Table 4). The fishing harbour of Teboulba is dominated by the bivalve *Cerastoderma glaucum* and the gastropods *Calliostoma zizyphinum* and *Gibbula ardens*. The marina of Monastir is dominated by the gastropods *Cerithium scabridum*, *Tritia cuvierii*, *Bulla striata*, and *Collumbella rustica* and the polychaetes *Cirratulus cirratus* and *Perinereis cultrifera*. The bivalve *Cerastoderma glaucum* contributes to the similarity within the fishing harbour and marina, accounting for percentages of 54.9% and 22.2%, respectively. While the percentage of similarity is 56.70% in the commercial harbour, the benthic assemblage is strongly represented by polychaete species (82%) such as *Cirratulus cirratus*, *Neanthes acuminata*, *Hediste diversicolor*, *Perinereis cultrifera*, *Capitella capitata*, and *Notomastus latericeus*. A dissimilarity exists between the three harbour groups (ANOSIM test, r = 0.65; $p < 0.1$), reflecting a significant difference between the three benthic assemblages (confirmed also by a PERMANOVA test).

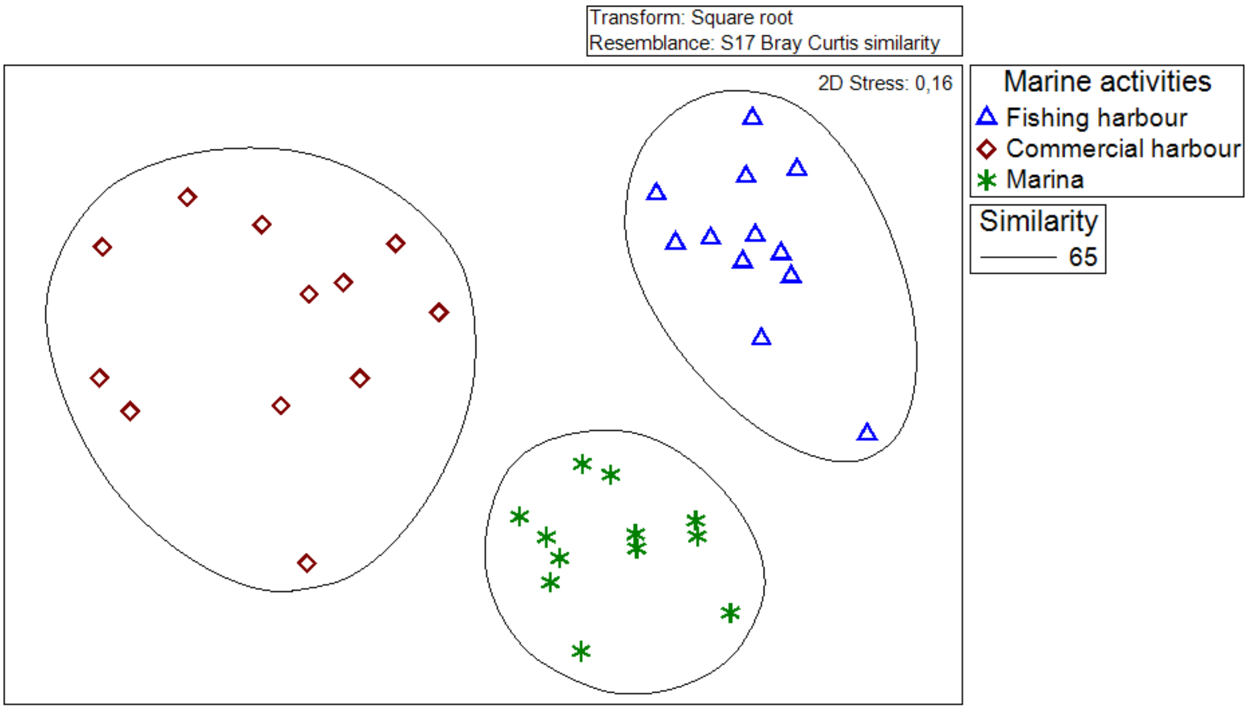

Figure 5. Spatial ordination of samples (3 × 4 replicates for each harbour) provided by n-MDS: the three site groups corresponding to the three main marine activities of each harbour are represented by continuous-line ovals representing the percentage of similarity measured by cluster analysis.

Table 3. Results from PERMANOVA using Bray–Curtis distance matrices to test differences between the three separate macrobenthic assemblages of the three harbours. Significant *p*-values are given in bold.

Pairwise Comparison				
Group	**t**	**P (Perm)**	**Unique Perms**	**P(MC)**
Fishing harbour, Marina	1.612	0.0421	22	0.0161
Fishing harbour, Commercial harbour	1.521	0.0670	18	0.0042
Commercial harbour, Marina	1.742	0.0152	10	0.0115

Table 4. MDS-formed groups, with indication of similarities within each group (%) and the most representative species (C%) contributing to the similarity within the group, determined by SIMPER analysis, with species mean abundance A (ind per 0.2 m^{-2}) for each assemblage.

Fishing Harbour			Marina			Commercial Harbour		
Similarity (%) 19.25			25.04			56.70		
Species	**C**	**A**	**Species**	**C**	**A**	**Species**	**C**	**A**
Cerastoderma glaucum	54.9	308	*Cerithium scabridum*	52.2	502	*Cirratulus cirratus*	61.6	188
Pinctada imbricata radiata	49.1	218	*Tritia cuvierii*	46.1	336	*Neanthes acuminata*	58.4	185
Calliostoma zizyphinum	38.3	204	*Bulla striata*	42.4	290	*Hediste diversicolor*	50.05	131
Notomastus latericeus	33.5	123	*Collumbella rustica*	38.2	229	*Perinereis cultrifera*	34.79	115
Dexamine spinosa	29.9	100	*Cirratulus cirratus*	34.1	204	*Capitella capitata*	32.21	112
Gammarus insensibilis	25.0	98	*Perinereis cultrifera*	30.8	144	*Notomastus latericeus*	30.32	104
Gibbula ardens	19.3	88	*Cerastoderma glaucum*	22.2	129	*Marphysa sanguinea*	25.48	98
Hexaplex trunculus	13.3	73	*Dexamine spinosa*	19.4	124	*Malacoceros fuliginosus*	20.04	80
Leucothoe incisa	6.1	72	*Marphysa sanguinea*	14.7	118	*Bittium latreillii*	16.50	78

3.6. Linking Macrobenthic Fauna and Environmental Factors

The BIOENV procedure indicates that the macrofaunal distribution pattern in the three harbours can be explained by a combination of several variables (correlation coefficient = 0.704). These variables correspond to sediment characteristics (OM, mud content, and heavy metals) and harbour characteristics (i.e., depth). Organic matter content individually shows the strongest correlation with species distribution (correlation = 0.228) (Appendix C).

Principal component analysis (Figure 6) was performed for the nine stations to identify the influence of each environmental parameter on the biological variables and benthic macrofauna abundance. This analysis shows that the distribution of benthic organisms is greatly influenced by several environmental factors. The first principal component (explaining 53.58% of the total variability) allows for distinguishing those stations sampled in the commercial harbour of Sousse (C1, C2, and C3). These stations are grouped together as containing mud sediment with the highest contents in organic matter together with high concentrations of heavy metals and other trace elements such as Fe, Cu, Cd, Mg, and Hg. The second axis (38.44%) opposes the high percentage of edaphic factors (OM% and heavy metal concentration), grouping together the stations in Monastir marina characterized by medium sand, which are positively correlated with physicochemical parameters such as chlorophyll, transparency, and dissolved oxygen. Conversely, the stations sampled in the fishing harbour are characterized by fine sand rich in silt and clay, and are positively correlated with temperature and pH along with chemical contamination by certain elements such as F, N, Cr, and Al.

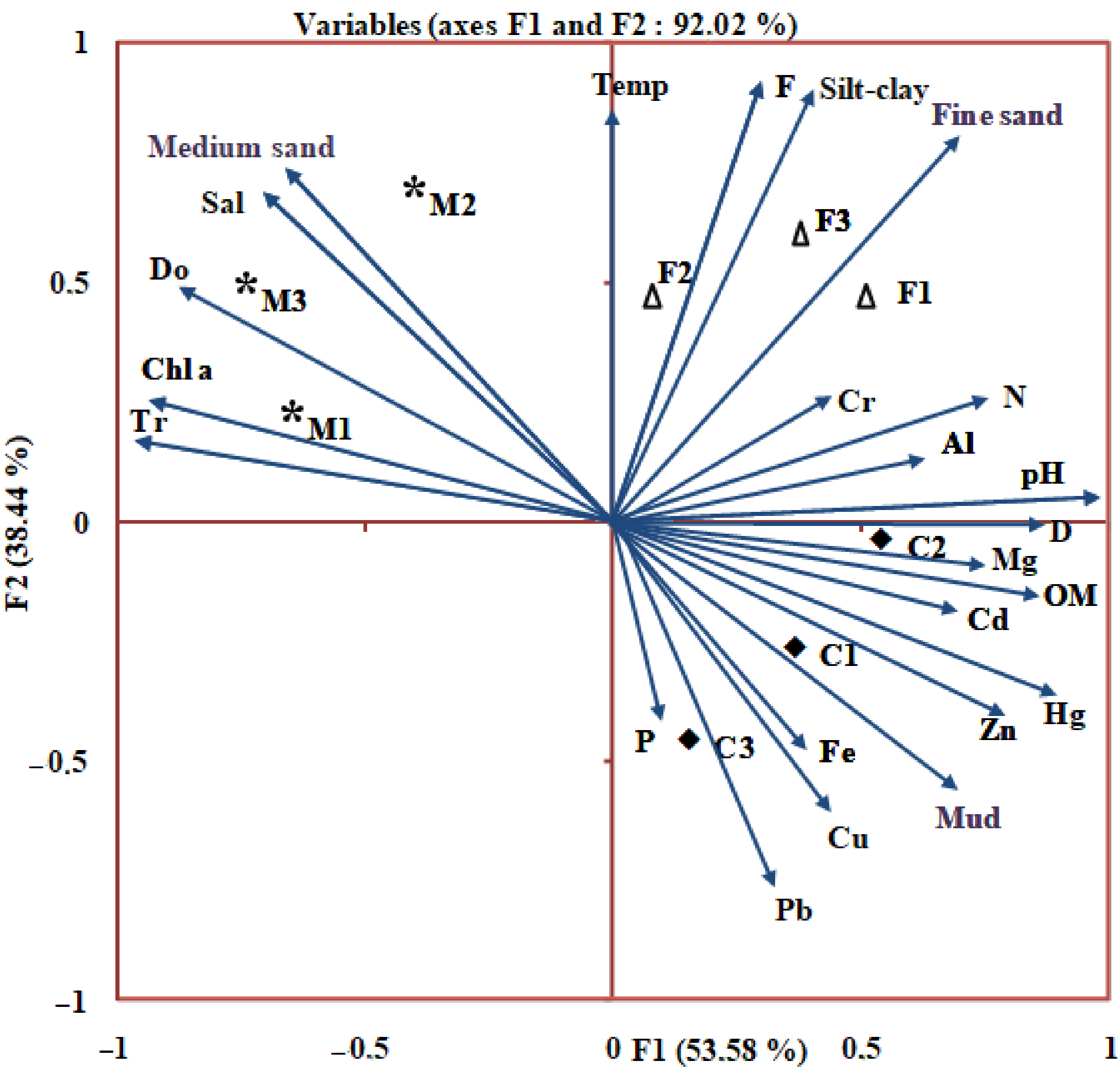

Figure 6. PCA established on values of the main environmental parameters recorded at the sampled stations: Temp: temperature, Sal: salinity, Do: dissolved oxygen, pH, Tr: transparency, D: depth, OM: organic matter, Zn: zinc, N: nitrogen, F: fluorine, Cd: cadmium, Cr: chromium, P: phosphorus, Cu: copper, Cr: chromium, Fe: iron, Hg: mercury, Al: aluminium, Mg: magnesium.

3.7. Ecological Quality Status (EcoQS)

All the sampling stations at the Monastir marina are classified by both biotic indices as having good ecological status (unpolluted). The fishing harbour is classified in a moderate ecological status, while the commercial harbour is classified as having a poor EcoQS, being strongly dominated by numerous tolerant and opportunistic polychaetes (Figures 3A and 7).

Biotic indices show a significant difference between the three different harbours (ANOVA; F = 2.4; $p < 0.05$ (for AMBI) and F = 1.2; $p < 0.05$ (for BO2A)) (Figure 7). Biotic indices suggest that the Monastir marina has a higher environmental quality compared with the fishing harbour of Teboulba and the commercial harbour of Sousse, both of which show very low diversity (number of taxa and abundance) and moderate ecological status as assessed by H′, J′, and the three biotic indices used here. The benthic diversity (number of species and abundance) and the biotic indices (H′, J′, AMBI, and BO2A) show a significant correlation with some environmental factors such as type of sediment, organic matter, and heavy metal contamination, mainly Cd, Hg, Fe, and Pb (Appendix D).

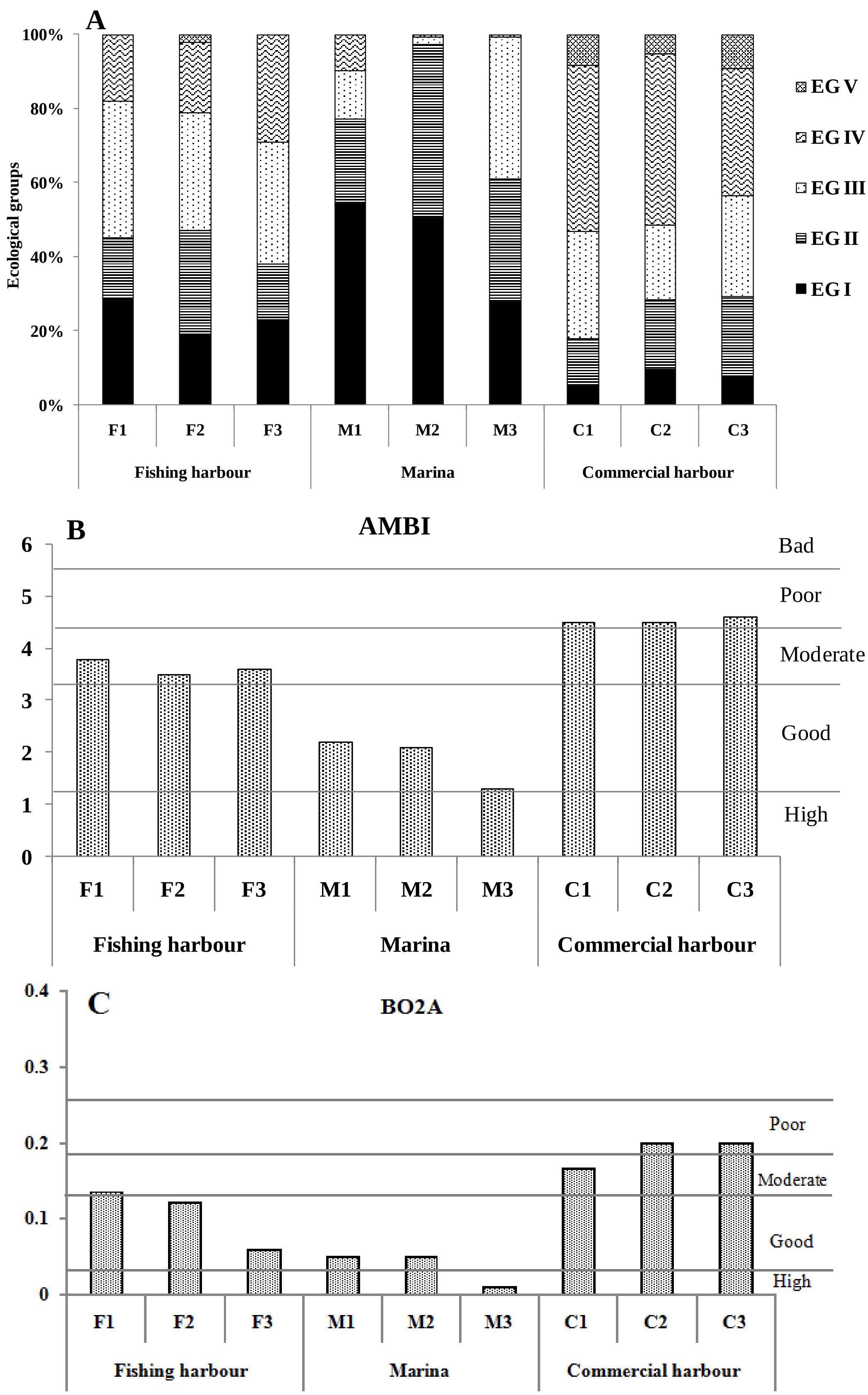

Figure 7. Spatial variability of the ecological groups (**A**) and biotic indices (**B**,**C**) in the harbours. EGI: sensitive species, EGII: species indifferent to organic matter enrichment, EGIII: species tolerant to excess organic matter enrichment, EGIV: second-order opportunistic species, EGV: first-order opportunistic species.

4. Discussion

Study of the impacts of anthropogenic perturbations and heavy metal pollution on harbour ecosystems is an important step in establishing appropriate environmental management and protection of marine biodiversity [40]. When the concentration of heavy metals in the coastal ecosystem reaches a certain level, it presents high impact to the structure, diversity, and abundance of the macrobenthic communities and the function of harbour ecosystems [9,21]. This paper illustrates the ecological risk assessment of heavy metals and environmental quality of central Tunisian harbours, central Mediterranean Sea.

4.1. General Patterns of the Benthos of the Three Harbours

In the present study, 94 taxa of benthic invertebrates are identified belonging to six zoological groups, including five nonindigenous species. In terms of number of species and abundance, the three harbours have higher percentages of molluscs and annelids, but the commercial harbour is dominated by polychaetes, whereas the marina and fishing harbour are dominated by molluscs followed by crustaceans. A predominance of polychaetes is usually observed in other harbour ecosystems (i.e., Gulf of Gabès harbours in Tunisia, Algerian harbours, Valdez harbour in Alaska, Mumbai harbour in India, and Mucuripe harbour in Brazil) [3,9,41–43]. Several authors have shown that polychaetes colonize fine-grained substrates and mud sediment. Since muddy sand sediments contain a high proportion of fine particles and a greater amount of organic matter, they host a wide variety of opportunistic polychaetes [32], and their presence appears to be linked to the availability of trophic resources (i.e., organic waste, dead animal corpses). These opportunist species proliferate in response to the excess organic matter enrichment in polluted environments [3,9,29].

4.2. Macrobenthic Assemblage Distribution and Heavy Metal Contamination

Three distinct macrofaunal assemblages are recognized corresponding to the three harbours, reflecting complex environmental gradients that involve abiotic factors (sediment characteristics, metal pollutants) and harbour activities (Figure 5). The first group includes the sampling stations in the marina characterized by medium sand and lower concentrations of organic matter and heavy metals, while the highest sedimentation rates and concentrations of pollutants are recorded for the second and the third group, respectively. These latter groups comprise the sampling stations of the commercial harbours distinguished by muddy sediment and high concentrations of heavy metals such as Zn, Pb, Hg, and Cu, but with lower Cd levels similar to those found in three other Mediterranean harbours, i.e., Cagliari (Italy), Heraklion (Greece), and El Kantaoui (Tunisia) [30]. Copper is one of the most toxic elements for macrobenthic communities when compared with mercury, cadmium, or zinc, and its presence can lead to a decrease in the number and the abundance of benthic species [30,44]. Mercury contamination is mainly related to the presence of organic matter, with the highest percentages recorded for mud and fine sediments of fishing and commercial harbours [9,30,45]. In many cases, mercury shows the highest concentrations both in seawater and sediments, and having a substantial effect on marine coastal habitats and macrobenthic communities [45–47].

Mercury is considered as a critical pollutant of aquatic ecosystems due to its elevated toxicity, with the highest concentrations recorded for commercial and fishing ports. This can be explained by mercury entering the marine environment via a number of sources, such as industrial wastewater discharges, antifouling paints widely used in the recent past on ships of all kinds, and atmospheric deposition [46,47]. According to the results of other published studies (Table 5), the concentrations of chemical pollutants are generally higher in industrial harbours, thus generally affecting the benthic diversity and EcoQS of harbour environments [5,9]. The industrial harbours of the Gulf of Gabès with the highest levels of organic matter and chemical contamination are classified as having moderate or bad ecological status, and higher values of trace metal contamination are strongly correlated with marine traffic and harbour activity [9]. Generally, the highest levels of metal contamination in harbour ecosystems are related to many anthropogenic effects.

Shipping traffic, loading, repairs, and dredging together with rainwater runoff and effluent discharges to coastal marine ecosystems during the last few decades have been considered to be one of the main drivers of marine ecological degradation [1,48].

Table 5. Comparison of trace metal concentrations in coastal and harbour environments from different regions (all data collected in previous studies are converted to the same unit (μg/g); (-) not assigned).

Locations		Cd	Cu	Pb	Cr	Zn	References
Hammamet Gulf	Fishing harbour	2.28	183.63	176.85	50.43	165.00	Present study
	Commercial harbour	4.83	183.20	727.36	32.96	302.48	
	Marina	0.86	53.40	18.55	24.90	101.04	
Harbours of Gabès Gulf		370.90	-	6.31	-	618.25	Mosbahi et al. (2021)
Harbours of Gulf of Tunis	Rades commercial	-	2.76	9.69	-	128	Chouba et al. (2013)
	Goulette fishing	-	2.39	11.66	-	125.33	
	Sidi Bousaid touristic	-	10.55	7.08	-	130	
Coast of Sfax		0.17	33.00	19.00	72.00	95.00	Gargouri et al. (2011)
Gabès Gulf (Tunisia)		73.01	2.56	6.13	16.76	545.03	Elzreli et al. (2015)
The southern coast of Sfax, Gabès Gulf		8.14	37.00	10.71	77.22	104.90	Neifar et al. (2018)
Skhira bay		266.87	-	-	36.7	-	Boudaya et al. (2019)
Asturian coastline sediments (North of Spain)		0.04	3.27	9.52	5.00	42.34	Lorena et al. (2020)
Northumberland Strait, NS, Canada (DL)		0.3	5.33	6.58	10.83	38.50	Chaudhary et al. (2020)
The port city of Busan (South Korea)		0.46	321.00	67.4	71.20	322.00	Jeong et al. (2020)
Nova Scotia, Canada		0.45	22.70	25.40	18.40	86.10	Zhang et al. (2019)
Veraval Harbour, Gujarat, Arabian Sea, India		0.63	33.0	220.00	171.00	603.00	Sundararajan et al. (2017)

Many ecological studies on benthic fauna highlight the importance of environmental factors in controlling the spatial distribution of benthic communities. The major factors include sediment characteristics such as granulometry [49,50], OM content, substrate type (presence or absence of vegetation), physicochemical parameters (i.e., salinity, dissolved oxygen, and pH), and hydrodynamics [3]. Similarly, Alessandro et al. [41] proved that depth is a crucial factor for the distribution of benthic communities in harbour environments, which are semienclosed areas characterized by a high concentration of organic matter and chemical contaminants. Equally, [40] showed that the EcoQS of three harbours located in the central Mediterranean Sea are correlated with the physical and chemical properties (metal contamination) of the sediment in each harbour ecosystem.

Harbour sediments in the studied area contain a record of different trace metal pollutants, with high concentrations especially in commercial and fishing harbours due to shipyard activities and the presence of very high organic matter contents, while the lowest values are recorded for the Monastir marina. These results are similar to those found in the Gulf of Tunis, such as in the commercial harbour of Rades, the fishing harbour of Goulette, and in the yachting harbour of Sidi Boussaid [51]. For the harbours of the Gulf of Gabès (see Table 5), Mosbahi et al. [9] revealed that the industrial and fishing harbours can be distinguished by high levels of metal pollutants mostly related to the intensity of shipping activities. The concentrations of Cu, Pb, Zn, and Cr reported in the present study appear very high in comparison with other areas, such as the coastal sediments of Asturia, North of Spain [52]; Northumberland Strait, Nova Scotia, Canada [53]; the harbour city of Busan (South Korea) [54]; and the Yangtze River, China [55]. However, Cu and Cr were not determined in the Gulf of Gabès by [9], while Pb shows the lowest concentration (6.31 μg/g) and Zn the highest (618.25 μg/g) [9] (Table 5). In the present study, Cd shows high values in the fishing and commercial harbours (2.28 to 4.83 μg/g, respectively) in comparison to values found in other ports such as in Asturia (North of Spain); Northumberland Strait, Nova Scotia (Canada); and the harbour city of Busan (South Korea) [52–54]. By contrast, the Gulf Gabès harbours are distinguished by their huge concentrations of Cd (370.90 μg/g). Similarly, in the present study, mercury attains a high concentration in the commercial and the fishing harbours (Table 5). These high concentrations of heavy metal pollutants seem to be related to the impact of urban wastewaters and industrial discharges that have not been treated [28,56]. In addition, emissions from vehicles due to the high volume of traffic near the commercial harbours may represent an important source of trace metal contamination [9,54]. The effects of these metal pollutants on aquatic ecosystems in different parts of the world have been pointed out by different authors such as Islam and Tanaka [57], who indicate a decrease in species diversity and a decline in abundance and biomass along with changes in benthic community structure, together with the degradation of marine habitats and the diminishing yield of marine resources. Hence, the monitoring of aquatic pollution has been recognized by several authors as an urgent priority for sustainable management of marine habitats and fisheries resources [5,9,30].

Owing to the specificity of harbour environments characterized by poor water exchange, such areas are considered as sinks for the accumulation of pollutants, especially heavy metals, which are classified as harmful due to their persistence in the environment together with their toxicity and ability to be absorbed into the food chain [21,22].

4.3. Ecological Quality Status of the Three Harbours

The use of biotic indices allows us to classify the three harbours as having poor to good ecological status. This study shows that the ecological status based on benthic macrofauna is associated, as expected, with anthropogenic activities and trace metal pollution taking place due to the maritime activities in the three harbours. Marina and fishing harbour areas tend to have good ecological status, while only the commercial harbour of Sousse is found to be severely impacted, being classified here as having a poor EcoQS. This finding has also been recently reported by several marine ecologists. Dimitriou et al. [5] demonstrated that harbour activities have an effect on the EcoQS of Mediterranean harbours. In fact, the EcoQS of the commercial harbour of Sousse is affected by a combined increase in mud sediment surface area, organic matter, and heavy metals, leading to heavily disturbed conditions that result in low macrobenthic diversity and a poor ecological status. These results are in agreement with those obtained from previous studies in neighbouring harbours [30].

Heavy metals are one of the most serious pollutants in the harbour environments and have attracted widespread attention worldwide due to their inherent toxicity, persistence, and bioaccumulation properties, which pose a great danger to the environmental conditions, benthic diversity, and the EcoQS of these coastal marine habitats [58,59].

5. Conclusions

The present study compares the heavy metal contamination and benthic indices of three harbours located on the eastern coast of Tunisia (central Mediterranean Sea) by using benthic fauna diversity and biotic indices to assess their EcoQS. High concentrations of heavy metals, fluorine, phosphorus, nitrogen, and organic matter in the industrial harbour of Sousse strongly influence the diversity, abundance, and structure of macrobenthic communities. The benthic ecological quality for the three harbours varies from poor to good ecological status. The reported results prove that the macrobenthic diversity and EcoQS of the three harbours are influenced by harbour activities related to major drivers such as depth, sediment type, and chemical contamination. This overview provides a valuable baseline database for harbour authorities to establish specific management programmes. Finally, it would be worthwhile carrying out seasonal and annual monitoring at selected stations in these harbours to follow the long-term evolution of benthic macrofauna in relation to anthropogenic pressures and especially harbour activities.

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Conflicts of Interest: The authors declare that they have no conflict of interest.

Appendix A

Table A1. Results of one-way ANOVAs with stations and harbours as factors for the 12 heavy metals (*: statistically significant difference; $p < 0.05$); *p* values in bold indicate a statistically very high difference. FH: fishing harbour; M: marina; CH: commercial harbour.

	Factors	*df*	*F*	*p*	Tukey Test
F	Stations	8	12.89	0.011 *	FH# M #CH
	Harbours	2	10.04	0.021 *	
P	Stations	8	14.1	0.018 *	FH# M #CH
	Harbours	2	8.02	0.024 *	
N	Stations	8	6.42	0.018 *	FH# M #CH
	Harbours	2	4.11	0.046 *	
Cu	Stations	8	14.2	0.012 *	FH# M #CH
	Harbours	2	5.02	**<0.001** *	
Cr	Stations	8	11.21	0.020 *	FH# M #CH
	Harbours	2	6.53	0.210 *	
Cd	Stations	8	11.07	0.013 *	FH# M #CH
	Harbours	2	4.28	**<0.001** *	
Mg	Stations	8	7.11	0.048 *	FH# M #CH
	Harbours	2	5.23	0.024 *	
Hg	Stations	8	7.02	**<0.001** *	FH# M #CH
	Harbours	2	5.32	**<0.001** *	
Pb	Stations	8	7.14	0.021 *	FH# M #CH
	Harbours	2	6.32	0.011 *	
Fe	Stations	8	11.54	**<0.001** *	FH# M #CH
	Harbours	2	4.21	0.032 *	
Al	Stations	8	9.24	0.012 *	FH# M #CH
	Harbours	2	6.42	0.020 *	
Zn	Stations	8	12.4	**<0.001** *	FH# M #CH
	Harbours	2	2.41	**<0.001** *	

Appendix B

Table A2. Abundance of macrobenthic species (number of individuals per 0.2 m^2) in the nine stations sampled in the three harbours during spring 2019.

Species	Fishing Harbour of Teboulba			Marina of Monastir			Commercial Harbourof Sousse		
	F1	F2	F3	M1	M2	M3	C1	C2	C3
Abra alba				3	7	5			
Actinia sp.	1		3						
Alvania geryonia				2		5			

Table A2. *Cont.*

Species	Fishing Harbour of Teboulba			Marina of Monastir			Commercial Harbourof Sousse		
	F1	**F2**	**F3**	**M1**	**M2**	**M3**	**C1**	**C2**	**C3**
Alvania sp.				2	7				
Amphiura mediterranea					2	1			
Antalis dentalis		3	2						
Balanus balanus					4	2			
Balanus sp.							17		43
Barbatia barbata		2	1						
Bittium reticulatum	4	2	8				2	3	
Bryozoa sp.							1	3	1
Bulla striata				4	15	8			
Calliosotoma granulatum				2	3	3			
Calliostoma conulus				1	2	1			
Calliostoma zizyphinum		37	8						
Capitella capitata		1	4				2	4	5
Carcinus aestuarii				1	4	3			
Carcinus sp.				4		1	2		1
Cerastoderma glaucum	18		10	2					
Cerithium scabridum	24	48	52	3	20	14	18		30
Cerithium sp.							19	11	6
Cerithium vulgatum	8							24	
Cirratulus cirratus				4	1	6			
Columbella rustica		2	1						
Conus mediterraneus					2				
Dexamine spiniventris		4	2						
Dexamine spinosa	3	1							
Diogenes sp.		2	1				1		2
Drilonereis filum							30	8	
Elasmopus pectenicrus		2						4	2
Eriphia verrucosa							2	2	
Euclymene lombricoides		3			2				
Euclymene oerstedi				6		4			
Euclymene sp.		2							
Fulvia fragilis				2	4	3	2	6	1
Gammarus insensibilis	8	7	4	12	6	4		2	
Gibbula ardens			2	9	44	12			
Glycera alba								6	3
Glycera tridactyla							1	5	
Haminoea navicula				2	1				
Hediste diversicolor	2		2						
Hexaplex trunculus				1		3			
Hilbigneris gracilis						4			3
Holothuria tubulosa	1		2		1	2			
Idotea balthica			2			4			
Idotea chelipes		2	4						
Leucothoe incisa	2		3		2				
Libinia dubia								2	2
Loripes orbiculatus		5	2			8	3	1	7
Lumbrineriopsis paradoxa		2			4				
Lumbrineris tetraura								3	3
Lysianassa pilicornis		5	3						
Macomangulus tenuis				3		4		1	
Malacoceros fuliginosus								6	
Marphysa sanguinea				3	1	4	12		7
Mesalia mesal							2		1
Microdeutopus gryllotalpa		2		3					
Moerchiella reticulata				2		4			
Mytillus galloprovibciallis								2	4
Mytilus sp.					2	1			

Table A2. *Cont.*

Species	Fishing Harbour of Teboulba			Marina of Monastir			Commercial Harbourof Sousse		
	F1	**F2**	**F3**	**M1**	**M2**	**M3**	**C1**	**C2**	**C3**
Naineris setosa				2		2			
Neanthes acuminata							1		3
Nephtys hombergii								3	
Nereis rava			4						
Nereis zonata			4				16		12
Ophiura sp.								2	
Orbinia cuvieri	2	6							
Ostriola stantina	1		1					2	
Paucibranchia bellii								3	27
Perinereis cultrifera							1		3
Peronidia albicans				2		2			
Phyllonotus trunculus					1	2			
Pilimnus minutus								2	2
Monodaeus couchii		2		2		3		4	2
Pinctada imbricata radiata	2	2	6	3	4	8	2	9	5
Platynereis dumerilii						2	12	22	17
Protocirrineris chrysoderma				2					
Raphitoma laviae					4	1			
Rissoa membranacea	2		4						
Rissoa sp.		8	2						
Ruditapes decussatus					2	3			
Ruditapes philippinarum				1		2			
Scoletoma impatiens				2		5			
Solemya togata					2	1			
Sphaeroma serratum			1					2	
Tricolia speciosa	5								
Tritia elongata		4	3						
Tritia corniculum		8	2			5			
Tritia cuvieri				2	3	10			
Tritia incrassata							2	4	8
Tritia louisi				4		7			
Tritia mutabilis					2				
Tritia reticulata				44	24	33			
Turritella communis								2	2

Appendix C

Table A3. Best correlations obtained between an increasing number of environmental variables and macrofaunal distribution in the three different harbours, as obtained from the BIO-ENV procedure. The maximum correlation (0.704) is obtained by associating six variables: OM: organic matter (highest individual correlation = 0.228); Do: dissolved oxygen; Tr: transparency.

N° of Variables	Correlation	Selections
1	0.141	Depth
1	0.182	Mud
1	**0.228**	OM
2	0.230	OM, Sand
2	0.261	OM, Mud
3	0.301	OM, Mud, Tr
3	0.420	OM, Mud, depth
4	0.501	OM, Sand, Pb, Cd
4	0.512	OM, Mud, Do, pH
5	0.558	OM, Depth, Sand, Hg, pH
5	0.612	OM, Mud, Cd, Pb, Hg
6	**0.704**	OM, Mud, Pb, Zn, Hg, depth
7	0.682	OM, Pb, P, Cd, Depth, Mud, Tr
8	0.662	Mud, OM, Depth, Cd, Hg, Cu, Pb, Fe

Appendix D

Table A4. Spearman's rank correlation coefficients (*r*) and associated significance values (*p*) for relationships between sediment characteristics (chemical contamination, OM, sand and silt) and taxonomic richness (S), abundance (N), Shannon–Wiener diversity (H′), Pielou's evenness (J′), AZTI marine biotic index (AMBI), and the benthic opportunistic annelids amphipods index (BO2A); significant correlations ($p < 0.05$) are highlighted in bold type.

	S		**N**		**H′**		**J′**		**AMBI**		**BO2A**	
	r	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***	***r***	***p***
Pb	0.022	−0.160	**0.016**	**0.010**	**0.224**	**0.001**	**0.230**	**0.001**	**0.022**	**0.012**	**0.110**	**0.001**
Cd	**0.212**	**0.020**	**0.020**	**0.021**	**−0.473**	**0.010**	**−0.506**	**0.013**	**0.015**	**0.010**	**0.580**	**0.018**
Cu	**0.031**	**0.001**	0.021	0.101	**0.242**	**0.037**	**0.183**	**0.001**	**−0.231**	**0.014**	**−0.169**	**0.009**
Zn	**0.341**	**0.020**	0.001	0.118	**−0.907**	**0.001**	**−0.910**	**0.001**	**0.230**	**0.002**	**0.904**	**0.001**
Cr	**0.124**	**0.010**	**0.324**	**0.004**	**−0.939**	**0.001**	**−0.931**	**0.040**	**0.017**	**0.001**	**0.916**	**0.001**
Mg	0.412	0.104	0.234	0.120	0.029	0.058	0.158	0.062	0.011	0.073	−0.136	0.076
Hg	**0.241**	**0.013**	0.110	0.211	**0.545**	**0.017**	**0.025**	**0.030**	**−0.561**	**0.005**	0.183	0.147
Fe	**0.452**	**0.004**	**0.142**	**0.011**	**−0.910**	**0.001**	**−0.967**	**0.001**	**0.192**	**0.001**	**0.177**	**0.001**
Al	**0.029**	**0.060**	0.341	0.118	**0.241**	**0.011**	0.172	0.361	0.321	0.060	**0.112**	**0.001**
P	**0.242**	**0.010**	0.011	0.080	**−0.907**	**0.001**	**0.410**	**0.001**	0.230	0.122	**0.204**	**0.001**
F	**0.018**	**0.030**	0.241	0.118	**0.244**	**0.011**	0.164	0.210	**0.021**	**0.002**	**0.102**	**0.001**
N	0.131	0.120	**0.101**	**0.028**	**0.306**	**0.002**	**0.201**	**0.001**	**0.230**	**0.001**	**0.204**	**0.002**
OM	0.025	0.200	0.401	0.055	**0.224**	**0.026**	0.127	0.340	**0.211**	**0.048**	0.112	0.221
Mud	**0.112**	**0.003**	**0.840**	**0.044**	0.109	0.001	**0.280**	**0.040**	**0.317**	**0.001**	0.126	0.102

References

1. Guerra-García, J.M.; García-Gómez, J.C. Soft-bottom mollusc assemblages and pollution in a harbour with two opposing entrances. *Estuar. Coast. Shelf Sci.* **2004**, *60*, 273–283. [CrossRef]
2. Caplat, C.; Texier, H.; Barillier, D.; Lelievre, C. Heavy metals mobility in harbour contaminated sediments, the case of Port-en-Bessin. *Mar. Pollut. Bull.* **2005**, *50*, 504–511. [CrossRef] [PubMed]
3. Dauvin, J.C.; Bakalem, A.; Baffreau, A.; Grimes, S. Benthic ecological status of Algerian harbours. *Mar. Pollut. Bull.* **2017**, *125*, 378–388. [CrossRef]
4. Romanelli, G.; Berto, D.; Calace, N.; Amici, M.; Maltese, S.; Formalewicz, M.; Campanelli, A.; Marini, M.; Magaletti, E.; Scarpato, A. Ballast water management system: Assessment of chemical quality status of several ports in Adriatic Sea. *Mar. Pollut. Bull.* **2019**, *147*, 86–97. [CrossRef] [PubMed]
5. Dimitriou, P.D.; Chatzinikolaou, E.; Arvanitidis, C. Ecological status assessment based on benthic macrofauna of three Mediterranean ports: Comparisons across seasons, activities and regions. *Mar. Pollut. Bull.* **2020**, *153*, 110–997. [CrossRef] [PubMed]
6. Miralles, L.; Ardura, A.; Arias, A.; Borrell, Y.J.; Clusa, L.; Dopico, E.; Hernandez de Rojas, A. Barcodes of Marine Invertebrates from North Iberian Ports, Native Diversity and Resistance to Biological Invasions. *Mar. Pollut. Bull.* **2016**, *112*, 183–188. [CrossRef]
7. Pejovic, I.; Ardura, A.; Miralles, L.; Arias, A.; Juan Borrella, Y.; Garcia-Vazqueza, E. DNA barcoding for assessment of exotic molluscs associated with maritime ports in northern Iberia. *Mar. Biol. Res.* **2015**, *12*, 168–176. [CrossRef]
8. Travizia, A.; Balkovića, I.; Bacci, T.; Bertasic, F.; Cuicchif, C.; Flander-Putrled, V.; Gratib, F.; Grossi, L.; Jaklin, A.; Lipej, L.; et al. Macrozoobenthos in the Adriatic Sea ports: Soft-bottom communities with an overview of non-indigenous species. *Mar. Pollut. Bull.* **2019**, *147*, 159–170. [CrossRef]
9. Mosbahi, N.; Pezy, J.P.; Neifar, L.; Dauvin, J.C. Ecological status assessment and non-indigenous Species in industrial and fishing harbours from the Gulf of Gabès (central Mediterranean Sea). *Environ. Sci. Pollut. Res.* **2021**, *28*, 65278–65299.
10. Cognetti, G.; Maltagliati, F. Port activities and international environmental control in the Mediterranean. *Mar. Pollut. Bull.* **2005**, *50*, 119–120. [CrossRef]
11. Gutperlet, R.; Capperucci, R.M.; Bartholomä Senckenberg, A. Benthic biodiversity changes in response to dredging activities during the construction of a deep-water port. *Mar. Biodivers.* **2015**, *45*, 819–839. [CrossRef]
12. Tavakoly, S.; Rosli, H.; Aishah, S. Ecological quality assessment based on macrobenthic assemblages' indices along West Port, Malaysia coast. *Environ. Earth Sci.* **2015**, *74*, 1331–1341.
13. Yan, W.; Zhou, Y.; Yanling, Q.; Chen, D.; Zhu, Z.; Jianfu, Z.; Bergman, Ǻ. Occurrence and risk assessment of trace metals and metalloids in sediments and benthic invertebrates from Dianshan Lake, China. *Environ. Sci. Pollut. Res.* **2017**, *24*, 14847–14856.
14. Borja, A.; Franco, J.; Perez, V. A marine Biotic Index to establish the ecological quality of soft–bottom benthos within European estuarine and coastal environments. *Mar. Pollut. Bull.* **2000**, *40*, 1100–1114. [CrossRef]
15. Dauvin, J.C.; Andrade, H.; De-La-Ossa-Carretero, J.A.; Del Pilar Ruso, Y.; Riera, R. Polychaete/Amphipod ratios: Towards validate simple benthic indices. *Ecol. Indic.* **2016**, *63*, 89–99. [CrossRef]

16. Dauvin, J.C. Twenty years of application of Polychaete/Amphipod ratios to assess diverse human pressures in estuarine and coastal marine environments: A review. *Ecol. Indic.* **2018**, *95*, 437–445. [CrossRef]
17. Zaghden, H.; Kallel, M.; Elleuch, B.; Oudot, J.; Saliot, A.; Sayadi, S. Evaluation of hydrocarbon pollution in marine sediments of Sfax coastal areas from the Gabès Gulf of Tunisia, Mediterranean Sea. *Environ. Earth. Sci.* **2014**, *72*, 1073–1082. [CrossRef]
18. Drira, Z.; Kmiha-Megdiche, S.; Sahnoun, H.; Hammami, A.; Allouche, N.; Tedetti, M.; Ayadi, H. Assessment of anthropogenic inputs in the surface waters of the southern coastal area of Sfax during spring (Tunisia, Southern Mediterranean Sea). *Mar. Pollut. Bull.* **2016**, *104*, 335–363. [CrossRef]
19. Boudaya, L.; Mosbahi, N.; Dauvin, J.C.; Neifar, L. Structure of the benthic macrofauna of an anthropogenic influenced area: Skhira Bay (Gulf of Gabès, central Mediterranean Sea). *Environ. Sci. Pollut. Res.* **2019**, *26*, 13522–13538. [CrossRef]
20. Mosbahi, N.; Serbaji, M.N.; Pezy, J.P.; Neifar, L.; Dauvin, J.C. Response of benthic macrofauna to multiple anthropogenic pressures in the shallow coastal zone south of Sfax (Tunisia, central Mediterranean Sea). *Environ. Pollut.* **2019**, *253*, 474–487. [CrossRef]
21. Galkus, A.; Joksas, K.; Stakeniene, R.; Lagunaviciene, L. Heavy Metal Contamination of Harbor Bottom Sediments. *Pol. J. Environ. Stud.* **2012**, *21*, 1583–1594.
22. Zaaboub, N.; Oueslati, W.; Helali, M.A.; Abdeljaouad, S.; Huertas, J.F.; Galindo, A.L. Trace elements in different marine sediment fractions of the Gulf of Tunis (Central Mediterranean Sea). *Chem. Speciat. Bioavailab.* **2014**, *26*, 1–12. [CrossRef]
23. Ennouri, R.; Zaaboub, N.; Fertouna-Bellakhal, M.; Chouba, L.; Aleya, L. Assessing trace metal pollution through high spatial resolution of surface sediments along the Tunis Gulf coast (southwestern Mediterranean). *Environ. Sci. Pollut. Res.* **2015**, *23*, 5322–5334. [CrossRef] [PubMed]
24. Helali, M.A.; Zaaboub, N.; Oueslati, W.; Added, A.; Aleya, L. Suspended particulate matter fluxes along with their associated metals, organic matter and carbonates in a coastal Mediterranean area affected by mining activities. *Mar. Pollut. Bull.* **2016**, *104*, 171–181. [CrossRef]
25. Gargouri, D.; Azri, C.; Serbaji, M.M.; Jedoui, Y.; Mabrouk, M. Heavy metal concentrations in the surface marine sediments of Sfax Coast, Tunisia. *Environ. Monit. Assess.* **2011**, *75*, 519–530. [CrossRef] [PubMed]
26. Bejaoui, B.; Ben Ismail, S.; Othmani, A.; Ben Abdallah-Ben Hadj Hamida, O.; Chevalier, C.; Feki-Sahnoun, W.; Harzallah, A.; Ben Hadj Hamida, N.; Bouaziz, R.; Dahech, S.; et al. Synthesis review of the Gulf of Gabes (eastern Mediterranean Sea, Tunisia): Morphological, climatic, physical oceanographic, biogeochemical and fisheries features. *Estuar. Coast. Shelf Sci.* **2019**, *219*, 395–408. [CrossRef]
27. Rabaoui, L.; El Zrelli, R.; Balti, R.; Mansour, L.; Courjault-Radé, P.; Daghbouj, N.; Tlig- Zouari, S. Metal bioaccumulation in two edible cephalopods in the Gulf of Gabès, south-eastern Tunisia: Environmental and human health risk assessment. *Environ. Sci. Pollut. Res.* **2017**, *24*, 1686–1699. [CrossRef]
28. Naifar, I.; Pereira, F.; Zmemla; Bouaziz; Elleuch, B.; Garcia, D. Spatial distribution and contamination assessment of heavy metals in marine sediments of the southern coast of Sfax, Gabes Gulf, Tunisia. *Mar. Pollut. Bull.* **2018**, *131*, 53–62. [CrossRef]
29. Aloui-Bejaoui, N.; Afli, A. Functional diversity of the macro invertebrate community in the port area of Kerkennah Islands (Tunisia). *Medit. Mar. Sci.* **2012**, *13*, 93–102. [CrossRef]
30. Chatzinikolaou, E.; Mandalakis, M.; Damianidis, P.; Dailianis, T.; Gambineri, S.; Rossano, C.; Scapini, F.; Carucci, A.; Arvanitidis, C. Spatio-temporal benthic biodiversity patterns and pollution pressure in three Mediterranean touristic ports. *Sci. Total Environ.* **2017**, *624*, 648–660. [CrossRef]
31. Chebaane, S.; Sempere-valverde, J.; Dorai, S.; Kacem, A.; Sghaier, Y. A Preliminary inventory of alien and cryptogenic species in Monastir Bay, Tunisia, spatial distribution, introduction trends and pathways. *Medit. Mar. Sci.* **2019**, *20*, 616–626. [CrossRef]
32. Dauvin, J.C.; Fersi, A.; Pezy, J.P.; Bakalem, A.; Neifar, L. Macrobenthic communities in the tidal channels around the Gulf of Gabès, Tunisia. *Mar. Pollut. Bull.* **2021**, *162*, 111–846. [CrossRef]
33. Rodier, J. *L'analyse de L'eau. Eaux Résiduaires, Eau de Mer*, 8éme ed.; Dunod: Paris, France, 1996.
34. Zaâbi, S.; Gillet, P.; Afli, A.; Boumaiza, M. Biodiversity of polychaetous annelids from the peninsula of Cap Bon, northeast coast of Tunisia. *Zoosymposia* **2009**, *2*, 587–600. [CrossRef]
35. Mosbahi, N.; Pezy, J.P.; Dauvin, J.C.; Neifar, N. Spatial and temporal structures of the macrozoobenthos from the intertidal zone of the Kneiss Islands (Central Mediterranean Sea). *Open J. Mar. Sci.* **2016**, *6*, 223–237. [CrossRef]
36. Yoshida, M.; Hamadi, K.; Ghrabi, A. Solid Waste Landfills and Soil/Sediment Contamination around Bizerte Lagoon: Possible Pollution Sources. *RPP-SEPMCL Initial Rep.* **2002**, 55–75.
37. Sharifuzzaman, S.M.; Rahman, H.; Ashekuzzaman, S.M.; Islam, M.M.; Chowdhury, S.R.; Hossain, M.S. Heavy metals accumulation in coastal sediments. In *Environmental Remediation Technologies for Metal-Contaminated Soils*; Hasegawa, H., Rahman, I., Rahman, M., Eds.; Springer: Tokyo, Japan, 2016. [CrossRef]
38. Rechnelty Tanjung, R.H.; Hamuna, B.; Novryanto Yonas, M. Assessing heavy metal contamination in marine sediments around the coastal waters of Mimika regency, Indonesia. *J. Ecol. Eng.* **2019**, *20*, 35–42. [CrossRef]
39. Tempesti, J.; Langeneck, J.; Maltagliati, F.; Castelli, A. Macrobenthic fouling assemblages and NIS success in a Mediterranean port: The role of use destination. *Mar. Pollut. Bull.* **2020**, *150*, 110768. [CrossRef]
40. Alessandro, M.; Porporato, E.; Esposito, V.; Salvatore, G.; Deidune, A.; Federica, N.; Larissa, F.; Rocco, A.; Bertof, D.; Renzig, M.; et al. Common patterns of functional and biotic indices in response to multiple stressors in marine harbours ecosystems. *Environ. Pollut.* **2020**, *259*, 113–959.

41. Blanchard, A.L.; Feder, H.M.; Shaw, D.G. Long-term investigation of benthic fauna and the influence of treated ballast water disposal in Port Valdez, Alaska. *Mar. Pollut. Bull.* **2002**, *44*, 367–382. [CrossRef]
42. Mandal, S.; Harkantra, S.N. Changes in the soft-bottom macrobenthic diversity and community structure from the ports of Mumbai. India. *Environ. Monit. Assess.* **2013**, *185*, 653–672. [CrossRef] [PubMed]
43. Moreira, L.B.; Castro, I.B.; Hortellani, M.A.; Sasaki, S.T.; Taniguchi, S.; Petti, M.A.V.; Fillmann, G.; Sarkis, J.E.S.; Bícego, M.C.; Costa-Lotufo, L.V.; et al. Effects of harbor activities on sediment quality in a semi-arid region in Brazil. *Ecotoxicol. Environ. Saf.* **2017**, *135*, 137–151. [CrossRef]
44. Weis, J.S.; Weis, P. Effects of contaminants from chromated copper arsenate treated lumber on benthos. *Arch. Environ. Contam. Toxicol.* **1994**, *26*, 103–109. [CrossRef]
45. Ram, A.; Rokade, M.A.; Borole, D.V.; Zingde, M.D. Mercury in sediments of Ulhas estuary. *Mar. Pollut. Bull.* **2003**, *46*, 846–857. [CrossRef]
46. Gworek, B.; Bemowska-Kałabun, O.; Kijeńska, M.; Wrzosek-Jakubowska, J. Mercury in Marine and Oceanic Waters—A Review. *Water Air Soil Pollut.* **2016**, *227*, 371. [CrossRef]
47. Dietz, R.; Fort, J.; Sonne, C.; Albert, C.; Bustnes, J.O.; Christensen, T.K.; Ciesielski, T.M.; Danielsen, J.; Dastnai, S.; Eens, M.; et al. A risk assessment of the effects of mercury on Baltic Sea, Greater North Sea and North Atlantic wildlife, fish and bivalves. *Environ. Int.* **2021**, *146*, 106178. [CrossRef]
48. Dong, J.Y.; Sun, X.; Zhang, Y.; Zhan, Q.; Zhang, X. Assessing benthic habitat ecological quality using four benthic indices in the coastal waters of Sanshandao, Laizhou Bay, China. *Ecol. Indic.* **2021**, *129*, 107980. [CrossRef]
49. Dauvin, J.C.; Ruellet, T.; Desroy, N.; Janson, A.L. The ecological quality status of the Bay of Seine and the Seine estuary: Use of biotic indices. *Mar. Pollut. Bull.* **2007**, *55*, 241–257. [CrossRef]
50. Afli, A.; Ayari, R.; Zaabia, S. Ecological quality of some Tunisian coast and Lagoon locations, by using benthic community parameters and biotic indices. *Estuar. Coast. Shelf Sci.* **2008**, *80*, 269–280. [CrossRef]
51. Chouba, L.; Mzoughi, N. Assessment of heavy metals in sediment and in suspended particles affected by multiple anthropogenic contributions in harbours. *Int. J. Environ. Sci. Technol.* **2013**, *10*, 779–788. [CrossRef]
52. Lorena, S.P.; García-Ordialesa, E.; Roqueñía, N.; Jose, A.G.G.; Loredoa, J. Geochemical distribution of selected heavy metals in the Asturian coastline sediments (North of Spain). *Mar. Pollut. Bull.* **2020**, *156*, 111–263.
53. Chaudharya, M.; Walkera Tony, R.; Willisb, R.; Oakesc, K. Baseline characterization of sediments and marine biota near industrial effluent discharge in Northumberland Strait, Nova Scotia, Canada. *Mar. Pollut. Bull.* **2020**, *157*, 111–372. [CrossRef] [PubMed]
54. Jeong, H.; Choia, J.Y.; Jaesoo, L.; Shimb, W.J.D.; Kime, Y.O.K.; Raa, K. Characterization of the contribution of road deposited sediments to the contamination of the close marine environment with trace metals: Case of the port city of Busan (South Korea). *Mar. Pollut. Bull.* **2020**, *161*, 111–717. [CrossRef] [PubMed]
55. Zhang, W.; Feng, H.; Chang, J.; Qu, J. Heavy Metal Contamination in Surface Sediments of Yangtze River Intertidal Zone: An Assessment from Different Indexes. *Envir. Pollut.* **2009**, *157*, 1533–1543. [CrossRef] [PubMed]
56. Arnason, J.G.; Fletcher, B.A. A 40+ year record of Cd, Hg, Pb, and U deposition in sediments of Patroon Reservoir, Albany County, NY, USA. *Environ. Pollut.* **2003**, *123*, 383–391. [CrossRef]
57. Islam, M.D.S.; Tanaka, M. Review Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. *Mar. Pollut. Bull.* **2004**, *48*, 624–649. [CrossRef]
58. Divya, M.; Ajay, Y.; Anirudh, R. Behaviour of trace metals in the anoxic environment of Veraval harbour, India. *Mar. Pollut. Bull.* **2018**, *129*, 645–654.
59. Wu, J.; Rong, S.; Wang, M.; Lu, R.; Liu, J. Environmental quality and ecological risk assessment of heavy metals in the Zhuhai Coast, China. *Front. Mar. Sci.* **2022**, *9*, 898423. [CrossRef]