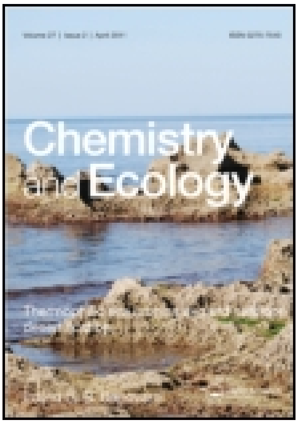

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Factors affecting the distribution of trace elements in harbour sediments

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Factors affecting the distribution of trace elements in harbour sediments

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A large number of data ($n = 488$) were acquired from 2003 to 2006 for five Italian harbours and three control areas to determine trace element (Al, As, Cd, Cu, Zn, Pb, Hg, Cr, and Ni) levels in sediments. Results were utilised to evaluate, on a multivariate statistical basis, pollution levels, significant relationships between observed levels and specific factors, and enrichment factors. Of the factors tested, main human use of harbour, was best able to determine segregations in the observed trace element fingerprints. Compared with the concentration limit approach, the evaluation of enrichment factors, even if affected by mathematical approximations, represented a useful tool for environmental studies, allowing evaluation of the presence of sediments enriched by human activities and reducing the occurrence of both false positives and false negatives due to natural differences in aluminosilicate levels.

Keywords: harbour; trace elements; sediments; enrichment factors

1. Introduction

Harbours are key structures of great economical and social importance in coastal areas. In future decades, harbours are expected to grow in both number and size due to worldwide changes in business practices and the globalisation of commerce [1]. These changes are likely to increase the adoption of procedures that maintain harbour functionality, and dredging operations are expected to become increasingly important in the management of coastal areas [2]. Nevertheless, it is also known that dredging has negative effects on marine ecosystems [3] due to both the procedure itself and the disposal phases [4]. The removal of substratum from the seafloor leads to changes in intertidal profiles, tidal flow and tidal direction [5], and to alterations in the structure of biological communities [6], causing significant damage to *Posidonia oceanica* meadows [7,8]. Greater environmental impacts arise from sediment resuspension following disturbance of the substratum [9]. In fact, the consequent increase in suspended solids reduces water transparency and changes natural sedimentation rates producing biological effects, for example, stressing

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photosynthetic organisms [8,10] and determining alterations in benthic population cover [11]. The increase in mineralisation rate reduces the available oxygen [12], causing the release of nutrients into water column [13,14]. In polluted harbours, sediment resuspension also increases the redox potential in sediments, leading to the desorption of trace elements with consequent adverse effects on communities [9,15,16]. Traditional techniques used to characterise harbour sediments are based on the concentration limit approach, a comparison of the sediment levels of pollutants with legislative limits. In European countries, results obtained from characterisations are compared with general reference levels of pollution established by each national government, and in Italy, defined by the Central Institute of Marine Applied Research [17]. Nevertheless, this approach has severe limitations. In fact, fine particles of silty fractions (mud, clay), which tend to accumulate pollutants at high concentrations, are also rich in feldspars and natural metals [18] and, for this reason, the measured concentrations of trace elements in sediments summarise both enrichment due to human activities (mining, domestic effluents, industry, agriculture) and levels due to natural local geomorphologic structures (rock weathering, volcanic eruptions). Oligo-elements, which are essential to biological communities (i.e. Cu, Zn, Ni, Cr) within an element-specific concentration range, and potential harmful elements that have no biological function (i.e. Pb, Cd, As, Hg) tend to accumulate in sediments. In recent decades, different ecotoxicological tests have been developed and applied to improve classic approaches based on concentration limits, promoting qualitative–quantitative evaluation of the biological effects of bioavailable fractions of trace elements [19]. Although these tests are of particular interest, providing useful data related to the biological effects of the bioavailable fraction of pollutants, little information can be obtained about the origin of the trace elements. For this reason, ecotoxicological studies require good preliminary knowledge of the biogeochemical characteristics of the studied area. Despite the large volume of literature providing reference data concerning local pollution levels of harbour sediments from all over the world [20,21], few papers have proposed and tested the application of indices to discriminate between the enrichment of trace elements derived from human activities and natural backgrounds [18,22]. Furthermore, typical trace element distribution patterns in sediments arising from different human activities that lead to harbour pollution (i.e. industrial, commercial, mixed, etc.) are not well known [23,24].

The aims of this study were to: (1) evaluate pollution levels in five Italian harbours, in particular exploring significant relationships between the observed pollution levels and specific harbour factors (i.e. main human use); (2) calculate enrichment factors for the trace elements considered; and (3) compare, on a multivariate statistical basis, sediment classifications obtained from application of the Italian legislative limits with results obtained from application of the calculated enrichment factors proposed in the literature. For these reasons, eight trace elements of ecotoxicological interest (Cu, Zn, Ni, Cr, Pb, Cd, As, Hg) were determined in a large and statistically significant number of sediment samples ($n = 488$), and Al levels were measured to perform normalisations of observed trace element levels with respect to the natural content due to the local geomorphologic structure.

2. Methods

2.1. *Study areas*

Vieste (V), Barletta (Barl), Molfetta (M), Bari (BA) and Brindisi (Br) were selected as harbour study sites, whereas Tricase (Tr), Costa Merlata (CM) and Open Adriatic Sea (OAS) were selected as controls. A summary of the main characteristics of each sampling site is reported in Table 1.

Table 1. Main characteristics of the sampling sites.

Geographical location	Sampling site	Geographical position East	Geographical position North	MHU	Sampling stations	Codification	
Gargano	Vieste	16°10′, 79	41°53′, 37	T	24	V	HS
Tavoliere	Barletta	16°17′, 52	41°19′, 95	C	57	Barl	HS
Tavoliere	Molfetta	16°35′, 46	41°12′, 76	T–C	37	M	HS
Murge	Bari	16°52′, 80	41°07′, 54	C	56	BA	HS
Salento	Brindisi[a]	17°59′, 38	40°39′, 56	I + C	21	Br	HS
Adriatic Sea – southern part	Tricase	18°22′00″	39°55′00″	N	9	Tr	CA
Coastal area of Ostuni	Costa Merlata	17°38′38″	40°46′33″	N	10	CM	CA
Open Adriatic Sea	Adriatic Sea[b]	18°07.017′ 18°21.402′	40°31.565′ 40°32.797′	N	6	OAS	CA

Notes: In general, in the Apulia region, summertime tourism, fishery and industry represent the most important economic resources. Harbours, in particular, are key areas of economic exchange in this region. The coastal geomorphology is variable from the north to the south: nearly 80% of the coast is cliff in Gargano (northern), whereas sandy coastlines are common from Manfredonia to Barletta and from Monopoli to Otranto (Tavoliere and Murge). Sediments and rocks are mainly constituted by carbonate (calcite and dolomite) minerals.
[a]The harbour at Brindisi is divided into three parts: internal, medium and external. In this study, we considered only the external harbour.
[b]Adriatic Sea, we considered only analytical values obtained from sampling stations located at the centre of the Adriatic Sea, along a transect corresponding to the route Brindisi–Tirana. East and north are reported as Gauss–Boaga geographical coordinates for each area. Coordinates concerning harbour sites were reported as the harbour basin centre, whereas coordinates for controls represent the centre of the sampling area. The first and the last sampling station coordinates along the selected transect were reported for OAS. MHU, main human use of the sampling area: tourism (T), commerce (C), mixed tourism + commerce (T–C), mixed industry + commerce (I + C), none (N). Sampling stations, number of sampling stations for each sampling site; Codification, internal codification of each study area; HS, harbour site; CA, control area.

2.2. *Sampling strategy*

In total, 463 sediments samples were collected at different levels from 195 harbour stations between 2003 and 2006. In each sampling area, pre-surveys were performed to localise, on a geo-referenced map, industrial effluents, effluents from municipal wastewater treatment plants, rainwater drainage effluents and other local pollution sources. Sampling stations were randomly distributed over a square grid $\sim$ 100 m in length, with additional stations located along transects near the presumed pollution sources. The position of the sampling station inside each harbour was recorded and considered as the *location factor*. Control areas ($n = 25$) were sampled randomly to evaluate the natural range of trace elements along the Adriatic coast in the Apulia region. Sediments were collected using a vibrocorer equipped with a penetrometer, which gave a precise measure of penetration rate and depth. Samples were retrieved in a pre-cleaned high-density polyethylene liner tubes (100 mm internal diameter) of different lengths, collected, homogenised, codified and stored in the dark in Teflon bottles at 4 °C prior to analyses. Cores were sampled at different levels to evaluate the relationship between depth and pollution level.

2.3. *Laboratory analyses*

Trace elements were determined after mineralisation with a mixture of H_2O_2 and HNO_3 in a microwave oven (Milestone, mod. ETHOS D Microwave Labstation), according to the US-EPA method [25]. Aluminium (Al), cadmium (Cd), lead (Pb), nickel (Ni), chromium (Cr) and copper (Cu) determinations were performed by atomic absorption spectrometry with electrotermic atomisation (GF-AAS, Perkin-Elmer, mod. AAnalyst 700), whereas zinc (Zn) was determined by atomic absorption spectrometry with flame atomisation (flame-AAS) according to the US-EPA methods [26,27]. Mercury (Hg) and arsenic (As) were determined by atomic absorption via cold vapour generation (CV-AAS) according to the US-EPA method [28], and by atomic absorption after hydride generation following the US-EPA method [29], respectively. Analytical results were reported as mg·kg^{-1} dry weight (d.w.).

2.4. *Quality assurance and quality control (QA/QC)*

Chemicals and reagents were analytical grade and glassware was carefully washed to avoid sample cross-over contamination. Recoveries and reproducibility were checked by analysing procedural blanks and reference materials purchased from the National Institute of Standards and Technologies (NIST; Estuarine Sediment SRM1646a) and the Department of Trade and Industry as a part of the National Measurement System of the UK (SRM; Harbour sediment). Analytical blanks were prepared prior to test samples using the same procedure. A solvent/matrices blank was checked every 15 samples to evaluate the detection instruments. Standard reference materials were analysed in statistical replicates ($n = 10$) to calculate averages and standard deviation of recoveries for all tested trace elements. Average recovery percentages were within 75.7–102.3% and analytical concentrations were not recovery corrected. The limit of detection (IDL) was defined as the average blank ($n = 10$) plus three standard deviations. Methodology detection limits were also calculated for the adopted procedures and were as follows: $1\,\text{mg}\cdot\text{kg}^{-1}$ (Al), $0.05\,\text{mg}\cdot\text{kg}^{-1}$ (Cd), $0.1\,\text{mg}\cdot\text{kg}^{-1}$ (Pb, Zn), $0.01\,\text{mg}\cdot\text{kg}^{-1}$ (Ni, Cu, Hg) and $0.5\,\text{mg}\cdot\text{kg}^{-1}$ (As, Cr).

2.5. *Normalisation criteria and calculation of enrichment factors*

To evaluate enrichment due to human activity, different normalisation methods have been proposed to improve sample homogeneity for trace elements [18,22]. Many different variables that mutually compete can modulate trace element levels in sediments, for this reason, normalisation techniques, which are arbitrary simplifications of complex systems, evidence some limits and might introduce significant errors. By contrast, environmental studies often require reduced system variability to highlight specific targets and normalisations might be helpful to evidence some dynamics. The choice of which techniques to adopt represents another difficulty, in fact, each method has advantages and limitations that must be considered in data evaluation. Some methods, such as physical normalisation, require the physical modification of samples and the selection of a specific fraction (often represented by the finer sediment fractions characterised by a particle diameter $< 63\,\mu\text{m}$) on which to perform analyses. These procedures are particularly useful in depositional systems where hydrological factors produce a natural gradient of silt deposition and evaluating the presence of local pollution sources might be difficult. Nevertheless, these procedures have some limits, namely the difficult laboratory pretreatment of samples, which increases analytical errors [30], and a reduction in the natural mineralogical variability of sands. Furthermore, most environmental studies on trace elements are performed on the chemical composition of the total sample (< 2 mm), and the concentration limit approach requires comparison of the obtained results with reference values, which are usually developed on total samples. Other normalisation methods, known as chemical normalisation techniques by representative elements [18], allow chemical analyses to be perfomed on total samples, selecting a normalising element in respect of which the normalised value for each pollutant is calculated by applying mathematical formulas. According to the literature, use of a normalising element allows notable reductions in the environmental variables of interest, excluding grain-size field data. The rationale on which this technique is based is represented by the fact that some elements such as Al and Fe evidence a geological origin linked to feldspars and a fixed local correlation with other trace elements. In fact, these elements represent aluminosilicates, the main group of minerals generally found in finer sediment fractions, are considered to normalise both for grain size and mineralogical variability [31–33], and only negligible amounts originate from human activity. Furthermore, current legislation requires that these elements be determined in total samples for the environmental characterisation of harbours and their acquisition does not represent an additional cost. However, it should be noted that these elements may change their chemical state and speciation in estuaries, and this might represent a limitation of this technique. Even though Fe in sediments that are anoxic and rich in organic substances might indicate the occurrence of remobilisation or precipitation-selective phenomena [34–36] and Al might indicate fluctuations due to changes in salinity, pH, temperature, etc., the obtained data should be used to enrich knowledge in environmental studies, and are not exhaustive evidence of the occurring dynamics.

In this study, we decided to perform normalisations with Al as the reference element. The Enrichment Factor is defined as the concentration ratio of the considered element to a reference element in a given sample, divided by the same ratio in the earth's crust [37]. To avoid miscalculations due to differences among local bedrock chemical composition and average values the earth's crust, which can occur in small areas [38], local background values, obtained by calculating the linear regression in local control areas, were utilised as references. Linear regression equations of trace elements versus Al natural values were calculated for controls [39], and equations were adopted to evaluate enrichment factors in harbour sites as the ratio between real and predicted values compared with Al concentrations according to the following formula:

$$EF = X_{\mathrm{HS}}/Y_{\mathrm{HS}}(X_{\mathrm{CA}}/Y_{\mathrm{CA}})^{-1},$$

where X_{HS} and X_{CA} are the normalising element values in harbour stations and control areas, and Y_{HS} and Y_{CA} are measured trace element values in harbour stations and control areas. To improve the significance, enrichment factors were calculated only for predicted values within the range of $1 \pm 2\sigma$, as reported in the literature [18]. Even with the possible useful advantages of chemical normalisation strategies, one should note that normalisations are mathematical calculations and might introduce approximation errors. Some authors suggest performing multiparameter normalisation to avoid both variability at the lower level and binding phenomena on organic matter, which could affect linear regression. Nevertheless, the organic matter as a normalising factor or cofactor was excluded in this work because control areas showed very low concentrations of organic matter and, in this case, the application of a multiparameter strategy for normalisation was excluded because it could be affected by large calculation errors.

2.6. *Statistical analyses*

Statistical analyses were performed separately on collected data and enrichment factors. Univariate analyses in terms of mean, standard deviation, minimum, maximum values, linear regressions and Pearson's [40] correlation matrices ($p < 0.05$, $n = 488$) were performed using Statistica 7.0 (StatSoft Inc., Padova, Italy), whereas multivariate analyses were developed using the Primer-E Software package v6.0 (Plymouth Marine Laboratory, UK) according to Clarke and Warwick [41]. Euclidean distance resemblance matrices were calculated after square root ($\surd$) and $\log(x + 1)$ function transformation, and successive normalisation of field data and enrichment factors [42]. Principal components analysis [43] was applied because it is generally considered to be an effective tool for exhibiting the main factors controlling geochemical data contained in a matrix [44]. Because of its weakness in distance-preserving properties [45], data sets were tested by nonmetric multidimensional scaling (nMDS) ordination techniques [46], performing runs by the application of the Kruskal stress formula 1, imposing a minimum stress of 0.01 and restarting the process 50 times. The significance of observed ordinations related to different factors of interest was tested using the analysis of similarities (ANOSIM) test statistic R one-way (otherwise defined by a single factor), which was performed by running 999 permutations in association with the pairwise test. This procedure allows us to test a hypothesis for differences between groups of samples (according to an *a priori* defined factor), using permutation/randomisation methods on a resemblance matrix, also evaluating the significance in discriminating differences among couples factors. In this study, three possible discriminating factors were considered: main human use of the harbour (MHU), geographical location of sampling stations inside the harbour (LSS), and depth of the collected samples (LeS). Concerning MHU, the specific codifications adopted are reported in Table 1. Geographical locations corresponding to the LSS factor were assigned as follows: inner (I), medium (Me), communicating channels (CC) and marine (M), relating to the distance of the sampling stations from the open sea; and finally far (F) and near (Ne) relating to distance of the sample from the presumed pollution source. Sediment cores were sampled at different depths (LeS) and levels of interest were codified as reported (level = code): $0-20\,\mathrm{cm} = 1$; $20-100\,\mathrm{cm} = 2$; $100-200\,\mathrm{cm} = 3$; $> 200\,\mathrm{cm} = 4$, to obtain LeS assigned values ranging from 1 to 4.

3. Results

3.1. *Definition of pollution levels in studied harbours*

In Table 2 mean (M), standard deviation (SD), maximum (Max) and minimum (Min) values calculated for harbour sites and control areas are reported. Aluminium levels in the harbour samples showed wide variations, ranging from ~ 0.1 to $\sim 29\%$, with minimum values measured in Br and maximum values in M; in control areas this element ranged within 0.06–1.6%. Maximum values for As, Cd, Cr, Hg, Ni, Pb, Cu and Zn were recorded in harbours. In particular, commercial harbours gave the highest values for all elements, with the exception of As, which was maximum in I–C harbour. Even though these data are not given in Table 2, the highest levels of Pb, Hg, Ni and Zn were recorded in BA harbour, whereas maximum concentrations for Cd, Cr and Cu were observed in Barl harbour. In Italy, the Central Institute of Marine Applied Research sets the concentration limits for trace elements in marine sediments. In particular, LCB is defined as the maximum value of natural concentrations in harbour coastal sediments, whereas LCL is the minimum level indicating sediment pollution. LCB and LCL are not defined for Al because its sediment levels are considered to be mainly natural in origin [17]. Concentration limits for each trace element, expressed as LCB and LCL, are reported in Table 2. Even though the Commission of the European Communities establishes specific environmental quality standards for priority substances and other selected pollutants in sediments, the proposed values are indicative of a good quality status and are adaptable for highly polluted areas such as harbours. For this reason, a national interest reference was selected in this article. Concerning maximum concentrations measured in both harbours and control areas, almost all the trace elements considered in this study showed levels higher than LCB, and frequently also higher than LCL. Maximum harbour As concentrations were always between LCB and LCL, except for Br harbour which showed a maximum level that was more than twice the LCL limit. Maximum Cr levels were always lower than LCL, but were higher than LCB in Barl and BA harbours. A maximum Ni value higher than LCL was reported in BA harbour, whereas Cd, Pb, Cu and Zn showed maximum levels higher than LCL in Barl and BA. Concerning Hg, maximum levels higher than LCL were measured in BA harbour. Maximum Zn and Ni levels were higher than LCB in all the areas studied, including control areas. In particular, in controls, maximum values of As ($29.5\,\mathrm{mg{\cdot}kg^{-1}}$), Hg ($0.55\,\mathrm{mg{\cdot}kg^{-1}}$), Ni ($41.50\,\mathrm{mg{\cdot}kg^{-1}}$) and Zn ($84.5\,\mathrm{mg{\cdot}kg^{-1}}$) were higher than LCB. This is of particular interest because, according to the concentration limit approach, controls in which observed levels exceed LCB have to be classified as ‘enriched’.

Considering averages, both harbours and controls were lower than LCL for As, Cr, Hg, and Ni; while in BA harbour, Pb, Cu and Zn showed average levels higher than LCL. Average values higher than LCB were observed for Ni (BA), Pb (Br), Cu (Br), Cd (Ba and Br), Hg (BA) and Zn (Br and Barl).

The relative percentage of As to other elements was similar in each area ($\sim 15\%$), although Al percentages showed wide fluctuations ($\sim 60\%$ in V, 1–2% in M). BA showed the highest relative percentages of Cu ($\sim 12\%$) and Pb ($\sim 21\%$). Concerning Zn, observed relative percentages

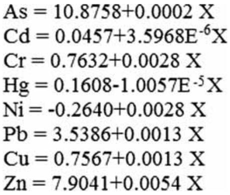
Table 2. Basic unvariate statistics performed on collected data.

	Al	As	Cd	Cr	Hg	Ni	Pb	Cu	Zn	
HS	21,517	10.4	0.26	29.1	0.10	27.11	43.8	34.50	114.8	M
	37,430	4.9	0.42	17.5	0.17	15.60	42.0	28.76	91.9	SD
	296,550	72.2	5.36	89.9	1.44	88.04	178.1	134.27	1,131.0	Max
	650	2.5	<MDL	4.1	<MDL	3.20	2.9	<MDL	5.1	Min
CA	4,563	11.7	0.06	13.7	0.11	12.31	9.3	6.67	32.4	M
	5,275	6.2	0.03	15.3	0.15	14.71	7.3	7.14	28.7	SD
	15,780	29.5	0.10	44.0	0.55	41.50	22.7	20.11	84.5	Max
	620	4.3	0.03	2.9	0.01	1.32	1.8	0.72	7.4	Min
C	14,907	11.1	0.32	34.0	0.13	31.96	58.1	45.32	145.9	M
	11,168	3.2	0.49	17.4	0.19	14.52	42.7	27.90	92.4	SD
	48,750	19.5	5.36	89.9	1.44	88.04	178.1	134.27	1131.0	Max
	2,124	3.3	<MDL	5.8	<MDL	6.00	5.2	4.94	30.4	Min
T	10,579	11.6	0.13	18.5	0.03	23.64	11.8	13.62	73.6	M
	6,835	8.0	0.14	11.3	0.04	16.13	6.9	10.99	58.8	SD
	29,184	27.3	0.68	39.2	0.13	58.31	33.5	43.66	173.1	Max
	2,767	2.5	0.01	5.4	0.01	4.43	2.9	1.02	5.1	Min
I + C	1,527	8.0	0.07	10.1	0.05	9.99	9.4	3.88	23.6	M
	1,484	10.4	0.10	8.2	0.04	8.66	18.6	4.94	12.3	SD
	9,821	72.2	0.68	45.4	0.14	39.90	95.0	33.44	50.8	Max
	650	3.6	0.02	4.1	<MDL	3.91	2.9	1.02	5.1	Min
T–C	66,413	8.4	0.17	22.6	0.03	17.52	12.8	12.79	48.4	M
	76,643	3.0	0.08	10.6	0.03	10.48	6.4	8.05	23.0	SD
	296,550	19.7	0.36	49.7	0.12	61.78	31.2	36.77	115.1	Max
	4,378	4.1	0.03	7.6	<MDL	3.20	5.2	<MDL	6.1	Min
LCB	nd	17	0.2	50	0.2	32	25	15	50	
LCL	nd	32	0.8	360	0.8	75	70	52	170	

Notes: Mean (M), standard deviation (SD), maximum (Max) and minimum (Min) values calculated in harbour sites (HS) considering the whole database and control areas (CA) are reported. Concerning HS, values obtained for different main human uses of the sampling area (MHU) were also highlighted. C, commerce; T, tourism; I + C, industry + commerce; T–C, mixed tourism + commerce. Concentration values of limits defined by the Central Institute of Marine Applied Research (APAT-ICRAM, 2006) are also reported and indicated as LCB (maximum value referring to natural concentrations in sediments), and LCL (minimum level indicating sediment pollution). Al, aluminium; As, arsenic; Cd, cadmium; Cr, chromium; Hg, mercury; Ni, nickel; Pb, lead; Cu, copper; Zn, zinc; nd, not defined. Data are expressed as mg kg^{-1} d.w. MDL, methodology detection limit.

ranged from 33 to $\sim$ 45% in all studied harbours. M harbour showed similar values ($\sim$ 10%) for As, Cr, Ni and Pb, with the lowest Cu ($\sim$ 4%) and Zn ($\sim$ 33%) percentages. Controls showed the highest relative percentage of As ($>$ 10%), and higher values were also reported for Cr ($\sim$ 12%) and Ni ($\sim$ 11%).

Reciprocal relationships between pairs of variables were explored separately in harbours ($n = 462$, $p < 0.01$) and control areas ($n = 24$, $p < 0.01$), applying Pearson's (1894) correlation matrices to evaluate the significance of the enrichment factors in harbours. Considering harbour sites, Al showed a positive, low but significant correlation with Cr (0.23) and Ni (0.13), and a negative correlation with Pb (-0.13). No significant relationships were recorded between the following pairs Al–Hg, Al–Cu, Al–Zn, Al–Cd and Al–As. With the exception of Al, all trace elements showed positive and significant reciprocal relationships, even though stronger correlations were reported only for the following pairs: Ni–Cr (0.74), Pb–Cu (0.81), Pb–Zn (0.71) and Cu-Zn (0.74). By contrast, in controls, results obtained from Pearson's correlation matrix suggested the occurrence of high positive correlations between Al–Cd (0.74), Al–Cr (0.98), Al–Ni (0.99), Al–Pb (0.92), Al–Cu (0.96) and Al–Zn (0.99), whereas As–Al and Hg–Al showed a weak positive (0.16) and significant negative (-0.35) relationship, respectively. As observed in harbours, in control areas, a strong positive cross-correlation ($>$ 0.70) between trace elements (Cd,



$As = 10.8758+0.0002\ X$
$Cd = 0.0457+3.5968E^{-6}X$
$Cr = 0.7632+0.0028\ X$
$Hg = 0.1608-1.0057E^{-5}X$
$Ni = -0.2640+0.0028\ X$
$Pb = 3.5386+0.0013\ X$
$Cu = 0.7567+0.0013\ X$
$Zn = 7.9041+0.0054\ X$

Figure 1. Linear regression functions calculated in control areas. As, arsenic; Cd, cadmium; Cr, chromium; Hg, mercury; Ni, nickel; Pb, lead; Cu, copper; Zn, zinc; X = aluminium levels.

Cr, Ni, Pb, Cu, Zn) was observed. With the exception of Al, Hg was not significantly correlated to any trace element and As was significantly positive related only to Cd (0.41).

3.2. *Enrichment factors*

Linear regressions of trace elements versus Al in controls were calculated using Statistica 7.0 and are summarised in Figure 1. Negative Al versus Hg and weak positive Al versus As relationships were also modelled. These linear functions allowed us to calculate enrichment factors in harbours using the formula suggested in the literature [18], with the exception of Cd and Hg, which showed no significance. The results obtained are reported in Table 3. Considering total averages, harbours showed enrichments only for Pb and Cu, whereas values calculated for the other elements were lower than predicted natural levels. Considering maximum values that referred to the most polluted areas, however, enrichments were observed for all calculated elements in all harbours, with the exception of M. A clear difference was observed when analysing single enrichment factors in each harbour site. Concerning averages, major enrichments were reported for BA harbour, particularly concerning Zn and Cu. Furthermore, BA and Br harbours showed a clear average enrichment for all trace elements considered in this study, with the exception of As. Barl and V showed enrichments only when considering maximum values and, on average, calculated enrichment factors were low,

Table 3. Enrichment factors calculated for harbours in respect of control areas.

	Total averages		BA			Barl		
Element	Mean	Max	Mean	Max	Min	Mean	Max	Min
As	0.70	4.81	1.00	1.58	0.60	0.66	1.14	0.28
Cr	0.48	1.53	1.34	2.70	0.33	0.66	1.57	0.18
Ni	0.46	1.53	1.62	4.25	0.29	0.54	1.20	0.21
Pb	1.38	5.85	6.85	13.41	0.99	0.94	3.80	0.17
Cu	1.20	4.86	5.42	12.28	0.88	1.23	4.98	0.18
Zn	0.93	9.46	3.98	23.38	0.91	0.93	2.33	0.26

	M			V			Br		
Element	Mean	Max	Min	Mean	Max	Min	Mean	Max	Min
As	0.35	0.81	0.17	0.89	2.10	0.19	0.71	6.46	0.32
Cr	0.12	0.27	0.04	0.61	1.29	0.18	2.01	9.01	0.80
Ni	0.09	0.33	0.02	0.81	1.99	0.15	2.49	9.95	0.97
Pb	0.14	0.35	0.06	0.68	1.94	0.17	1.70	17.20	0.52
Cu	0.15	0.42	0.00	0.94	3.01	0.07	1.41	12.20	0.37
Zn	0.13	0.31	0.02	1.13	2.66	0.08	1.46	3.14	0.31

Notes: Enrichment factors are reported as total averages (Mean) and maximum values (Max) considering the whole database collected in harbour sites and considering each harbour separately. Mean, average enrichment factor value; Max, maximum enrichment factor value; Min, minimum enrichment factor value; BA, Bari; Barl, Barletta; M, Molfetta; V, Vieste; Br, Brindisi; As, arsenic; Cr, chromium; Ni, nickel; Pb, lead; Cu, copper; Zn, zinc. Only elements significantly correlated to aluminium are reported. Values evidencing enrichments are underlined.

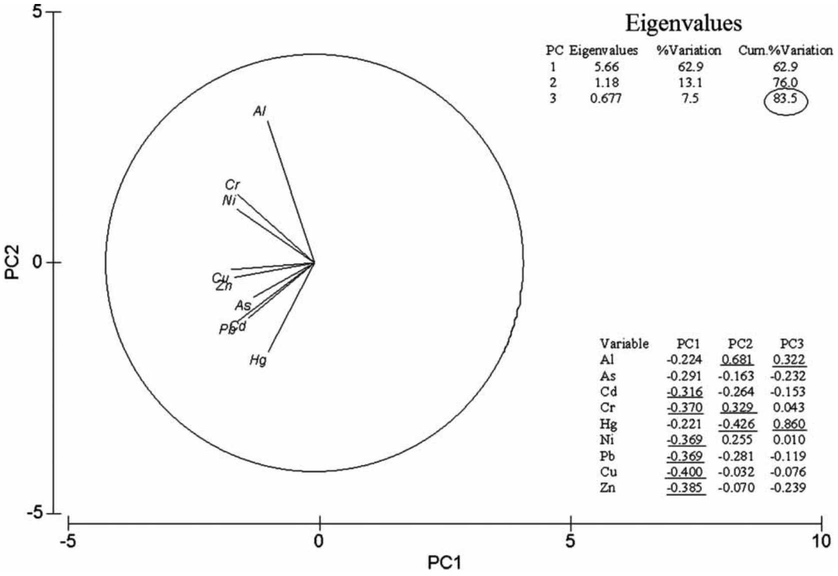
with the exception of Cu (Barl) and Zn (V) for which moderate enrichment levels were observed. M harbour showed no enrichments when considering either averages or maximum levels. The data support the presence of hotspots and localised human impact inside each harbour site, suggesting that the major environmental problems are related to BA and Br harbours.

Even where the data are not reported in the tables, considering results obtained for each sample, the number of samples with values higher than the LCL limits for Zn (111) and Pb (221) was similar to the number with enrichment factors values > 1 (124 for Zn, and 229 for Pb). Regarding Cu, a difference of $\sim$ 24% was observed, whereas concerning As, Cr and Ni, the number of samples shown to exceed the LCL was quite different from the number of samples with an enrichment factor > 1.

3.3. *Comparison among sediment classifications: classical approaches versus enrichment factors*

Concerning field data, principal component analyses (PCA) were performed and the results obtained are summarised in Figure 2. The first three components (62.9, 13.1 and 7.5%) accounted for 83.5% of the total variance. Analyses of the coefficients in the linear combinations of variables show that the first axis correlates highly with Cu and Zn, and is negatively correlated with all trace elements. The second axis is positively correlated with Al, Cr and Ni, even though stronger correlations were observed for Al and Hg. Two-dimensional plots of nMDS performed on collected data are reported in Figure 3, and results obtained by application of the ANOSIM test are summarised in Table 4. Under the tested conditions, the null hypotheses: (1) there is no segregation among stations related to MHU factor, and (2) there is no segregation among stations related to LSS factor are rejected. By contrast, the null hypotesis has to be accepted for the LeS factor.

Enrichment factors data were also analysed statistically to evaluate their performance in describing the studied systems. The PCA showed that the first three components (88.1, 6.1 and 2.0%) accounted for 96.2% of the total variance (Figure 4). Most of the total variance was explained



Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	5.66	62.9	62.9
2	1.18	13.1	76.0
3	0.677	7.5	83.5

Variable	PC1	PC2	PC3
Al	-0.224	0.681	0.322
As	-0.291	-0.163	-0.232
Cd	-0.316	-0.264	-0.153
Cr	-0.370	0.329	0.043
Hg	-0.221	-0.426	0.860
Ni	-0.369	0.255	0.010
Pb	-0.369	-0.281	-0.119
Cu	-0.400	-0.032	-0.076
Zn	-0.385	-0.070	-0.239

Figure 2. Principal component analyses performed on collected field data. Eigenvalues and eigenvectors are also reported; significant correlations are underlined. Al, aluminium, As, arsenic; Cd, cadmium; Cr, chromium; Hg, mercury; Ni, nickel; Pb, lead; Cu, copper; Zn, zinc.

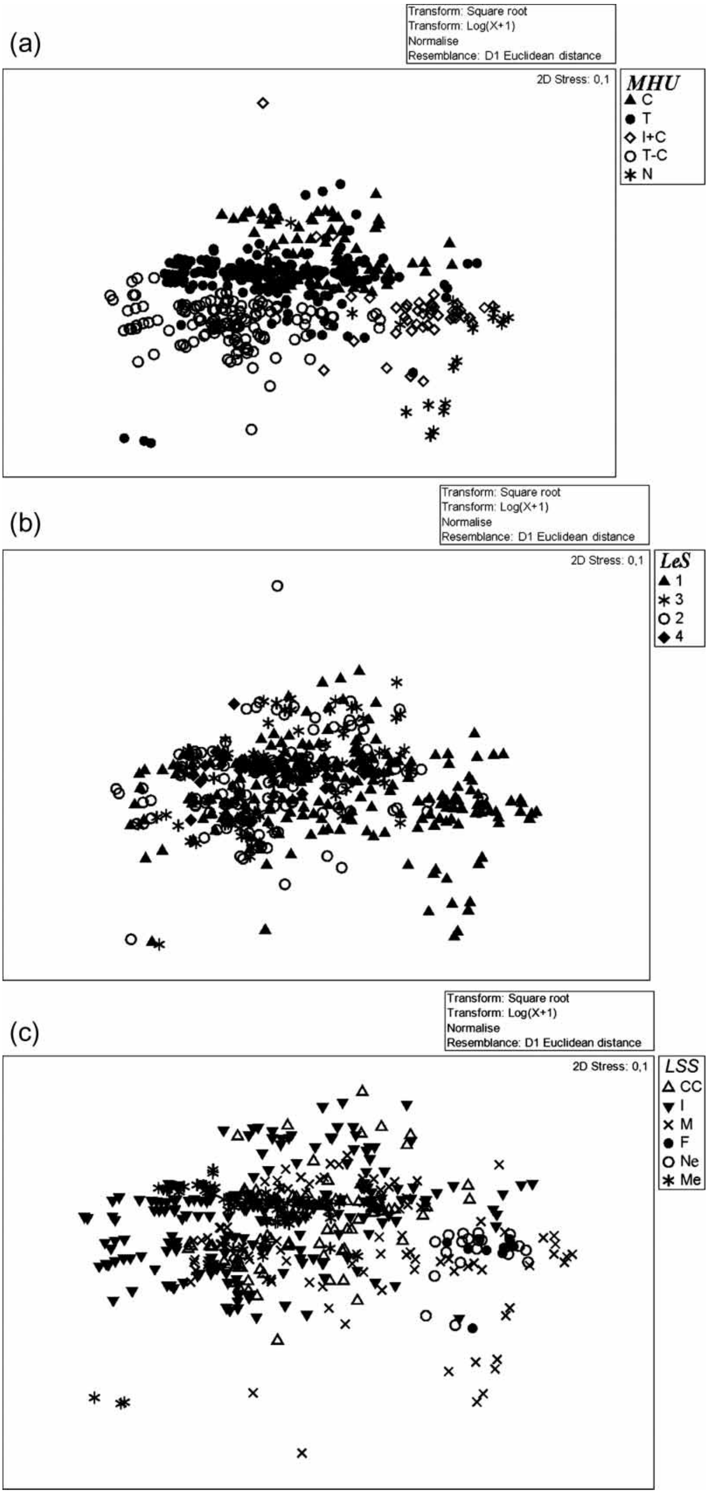
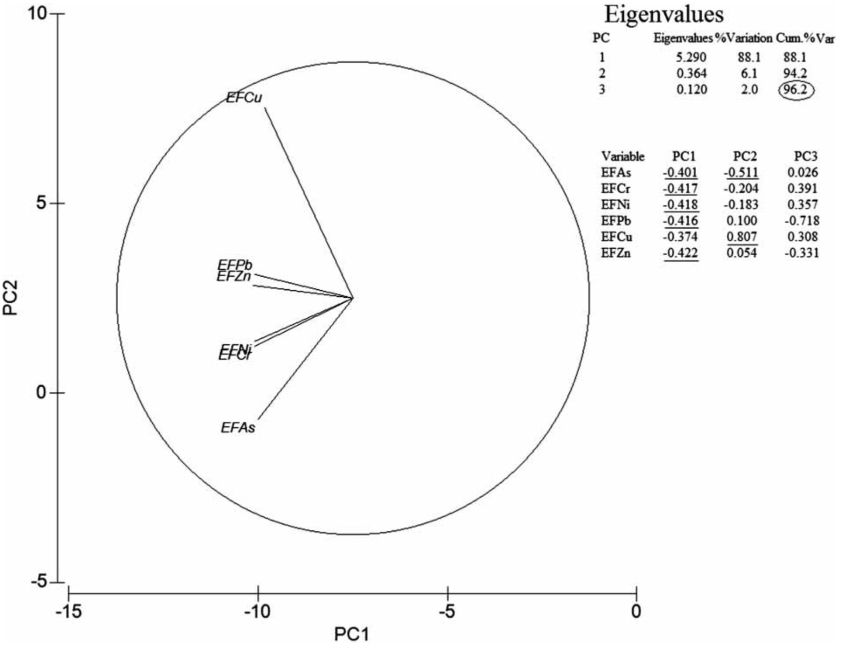


Figure 3. Non-metric multidimensional scaling (nMDS) performed on collected field data. Distribution according to factors (a) MHU, (b) LeS and (c) LSS are shown.

Table 4. Results obtained by the Anosim test one-way. Multivariate statistical analyses results obtained by the application of the Anosim test one-way (999 runs) are reported respectively for the trace element database and calculated enrichment factors.

	Factor	Global *R*	s (%)	NPS	Null hypothesis
Trace element database	MHU	0.461	0.1	0	rejected
	LSS	0.117	0.1	0	rejected
	LeS	−0.030	98.4	983	accepted
Enrichment factors database	MHU	0.694	0.1	0	rejected
	LSS	0.023	6.7	66	rejected
	LeS	0.032	3.9	39	rejected

Notes: Tested factors: MHU, main human use of the sampling area; LSS, geographical location of sampling stations inside the harbour; LeS, level of depth of collected samples; s, significance level of sample statistic; NPS, number of permuted statistics greater than or equal to global R. Effect on the null hypothesis of no significance of the observed segregation.



Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Var
1	5.290	88.1	88.1
2	0.364	6.1	94.2
3	0.120	2.0	96.2

Variable	PC1	PC2	PC3
EFAs	-0.401	-0.511	0.026
EFCr	-0.417	-0.204	0.391
EFNi	-0.418	-0.183	0.357
EFPb	-0.416	0.100	-0.718
EFCu	-0.374	0.807	0.308
EFZn	-0.422	0.054	-0.331

Figure 4. Principal component analyses performed on EF. Eigenvalues and eigenvectors are also reported; significant correlations are underlined. EFAs, enrichment factor of arsenic; EFCd, enrichment factor of cadmium; EFCr, enrichment factor of chromium; EFHg, enrichment factor of mercury; EFNi, enrichment factor of nickel; EFPb, enrichment factor of lead; EFCu, enrichment factor of copper; EFZn, enrichment factor of zinc.

by the first axis. The coefficients for the linear combinations of variables were between 0.37 and 0.42 for almost all enrichment factors. The second axis directly correlates to EFCu (0.807) and was negatively related to the EFA variable (−0.511). Two-dimensional plots of nMDS performed on enrichment factors are reported in Figure 5, and the results obtained using the ANOSIM test are reported in Table 4. In this case, the null hypothesis was not significant according to the tested factors and could be rejected for all of them. The pairwise test performed on the enrichment factors data indicated that the greatest significance concerning the LSS factor was observed for the following pairs: CC–I ($s = 0.09\%$, NPS = 0), CC–Me ($s = 0.05\%$, NPS = 0), F–M ($s = 0.1\%$, NPS = 0), M–N ($s = 0.01\%$, NPS = 0). For the LeS factor, major significance was observed for the pairs: 1–3 ($s = 0.1\%$, NPS = 0), 3–2 ($s = 2.2\%$, NPS = 22) and 1–2 ($s = 8.7\%$, NPS = 87). Furthermore, the pairwise test gave a value of $s = 0.1\%$ (NPS = 0)

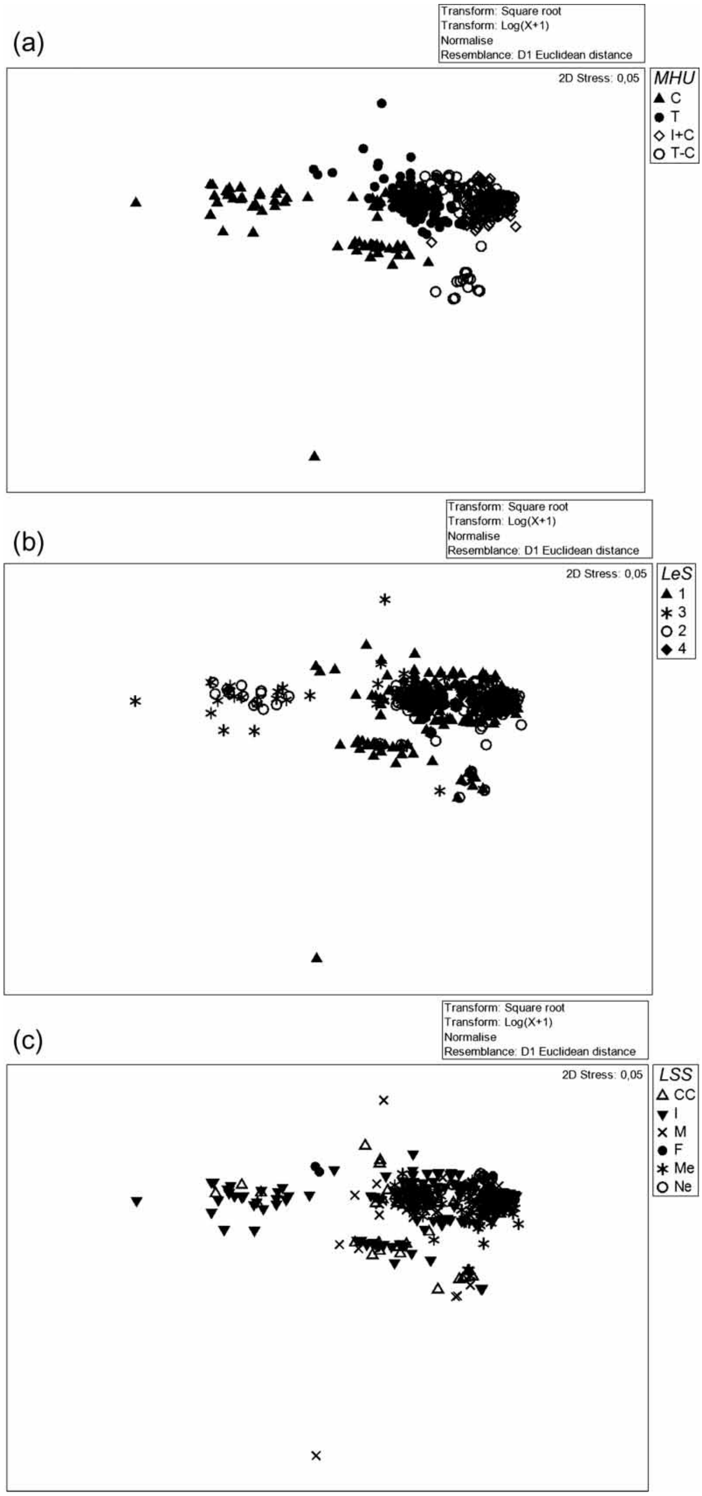


Figure 5. Non-metric multidimensional scaling (nMDS) performed on EF values. Distribution according to factors (a) MHU, (b) LeS and (c) LSS is shown.

for all possible pairs of the factor MHU. Compared with field data results, PCA performed on enrichment factors data gave an increase in total explained variance for the first three components of +8.7, with a net increase at the first axis of +15.4. The ANOSIM tests showed a higher significance for results obtained by performing analyses on enrichment factors for both MHU and LSS factors if compared with the results obtained from field data. Furthermore, data distribution according to the LeS factor showed significance for the enrichment factors database, whereas when performing the same test on field data no significance was observed. These results indicate that enrichment factors might provide additional information about sediment characteristics in harbour systems compared with field data in discriminating the variables distribution according to tested factors (MHU, LeS, LSS).

4. Discussion

Pollution levels differ among harbour sites, indicating the presence of a pollution hotspot of local interest for each harbour. Trace elements showed wide fluctuations due to the large differences between harbour sediment characteristics; these data were in agreement with results given in the literature [24]. Considering measured levels, even though little bibliographical data is available on the chemical composition of Mediterranean sediments, commercial harbours shows Hg levels (average 0.13 $mg{\cdot}kg^{-1}$) similar to values reported in the literature for a highly polluted area: the Gulf of Naples (0.18 $mg{\cdot}kg^{-1}$) [47]. Concerning Cd, concentrations measured in sediments from the southern Adriatic Sea are lower than values recorded in BA and Barl harbours [48]. Nevertheless, levels of Pb, Cr, Cu and Zn in BA sediments were lower than values reported from the Gulf of Naples [47], but higher than levels reported for the Adriatic Sea [48]. The contribution made by the natural sediment composition is evaluated by comparing results with levels observed in control areas. Apulia control areas, which were selected in this study, showed Cd levels lower than the averages reported in the literature for the Gulf of Naples [47], the Aegean Sea [49] and the southern Adriatic Sea [48] of 0.27, 0.53 and 0.20 $mg{\cdot}kg^{-1}$ d.w., respecively, suggesting lower levels for this element in the selected controls. By contrast, maximum Cr values were higher in selected controls than in sediments from the Gulf of Naples (32 $mg{\cdot}kg^{-1}$). Also Hg and Cu showed maximum values higher than averages reported for the southern Adriatic Sea (of 0.30 and 16.09 $mg{\cdot}kg^{-1}$, respectively), whereas Zn levels were similar to southern Adriatic Sea values (95.8 $mg{\cdot}kg^{-1}$). Concerning Pb, Apulia controls showed maximum values higher than levels found for the Aegean Sea (19 $mg{\cdot}kg^{-1}$) and the southern Adriatic Sea (4.43 $mg{\cdot}kg^{-1}$), whereas the Gulf of Naples was characterised by noticably higher values (168 $mg{\cdot}kg^{-1}$). The differences in trace element fingerprints observed among the study areas are due to the presence of multiple pollutant sources and to the natural diversity of these systems, mainly determined by variability in grain-size and feldspar content among the sampling sites. A significant difference in trace elements concentrations was observed related to the main human use of the harbour sites. In fact, whereas commercial (BA and Barl) harbours were dominated by high levels of Pb, Zn, Ni, Cu, Hg, Cd and Cr, the mixed (industrial + commercial) harbour (Br) was dominated by As, Cr and Pb. Statistical multivariate analyses confirmed the observed differences, indicating significant segregations related to the MHU. This result suggests that human activities lead to different trace element fingerprints in harbour sediments because of the occurrence of a different pattern of pollution. The observed significance related to the factor LSS indicates a difference in trace element pollution related to the position of the sampling station inside the harbour basin. Harbours are semi-enclosed marine areas, characterised by large man-made pressures and the presence of freshwater influents (rivers, urban discharges, etc.) which determine the occurrence of particular water flux dynamics, and also due to internal currents and salinity differences between harbour areas. The general presence of major sources of pollution in inner areas determines a significant difference in pollutant release/adsorption dynamics and distribution inside each basin.

Traditional characterisation approaches applied to the risk assessment of harbour sediments and based on the concentration limit approaches are not exhaustive in management practices. In fact, especially with regard to trace elements, comparison of measured levels with standard legislative limits does not allow us to distinguish between enrichments due to human activities and the natural sediment content of organic substances and feldspars. Sediments from Mediterranean harbours and ports are characterised by high percentages of small-size muddy-clay particles because of their reduced hydrodynamic energy and high terrestrial and river inputs [5]. In these systems, the deposition of complexes formed by organics, pollutants and muddy-clay particles (< 2–$20\,\mu$m diam.) commonly occurs and leads to significant enrichment, particularly in inner harbour areas which represent a trap for silt sedimentation. As reported in the literature, BA and Barl harbours are characterised by high percentages of silts in sediments, whereas V and Br show a sandy bottom [24] which might lead to differences in the accumulation dynamics of trace elements related to grain-size and differences in organic substances among the study areas. If these phenomena occur, performing evaluations considering only the legislative limits could be misleading. In fact, this does not take into account the total aluminosilicate content and natural structural differences in sediment among sampling stations. Our results suggest that an approach based on calculation of the enrichment factor might represent a useful tool to evaluate the occurrence of trace element enrichment in harbour sediments compared with local controls. In fact, even considering the methodological limits represented by the application of normalisation techniques, multivariate analyses performed on both field data and enrichment factors lead to a significant increase in system knowledge by the application of data normalisation, particularly concerning the evaluation of variable distribution related to LSS and LeS. However, it should be noted that as the concentration limit approach has some severe limitations. In fact, considering the data proposed in this study, selected controls showed values higher than LCB for As, Ni, Zn and Hg. Furthermore, considering As, measured values were close to the LCL. Concerning As in the Apulia region, application of the concentration limit approach might lead to an excess of false positive in harbours compared with controls. Furthermore, the occurrence of a false negative was found in this study, in fact, even though the average field data recorded for V harbour were notably lower than LCL, enrichment factors calculated for Zn suggested a clear enrichment.

As a further consideration, even though the tested approach based on enrichment factors was more informative than classic methods and represents a significant tool for use in harbour management, it should be included within a multi-approach strategy to reduce misleading information due to the intrinsic limits of each individual strategy.

5. Conclusions

This article indicates a significant segregation of the observed trace element fingerprints related to the considered factors. In particular, significance was reported for the factors main human use and geographical location of sampling stations inside the harbour. Statistical analyses performed on the enrichment factors data suggested that, for trace element pollution, classical techniques based on the concentration limit approach could be improved significantly.

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