



## Assessment of the metal pollution, potential toxicity and speciation of sediment from Algeciras Bay (South of Spain) using chemometric tools

M. Díaz-de Alba, M.D. Galindo-Riaño\*, M.J. Casanueva-Marenco, M. García-Vargas, C.M. Kosore

Department of Analytical Chemistry, Faculty of Sciences, University of Cádiz, Campus Río S. Pedro, 11510 Puerto Real, Cádiz, Spain

### ARTICLE INFO

#### Article history:

Received 1 December 2010  
Received in revised form 7 March 2011  
Accepted 8 March 2011  
Available online 15 March 2011

#### Keywords:

Available metal  
Sediment  
Total metal  
Speciation  
Anthropogenic pollution

### ABSTRACT

A study to determine total and mobile heavy metals concentrations in sediments from Algeciras Bay was performed and pollution hotspots were identified. The effects on aquatic organisms were established using sediment quality guidelines (SQGs). Ni and Cr exceeded the effect range medium and low levels, respectively, around industrial area. Potential toxicity of metals was determined by diethylenetriaminepenta-acetic acid (DTPA) extractions and low alert levels of Co, Cu, Zn, Cd, Ni and Pb were exceeded at most sampling sites. Three pollution indicators were used showing significant values for As, Ni, Cr, Pb and Cd. Sediment speciation using the sequential extraction BCR procedure was carried out, being Cd, Zn, Pb and As the most available metals. Principal component, cluster and ANOVA analyses were performed in order to assess the sources of metals and the influence of seasonality and anthropogenic activities on the sediment quality. Two principal component analysis (PCA) factors were obtained identifying the sampling sites affected by anthropogenic activities; Ni–Cr and Zn–Cu–V clusters were also obtained associated with stainless steel and petrochemical industrial activities. ANOVA showed the outstanding sites because of total metal concentration and significant differences among sampling sites by the acid extractable and reducible fractions for all metals except for Ba and V.

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### 1. Introduction

The distribution of metals within the aquatic environments is governed by complex processes affected by anthropogenic activities or natural processes including riverine or atmospheric inputs, floor erosion, biological activities, water drainage and discharge of urban and industrial wastewater [1]. Sediments accumulate higher levels of hazardous trace elements than water, causing serious problems due to their toxicity and bioaccumulation [2,3].

#### 1.1. Mobile metal and speciation of sediment

The behavior of metals in sediments depends on their chemical form affecting their mobility, bioavailability and toxicity. Therefore, quantifying metal speciation in sediments provides a good indicator of ecosystem quality [4].

Soluble, exchangeable and weak chelated metal species constitute the most mobile fraction and are usually assessed by complexing agents [5]. Sequential extraction procedures are accomplished to evaluate all metal fractions in sediments; although time-consuming, they provide detailed information concerning the

origin, biological and physico-chemical availabilities, mobilization and transport of trace metals. The procedure simulates the mobilization and retention of these species in the natural environment by changes such as pH, redox potential and organic matter degradation [6,7]. A three-stage sequential extraction procedure proposed by the European Community Bureau of Reference (BCR) was developed in order to standardize the various schemes described in the literature [8,9]. This method provides the acid extractable (water soluble, exchangeable and bound to carbonates metal), reducible (bound to Fe and Mn oxides), oxidizable (bound to sulfides and organic matter) and residual (inert) fractions of metal in the sediment.

#### 1.2. Assessment of sediment quality: sediment quality guidelines (SQGs)

Numerous sediment quality guidelines are used to protect aquatic biota from the harmful and toxic effects related with sediment-bound contaminants; they are useful assessment tool of the potential pollution [10–12]. These guidelines evaluate the degree to which the sediment-associated chemical status might adversely affect aquatic organisms and are designed for the interpretation of sediment quality. They are also used to rank and prioritize contaminated areas for further investigation [13–16]. Some of the SQGs are given in Table 1.

\* Corresponding author. Tel.: +34 956016362; fax: +34 956016460.  
E-mail address: [dolores.galindo@uca.es](mailto:dolores.galindo@uca.es) (M.D. Galindo-Riaño).

**Table 1**  
Sediment quality guidelines (SQGs) (mg kg<sup>-1</sup>).

Metal	Effect range low (ERL) <sup>a</sup> [14]	Effect range medium (ERM) <sup>a</sup> [14]	Low alert level (LAL) <sup>a</sup> [15]	High alert level (HAL) <sup>a</sup> [16]
As	8.2	70	0.5	70
Ba	NA	NA	50	1000
Cd	1.2	9.6	0.04	9.6
Co	NA	NA	0.5	120
Cr	81	370	4	370
Cu	34	270	2	270
Fe	NA	NA	NA	NA
Mn	NA	NA	NA	NA
Ni	21	52	3	50
Pb	47	220	2	218
V	NA	NA	5	350
Zn	150	410	5	410

<sup>a</sup> NA: not available.**Table 2**  
Metal concentrations in average continental shale (mg kg<sup>-1</sup>).

Metal	Average continental shale
As	13
Ba	580
Cd	0.3
Co	19
Cr	90
Cu	45
Fe	47000
Mn	850
Ni	68
Pb	20
V	130
Zn	95

### 1.3. Assessment of sediment quality: pollution indicators

The anthropogenic metal contribution in sediments can be estimated from the enrichment relative to unpolluted reference materials or widely accepted background (pre-industrial) levels, such as the average values for continental shale (Table 2) or crust abundances [17–19]. Different pollution indicators can be used such as enrichment factor (EF), contamination factor (CF) and geoaccumulation index ( $I_{geo}$ ), providing a relative ranking of sampling sites.

The enrichment factor estimates the anthropogenic impact on sediments by using the following equation:

$$EF = \frac{M_x \cdot Fe_b}{M_b \cdot Fe_x} \quad (1)$$

where  $M_x$ ,  $M_b$ ,  $Fe_x$  and  $Fe_b$  are the concentrations of metal (M) and Fe in the sample and in the background reference, respectively. Iron or aluminum is used as a normalization element to reduce the variations produced by heterogeneous sediments; the reference element must have minimum variability of occurrence or large concentrations in the studied environment [18,20].

**Table 3**  
Criteria of pollution indicators in sediment based on the EF, CF and  $I_{geo}$  values.

EF	Pollution status	CF	Pollution status	$I_{geo}$	Pollution status
>1	With anthropogenic sources	≥6	Very high	>5	Extreme
		3 ≤ CF < 6	Considerable	4–5	Strong to extremely strong
		1 ≤ CF < 3	Moderate	3–4	Strong
<1	Without anthropogenic sources	<1	Low	3–3	Moderate to strong
			1–2	Moderate	
			0–1	Unpolluted to moderate	
			<0	Unpolluted	

Contamination factor is an effective tool for monitoring pollution over a period of time and evaluating the pollution of the environment single substances [17,21]. It is calculated using the following ratio:

$$CF = \frac{\text{Measured concentration of the metal}}{\text{Background level of the metal}} \quad (2)$$

The geoaccumulation index is based on the geochemical data that makes possible to map the areas according to their pollution degree.  $I_{geo}$  values are calculated using the following equation:

$$I_{geo} = \log_2 \left( \frac{C_n}{1.5 \cdot B_n} \right) \quad (3)$$

where  $C_n$  is the measured concentration of the element and  $B_n$  is the geochemical background concentration for the average continental shale. The 1.5 factor includes possible variations of the background values due to lithogenic effects [18,20–22]. Criteria for these pollution indicators are given in Table 3.

## 2. Materials and methods

### 2.1. Study area

Algeciras Bay (Fig. 1) is an important industrial area located on the south-western Spanish coast (36° 11' 0" N; 5° 26' 45" W) [2,23]. The bay has a 10-km interior and 8-km mouth, covering about 75 km<sup>2</sup>, with a depth of up to 400 m. Large amounts of pollutants are discharged into the bay from major population centres such as the cities of Algeciras, Los Barrios, San Roque, Línea de la Concepción and Gibraltar receiving heavy urban sewage. The bay also receives the water discharge of Guadarranque and Palmones rivers. The intense activity from Algeciras harbour, one of the largest Spanish ports, is another impact factor. There are a significant number of industries around the coast of Algeciras Bay, including stainless steel manufacturing plant, petrochemical and petroleum refineries, paper mills, thermal power plants, ironworks, shipyards, piers and breakwaters [23]. The bay's water has a high turnover because of its proximity to the Strait of Gibraltar, where the Mediterranean Sea and the Atlantic Ocean meet with strong currents. These geographical conditions could disperse the pollutants in the water [2]. Nevertheless, the anthropogenic effect over the sediment quality in this bay is likely to be a serious problem and must be studied.

### 2.2. Sampling and sample pretreatment

Surface sediment samples for the preliminary study were collected from 17 sampling sites of Algeciras Bay in April 2007 (Fig. 1). Sediments for speciation were collected in autumn 2007–2008 and spring 2008–2009 from site 1.2 (Getares beach, a control site with scarce urban influence) and the four pollution hotspots at sites 2.1 (Isla Verde, with urban influence), 6.1 (Palmones, with the Acerinox steel manufacturing plant, urban and river influences), 7.1 (Guadarranque, with river, urban and industrial influences) and 8.1 (Puente Mayorga, with the Cepsa oil refineries and thermal power plants influences) using Ekman–Birdge grab. The samples

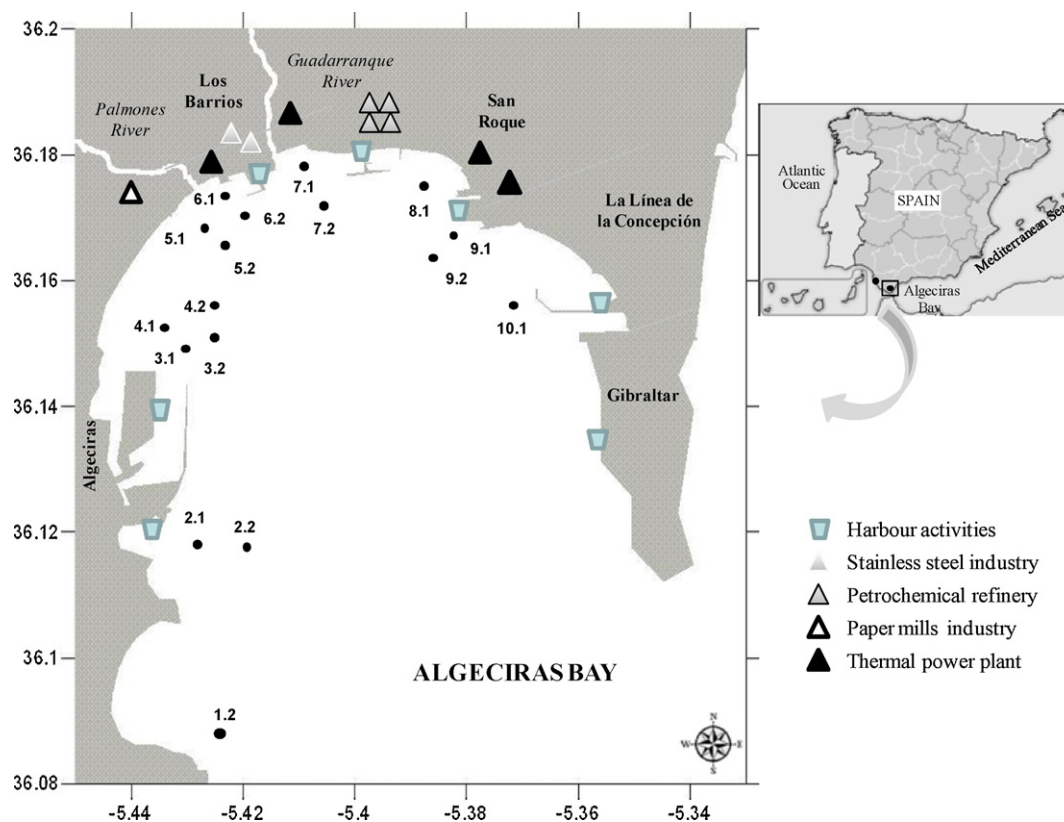


Fig. 1. Map of Algeciras Bay showing the sampling sites and the principal anthropogenic activities of the area.

Table 4

The BCR sequential extraction scheme used for operational speciation of metals.

Step	Sediment fraction	Extractant	Conditions <sup>b</sup>
F1	Water soluble, exchangeable or bound to carbonates	40 mL of 0.11 M CH <sub>3</sub> COOH	Shake 16 h at RT
F2	Reducible	40 mL of 0.5 M NH <sub>2</sub> OH-HCl (pH 1.5)	Shake 16 h at RT
F3	Oxidizable	10 mL of 8.8 M H <sub>2</sub> O <sub>2</sub> (twice) 50 mL of 1 M NH <sub>4</sub> OAc (pH 2)	Shake 1 h at RT and 1 h at 85 °C 16 h at RT
F4	Residual <sup>a</sup>	5 mL 48% HF (twice) 5 mL 65% HNO <sub>3</sub> (twice) 20 mL 3.86 M HCl	Heat until dryness Heat until dryness Heat and stir 1 h

<sup>a</sup> Digestion of the residual material has been included.

<sup>b</sup> RT: room temperature.

were oven-dried at 40 °C, grinded and sieved in order to obtain fine particle-size fractions (<0.063 mm).

### 2.3. Analytical methods

Organic matter content (%OM) was estimated by measuring the loss of weight on ignition at 550 °C. Sediment speciation analyses for the 5 selected sites were performed using BCR sequential extraction procedure [9] according to previously reported [24], using conditions given in Table 4 as a brief summary. Total acid digestions of sediments from all sediments were performed using the same procedure employed with the residual fraction. The extraction of diethylenetriaminepenta-acetic acid (DTPA)-extractable metals from all sediments was performed using 0.005 M DTPA + 0.01 M CaCl<sub>2</sub> + 0.1 M triethanolamine (TEA) [25]. Determination of total metal, DTPA-extractable and BCR fractions were performed using X-Series ICP-MS. Solaar-M Series atomic absorption spectrometer (FAAS) was used only for analysis of total and DTPA-extractable Fe and Mn (Thermo Elemental, UK). In all cases, blank samples were analyzed and taken into account.

The quality of total metal analysis was checked by using the estuarine sediment standard reference material NIST-SRM 1646a,

yielded good accuracy of analysis (80–106%, *n* = 5). Standard sediment reference material BCR-701 was used to check the BCR sequential extraction, giving very good recoveries (Table 5). No reference material was available for DTPA extraction; however, good recoveries ranging from 85% to 105% were obtained from a spiked sample, indicating the accuracy of method for the mobile metal analysis.

All reagents were of Suprapur and Pro analyze quality from Merck (Germany) or Panreac (Spain). Ultrapure water from

Table 5

Recoveries of Standard Sediment Reference Material BCR-701 by applying BCR sequential extraction method (*n* = 4).

Metal	Recovery (%)		
	Fraction 1	Fraction 2	Fraction 3
Cd	101 ± 2	96 ± 6	109 ± 10
Cr	120 ± 8	96 ± 8	88 ± 5
Cu	127 ± 5	72 ± 4	119 ± 10
Ni	104 ± 1	93 ± 9	90 ± 9
Pb	90 ± 8	99 ± 6	111 ± 8
Zn	93 ± 2	94 ± 7	95 ± 7

a Millipore Milli-Q<sup>50</sup> system was used. Samples and solutions handling were performed under a class 100 laminar flow hood cabinet Crusair 9005-FL (Cruma, Spain). Metal standard working solutions were prepared from 1000 mg L<sup>-1</sup> Merck stock solutions. All laboratory glassware was cleaned using a HNO<sub>3</sub> (20%) bath overnight followed by rinsing with ultrapure water.

Data statistical treatments were performed using STATISTICA 7 software package (2004, Statsoft Inc., USA).

### 3. Results and discussions

#### 3.1. Total metals and sediment quality

The distribution patterns of total metal concentrations and %OM in sediments from Algeciras Bay are given in Fig. 2 and compared

with SQGs (ERM: effect range medium; ERL: effect range low; HAL: high alert level; LAL: low alert level). The organic matter values ranged from 7% to 15%, with a mean value of 10%, obtaining the highest percentages in sediment from the sites 5.1; 5.2; 6.1; 7.1 and 7.2. The common values for coastal sediment are from 0.32% to 1.76%. Therefore, the studied sediments showed a high organic matter content that could decrease the metal availability by complexing. The ranges and average metal concentrations are included in Table 6 giving the following rank:

$$[\text{Fe}] > [\text{Mn}] > [\text{Ba}] > [\text{Cr}] > [\text{Zn}] > [\text{V}] > [\text{Ni}] > [\text{Pb}] > [\text{Cu}] \\ \approx [\text{Co}] \approx [\text{As}] > [\text{Cd}]$$

Pollution hotspots can be identified, principally in sampling sites 2.1, 3.2, 6.1, 6.2, 7.1 and 7.2 located around Isla Verde, Palmones and Guadarranque areas, whereas Getares beach (1.2) retained its

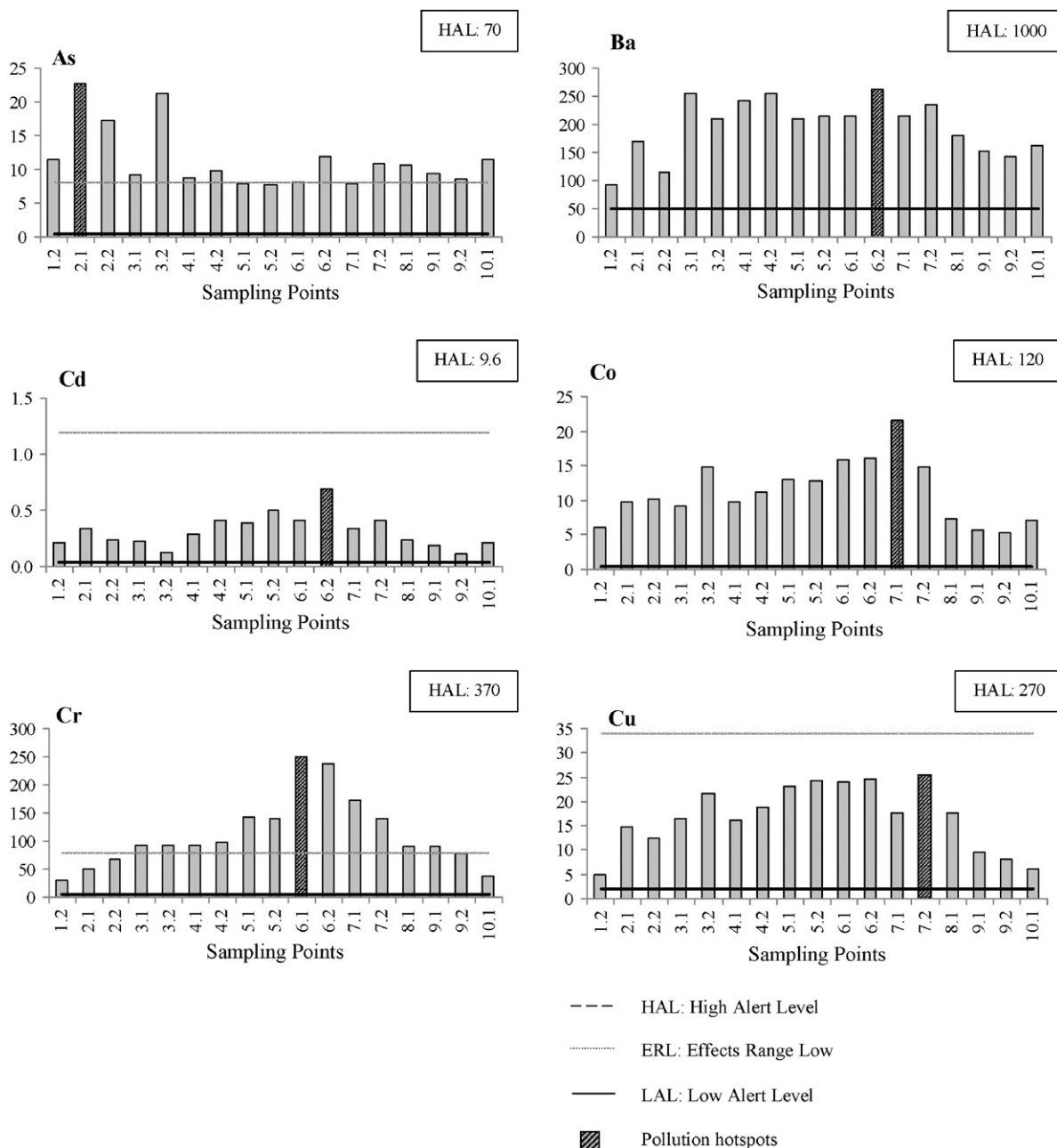


Fig. 2. Distribution patterns of total metal concentrations (mg kg<sup>-1</sup>), %OM and sediment quality guidelines.

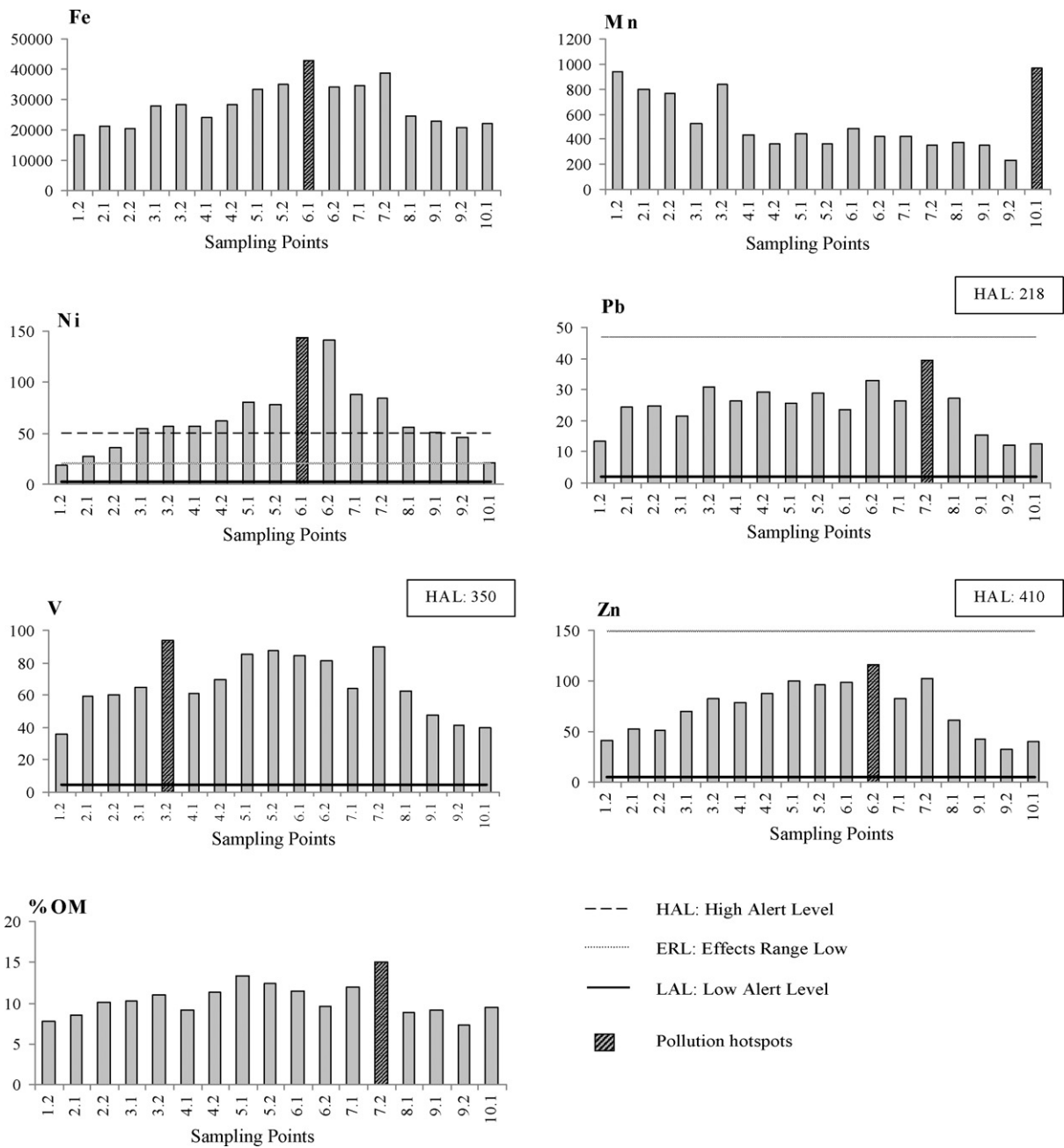


Fig. 2. (Continued).

pristine state being used as a control site. Ni and Cr extremely surpassed the ERM and ERL, respectively, at sampling sites 6.1 and 6.2, located around Acerinox stainless steel industry and the Palmones estuary, implying that biota might be regularly and occasionally exposed to toxic effects. The highest values observed for As at Isla Verde could have resulted from the maritime activities at the Algeciras harbour. The highest values for Ba and Mn may have been due to atmospheric deposition from the industrialized regions and sewage overflow urban-based industrial processes [26].

Further investigations about metal speciation were carried out during two seasonal sampling periods (autumn 2007–2008 and spring 2008–2009) at the identified pollution hotspots and the control site. For these samplings, the evaluation of the total metal concentrations was also carried out (Table 7) producing similar values than those obtained in April 2007.

Fig. 3 shows the pollution indicators for each metal based on the average shale content (Table 2) and using iron content to normalize the data. EF values were principally higher than 1 except for Ba, Fe, Co, Cu, Zn and V, meaning the anthropogenic influence on sediments. The highest values were found for Cr, Ni and Cd at sampling sites 6.1 and 6.2, indicating significant industrial influences for Cr and Ni and pollution from the oil based power generation, ship wastes and anticorrosive paints applied on ships for Cd; and for Pb and As at sites 2.1 and 2.2 with port activities. Mn had high EF values at 1.2 and 10.1 sites related to atmospheric deposition.

The CF values allowed to define the sediments from sites 5, 6 and 7 for Cr, Ni and Cd, from sites 2 and 3 for As and from almost all sites for Pb as moderately polluted ( $1 \leq CF < 3$ ). The rest of metals remained within the low contamination level of the sediment ( $CF < 1$ ).

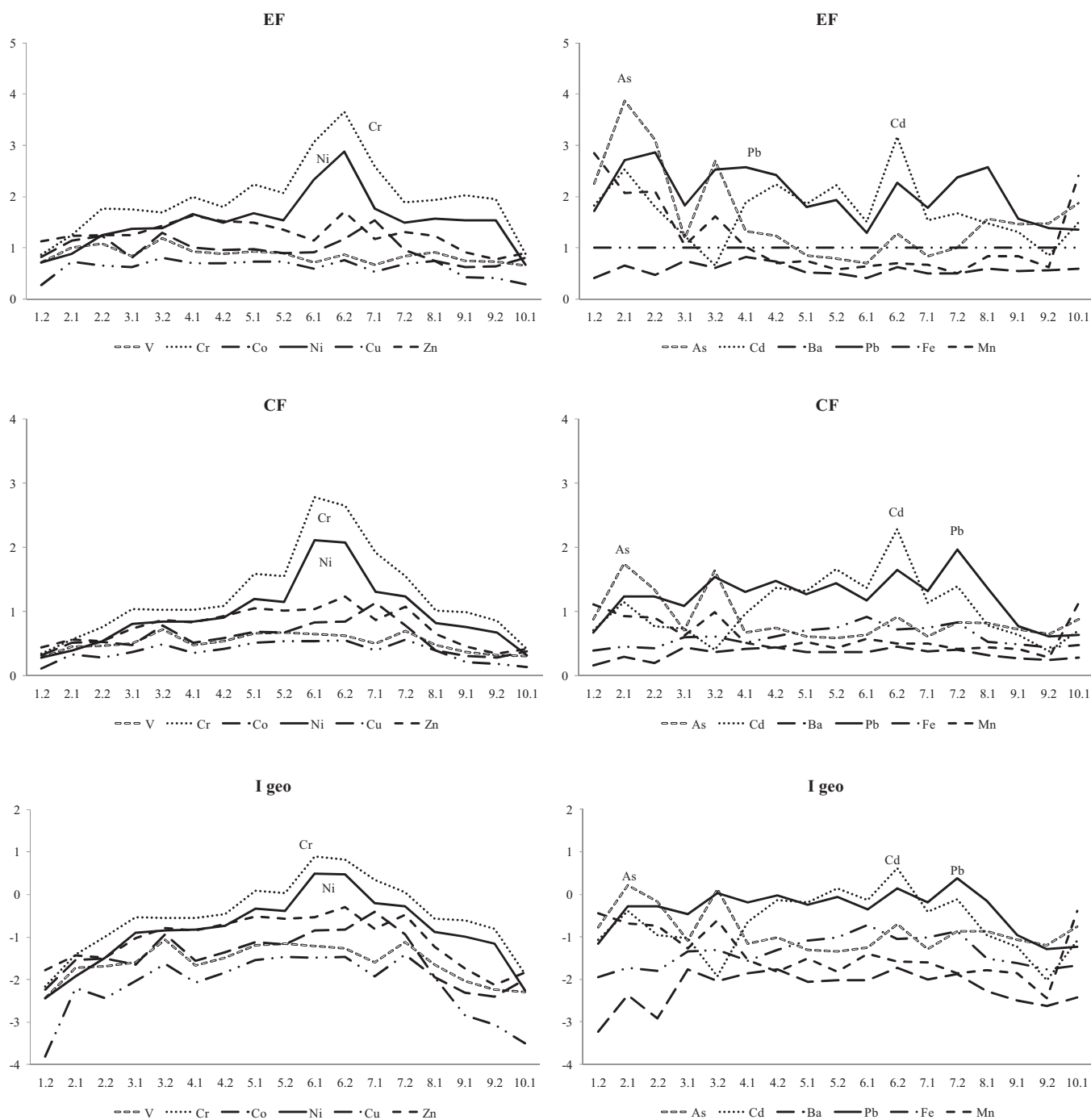


Fig. 3. Enrichment factor (EF), contamination factor (CF) and geoaccumulation index ( $I_{geo}$ ) in sediments from sampling sites of Algeciras Bay collected in April 2007.

The results of  $I_{geo}$  were more restrictive, characterizing the sediments as unpolluted except for Cr (sites 6.1, 6.2, 7.1), Ni (sites 6.1, 6.2), As (site 2.1), Cd (site 6.2) and Pb (sites 6.2 and 7.2) which were “unpolluted to moderately polluted”, with index values ranging from 0 to 1.

These pollution indicators indicated that Algeciras Bay sediments are still in their pristine state with respect to metal pollution except for As, Ni, Cr, Pb and Cd. The strong currents that predominate in the area, together with its deep waters, mean that pollutants are dispersed in a large body of water and their effects are considerably diminished, even with great anthropogenic activity in this area. It is noteworthy that despite this, there is moderate pollution of these five toxic metals.

### 3.2. Potential toxicity of metals in sediment

Table 8 shows the results of 0.005 M DTPA + 0.01 M  $\text{CaCl}_2$  + 0.1 M TEA extraction to evaluate the mobile metal concentration at each sampling site. Significant values were obtained for Cd, Co, Cu, Ni, Pb and Zn following this ranking:  $\text{Cu} > \text{Cd} \approx \text{Pb} > \text{Zn} > \text{Co} > \text{Ni}$ . The mobile metal fractions in site 6.1 were: Cu = 71%, Cd = 66%, Pb = 64%, Zn = 55%, Co = 15% and Ni = 6%, showing their high potential toxicity. V, Cr, As, Fe, Mn and Ba yielded insignificant percentage (<3%). The use of complexing ligands DTPA and TEA and the ion  $\text{Ca}^{2+}$  allows the fast exchange with bivalent cations, especially for  $\text{Cd}^{2+}$  and  $\text{Zn}^{2+}$  when they are mobile [27] and for  $\text{Cu}^{2+}$  even when complexed by organic matter with high stability constant of organic–Cu com-

**Table 6**  
Average total metal concentrations in sediments from 17 sites of Algeciras Bay collected in April 2007.

Metal	Concentrations (mg kg <sup>-1</sup> )			
	Min	Max	Mean	Stand. Dev.
As	8	23	11	5
Ba	93	263	196	50
Cd	0.1	0.7	0.3	0.1
Co	5	22	11	4
Cr	30	251	112	63
Cu	5	25	17	7
Fe	18285	42756	28129	7216
Mn	235	967	534	231
Ni	19	144	65	36
Pb	12	39	24	7
V	36	94	66	18
Zn	33	117	73	26

pounds [28,29]. This Cu mobility found at site 6.1 may be related to the discharge of organic matter entering the bay from Palmones River [23].

In order to predict the potential bioavailability (toxicity) of studied metals, the means of mobile metals extracted in DTPA were compared with LAL SQGs. LAL refers to concentration range limits normally found in uncontaminated sediments. The results revealed that LAL values for Co, Cu, Zn, Cd, Ni and Pb were exceeded at most sampling sites, principally at sampling site 6.1.

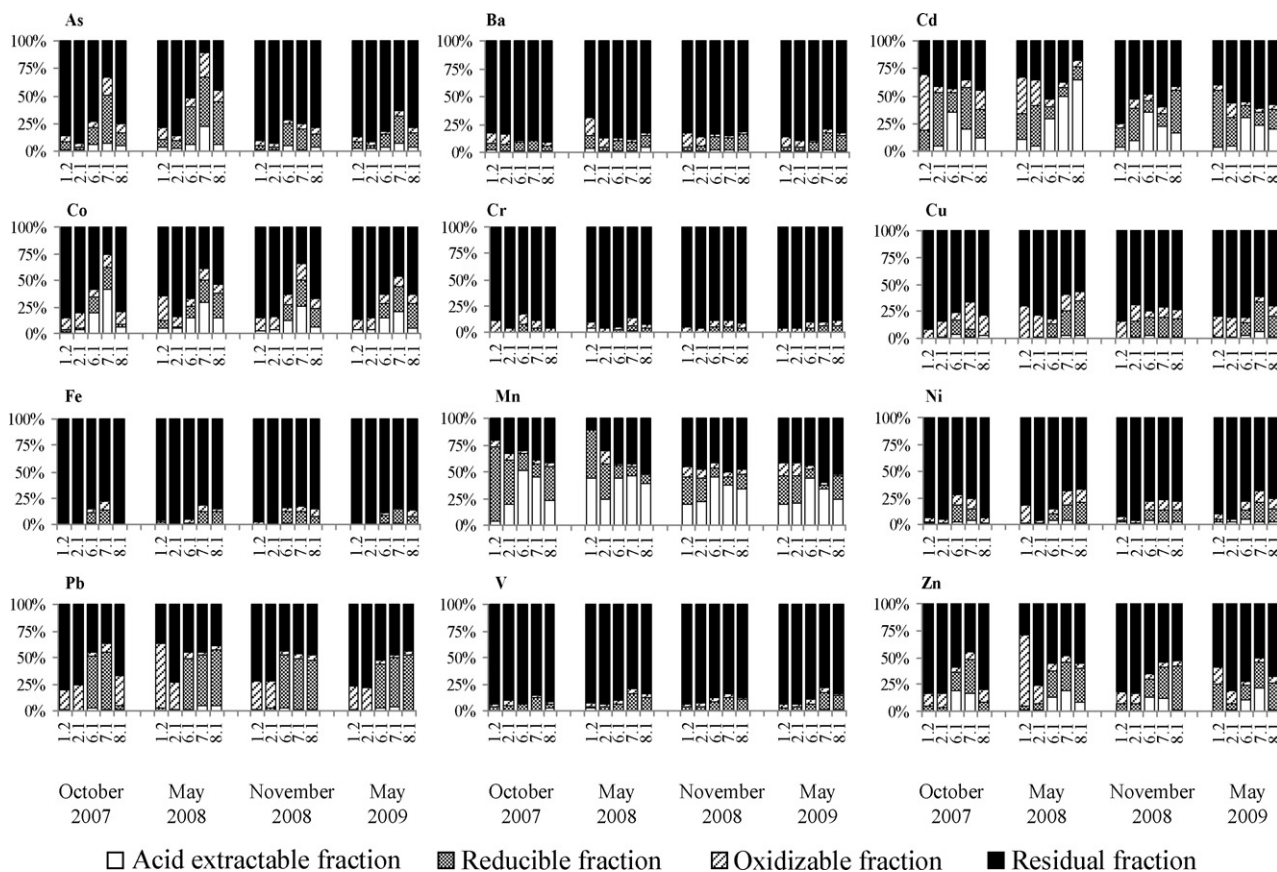
3.3. Sequential extraction of metals

The distribution of metals in sediment (in percentage) following BCR procedure is shown in Fig. 4. It was observed that Cd was the most mobile metal mainly extractable by weak acids (0–64%). The results were also in agreement with the fact that Cd has been reported as being among the easily removed and most labile metal [30,31]. Mn was also found to be highly extractable by weak acids (4–51%). The high percentage of Mn in the acid-soluble and carbonates fraction was probably due to its known close association with carbonates [2,32]. Mn was also recorded to have the highest percentage (8–67%) in the reducible fraction probably due to the presence Mn oxides in this fraction. The results also showed that Pb had a higher proportion in the reducible fraction (0.4–53%), followed by Cd (7–51%), As (3–43%) and Zn (4–41%). The results for Pb were in agreement with reported findings for regions influenced by industrial effluents, like coastal sediments in Spain [33] or marine sediments in Singapore [34], where Fe and Mn oxides were found to be important carriers of the metal in the sediments. It was observed that Zn, Pb, Cd, Cu and As recorded the highest contents (3–65%; 2–61%; 3–50%; 0–29% and 2–23% respectively) in the oxidizable fraction. The results for Pb, Cd and Cu may be due to their strong complexation with sediments being released after degradation of organic matter or oxidation from sulfides to sulfates. The observed behavior was probably due to the affinity of metals for the organic matter present in water, given the high anthropogenic loadings in the study region. Higher metal contents were found in the residual fraction for Fe (77–98%), Cr (83–97%), V (78–94%), Ba (69–91%) and Ni (67–96%). Other metals (Pb, Cu, Ba, As, Co and Zn) also showed significant contents. These fractions were less bioavailable because metals in the residual fraction are strongly bound to silicates. Low contents of residual fraction were obtained for Mn (9–52%) and Cd (17–75%) being more available.

**Table 7**  
Total metal concentrations (Mean values ± SD; mg kg<sup>-1</sup>; n = 4) in sediments for five selected sites.

Date	Site	As	Ba	Cd	Co	Cr <sup>a</sup>	Cu	Fe	Mn	Ni <sup>a</sup>	Pb	V	Zn
Oct 2007	1.2	12.8 ± 2.3	136.3 ± 10.4	0.003 ± 0.000	9.1 ± 0.1	45.5 ± 0.7	6.5 ± 0.3	17663.4 ± 697.1	896.7 ± 63.37	0.8 ± 0.1	12.4 ± 1.1	44.9 ± 0.5	27.5 ± 1.3
	2.1	24.3 ± 0.9	204.9 ± 10.8	0.1 ± 0.1	14.3 ± 0.5	106.4 ± 1.5	12.58 ± 0.03	21668.7 ± 450.6	437.0 ± 11.66	1.0 ± 0.2	22.2 ± 3.6	112.4 ± 17.7	49.9 ± 0.9
	6.1	7.7 ± 0.2	259.7 ± 1.9	0.2 ± 0.2	20.15 ± 0.99	286.8 ± 13.2	24.2 ± 0.8	32081.1 ± 84.7	314.2 ± 9.21	57.8 ± 7.9	30.4 ± 2.2	145. ± 14.8	103.2 ± 0.4
	7.1	7.9 ± 0.1	222.6 ± 3.0	0.067 ± 0.003	30.1 ± 0.9	156.1 ± 6.1	15.5 ± 0.2	22677.6 ± 291.4	224.4 ± 6.5	0.4 ± 3.7	24.4 ± 0.1	87.3 ± 22.4	68.8 ± 1.6
May 2008	8.1	12.62 ± 0.1	170.3 ± 32.5	0.0 ± 0.0	9.8 ± 0.6	102.4 ± 4.2	10.9 ± 0.8	18919.3 ± 489.8	285.2 ± 10.067	1.3 ± 1.3	19.3 ± 5.9	89.6 ± 1.0	34.7 ± 1.8
	1.2	11.7 ± 0.5	332.5 ± 34.4	0.02 ± 0.00	8.1 ± 0.3	44.7 ± 1.3	4.3 ± 0.1	17579.0 ± 217.7	682.3 ± 11.27	0.3 ± 0.4	12.2 ± 4.6	51.0 ± 0.9	31.9 ± 0.9
	2.1	15.8 ± 0.8	221.6 ± 2.7	0.0 ± 0.0	8.7 ± 0.2	85.5 ± 0.2	8.82 ± 0.2	17023.1 ± 149.1	365.0 ± 1.5	40.1 ± 0.4	16.1 ± 2.0	63.8 ± 1.8	38.8 ± 0.4
	6.1	5.0 ± 0.3	226.2 ± 24.0	0.2 ± 0.0	18.5 ± 0.8	394.7 ± 27.5	15.8 ± 1.0	28854.5 ± 76.2	330.8 ± 24.19	8.1 ± 19.1	19.2 ± 5.6	117.3 ± 12.8	69.8 ± 3.9
Nov 2008	7.1	3.5 ± 0.5	241.87 ± 0.01	0.13 ± 0.01	11.2 ± 0.4	169.8 ± 0.7	15.2 ± 0.6	20269.7 ± 811.1	227.6 ± 1.3	41.1 ± 0.1	11.4 ± 0.7	47.6 ± 0.3	42.3 ± 0.6
	8.1	5.7 ± 0.3	189.1 ± 1.0	0.09 ± 0.01	9.0 ± 0.2	176.5 ± 13.8	10.1 ± 1.1	17111.6 ± 189.9	250.4 ± 2.5	45.8 ± 2.8	11.2 ± 0.1	42.1 ± 0.7	33.2 ± 0.1
	1.2	4.5 ± 0.2	186.4 ± 6.2	0.072 ± 0.004	3.6 ± 0.1	31.8 ± 0.1	5.6 ± 0.1	22089.8 ± 1195.6	369.1 ± 21.3	8.2 ± 0.3	9.7 ± 0.1	22.6 ± 0.5	22.9 ± 0.7
	2.1	10.7 ± 1.6	192.5 ± 6.6	0.26 ± 0.04	11.0 ± 0.2	124.2 ± 0.7	24.16 ± 0.02	22492.1 ± 717.2	308.8 ± 7.9	47.2 ± 1.1	23.4 ± 0.9	82.7 ± 7.3	53.4 ± 1.2
May 2009	6.1	4.3 ± 0.4	288.9 ± 10.2	0.3 ± 0.1	17.2 ± 0.5	314.7 ± 1.4	29.2 ± 0.5	37107.9 ± 272.8	355.4 ± 6.9	19.2 ± 2.9	23.0 ± 1.9	116.0 ± 2.0	75.8 ± 3.0
	7.1	6.0 ± 0.0	271.1 ± 3.2	0.20 ± 0.06	24.9 ± 0.0	214.3 ± 35.1	23.2 ± 0.1	31765.2 ± 3224.9	303.5 ± 36.8	6.1 ± 3.4	24.2 ± 0.3	88.7 ± 0.0	74.3 ± 0.8
	8.1	7.5 ± 0.2	269.3 ± 18.6	0.13 ± 0.08	11.8 ± 0.5	132.8 ± 8.4	19.7 ± 1.0	29515.2 ± 131.3	276.3 ± 6.3	6.0 ± 7.5	18.9 ± 1.0	94.5 ± 16.5	48.1 ± 2.0
	1.2	4.4 ± 0.1	176.1 ± 7.3	0.07 ± 0.00	4.0 ± 0.1	39.6 ± 0.1	8.4 ± 0.4	15256.2 ± 1344.3	312.3 ± 15.2	10.4 ± 0.5	7.7 ± 0.1	26.2 ± 0.1	22.3 ± 1.1
May 2009	2.1	6.87 ± 0.01	200.6 ± 7.0	0.3 ± 0.1	5.0 ± 0.2	60.5 ± 2.4	12.14 ± 0.1	14578.1 ± 809.1	294.6 ± 2.2	13.7 ± 0.1	11.1 ± 0.04	30.1 ± 0.6	31.9 ± 0.2
	6.1	5.5 ± 2.5	259.6 ± 5.3	0.256 ± 0.002	12.5 ± 0.2	170.3 ± 8.4	27.8 ± 1.0	36721.3 ± 1684.9	317.0 ± 15.3	7.7 ± 0.9	14.9 ± 1.8	77.8 ± 3.4	71.1 ± 1.8
	7.1	11.5 ± 0.3	355.1 ± 20.8	0.26 ± 0.02	14.1 ± 0.4	348.7 ± 8.9	15.7 ± 0.6	18661.3 ± 348.7	277.7 ± 1.0	25.3 ± 2.0	25.1 ± 0.4	74.5 ± 6.4	50.0 ± 0.9
8.1	16.1 ± 0.4	353.6 ± 32.5	0.28 ± 0.01	13.6 ± 1.3	219.7 ± 18.2	22.8 ± 3.2	25457.8 ± 66.0	282.9 ± 3.1	10.6 ± 1.1	26.5 ± 3.7	100.3 ± 8.5	60.8 ± 4.3	

<sup>a</sup> The underlined concentration highly exceeded the HAL values.



**Fig. 4.** Distribution of heavy metals in sediment from Algeciras Bay obtained by sequential chemical extraction (BCR procedure). Sampling sites: 1.2 (Getares beach); 2.1 (Isla Verde); 6.1 (Palmones); 7.1 (Guadarranque); 8.1 (Puente Mayorga).

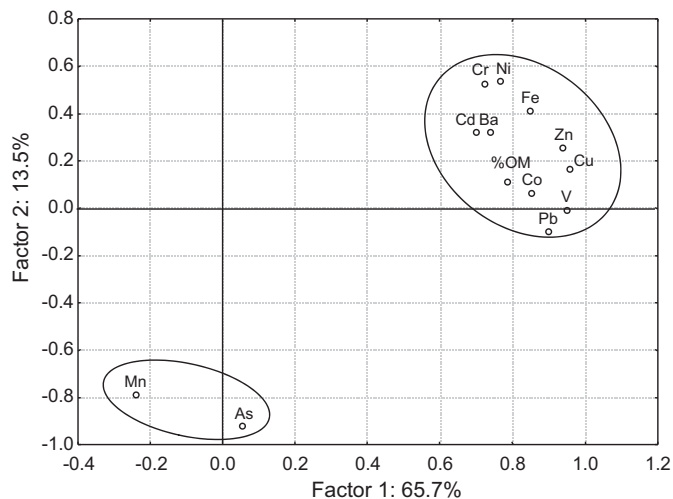
### 3.4. Potential sources of metals

Metal concentration and organic matter values in sediment from the 17 sampling sites were further investigated by applying principal component analysis (PCA) with Varimax rotation to obtain an overview of their behaviors and possible metal sources. Data were auto-scaled (standardized) in order to be treated with equal importance. Two-factor principal components for the loads (chemical variables) were extracted for the 13 variables in sediments represented by 17 cases corresponding to eigenvalues >1 and cumulative variance of 79.2%. Factor 1 accounted for 65.7% of the total variance explained and factor 2 explained 13.5%. The factor loadings plot (Fig. 5) was split into two groups: Group I containing Ba, Cd, Cr, Co, Cu, Fe, Ni, %OM, Pb, V and Zn, while Group II contained As and Mn. These groupings indicated similarities in behavior among these metals and organic matter, influencing the distribution of metals in sediments [35].

The scores (sampling sites) plots of the PCA of the sediment samples (Fig. 6) were used to identify the potential sources of pollution, considering that each factor (with its chemical variable associated) could be related with different sites. The principal group in the plot included the sampling sites from the inner of the bay with greatest influence of the anthropogenic activities; sites 1.2 and 10.1 from the outer of the bay with a low influence of PC1; sites 2.1 and 2.2 related to As and Mn; and sites 9.1 and 9.2 with the opposite behavior. The site 7.1 close to Guadarranque Estuary presented the highest contribution of PC1 and the site 3.2 with Algeciras Port influence showed the influence of PC2.

Hierarchical cluster analysis (CA) was applied on standardized data sets sorted by metals and sampling sites to understand the similarities among them. The dendrograms with single linkage

Euclidean distances are shown in Figs. 7 and 8. Two groups of metals formed clusters: Ni–Cr and Zn–Cu–V clusters with the most significant short linkage distance. The next joining included Fe–(Ni–Cr) and Pb–(Zn–Cu–V). These groups can be associated with stainless steel and petrochemical industrial activities, respectively. As and Mn showed the furthest cluster. Fig. 8 includes the sites clusters with short distances for 5.1–5.2, 4.1–3.1 and 9.1–9.2. The next clusters were formed among these groups and 7.2, 4.2 and 8.1 sites;



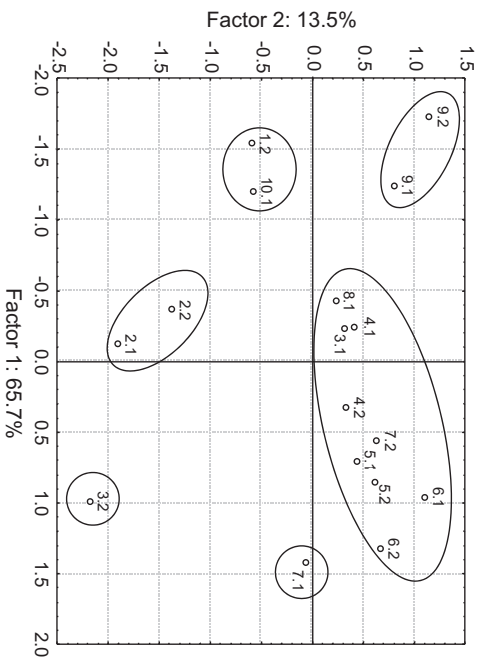
**Fig. 5.** Loadings of variables on the plane defined by the principal components of PCA analysis for total metal concentrations and %OM in sediments from Algeciras Bay.



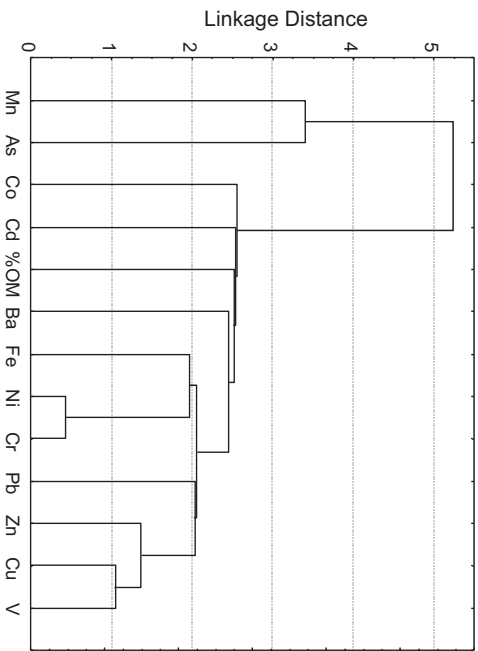
**Table 8**  
Concentrations (Mean values ± SD; mg kg<sup>-1</sup>; n = 4) of metal extracts in 0.005 M DTPA + 0.01 M CaCl<sub>2</sub> + 0.1 M TEA from 17 sites.

Site	As	Ba	Cd <sup>a</sup>	Co <sup>a</sup>	Cr	Cu <sup>a</sup>	Fe	Mn	Ni <sup>a</sup>	Pb <sup>a</sup>	V	Zn <sup>a</sup>
1.2	0.07 ± 0.01	0.21 ± 0.01	0.005 ± 0.002	0.135 ± 0.004	0.02 ± 0.00	0.34 ± 0.00	80.8 ± 0.0	19.7 ± 0.2	0.15 ± 0.02	2.75 ± 0.07	0.13 ± 0.01	2.2 ± 0.1
2.1	0.09 ± 0.02	0.27 ± 0.01	0.012 ± 0.002	0.28 ± 0.01	0.019 ± 0.001	1.6 ± 0.1	98.3 ± 0.0	29.2 ± 0.1	0.28 ± 0.02	4.5 ± 0.2	0.18 ± 0.01	3.0 ± 0.2
2.2	0.09 ± 0.01	0.18 ± 0.02	0.016 ± 0.002	0.20 ± 0.02	0.04 ± 0.00	1.23 ± 0.03	82.2 ± 0.2	25.9 ± 0.2	0.35 ± 0.02	4.3 ± 0.2	0.18 ± 0.01	2.2 ± 0.1
3.1	0.08 ± 0.01	0.15 ± 0.01	0.019 ± 0.002	0.37 ± 0.02	0.02 ± 0.00	3.1 ± 0.1	167.3 ± 0.0	6.2 ± 0.2	0.51 ± 0.03	5.5 ± 0.1	0.25 ± 0.01	6.7 ± 0.2
3.2	0.12 ± 0.01	0.16 ± 0.01	0.019 ± 0.001	0.46 ± 0.02	0.03 ± 0.00	3.3 ± 0.1	149.07 ± 0.03	17.9 ± 0.1	0.58 ± 0.02	6.8 ± 0.2	0.28 ± 0.01	6.5 ± 0.3
4.1	0.08 ± 0.01	0.14 ± 0.01	0.063 ± 0.002	0.45 ± 0.01	0.014 ± 0.003	3.7 ± 0.1	176.5 ± 0.0	5.8 ± 0.1	0.59 ± 0.01	7.2 ± 0.1	0.2 ± 0.0	12.5 ± 0.3
4.2	0.10 ± 0.01	0.18 ± 0.01	0.084 ± 0.002	0.55 ± 0.01	0.033 ± 0.003	4.8 ± 0.2	169.7 ± 0.4	6.3 ± 0.1	0.96 ± 0.02	11.8 ± 0.2	0.47 ± 0.01	18.7 ± 0.6
5.1	0.04 ± 0.01	0.10 ± 0.01	0.068 ± 0.001	0.83 ± 0.02	0.02 ± 0.00	6.0 ± 0.1	248.1 ± 0.7	15.58 ± 0.01	1.53 ± 0.01	6.3 ± 0.1	0.13 ± 0.01	16.8 ± 0.3
5.2	0.06 ± 0.01	0.15 ± 0.01	0.081 ± 0.002	0.90 ± 0.01	0.028 ± 0.001	6.9 ± 0.2	210.9 ± 3.2	16.9 ± 0.2	1.91 ± 0.03	8.6 ± 0.3	0.25 ± 0.01	19.0 ± 0.6
6.1	0.08 ± 0.02	0.28 ± 0.02	0.26 ± 0.01	2.38 ± 0.06	0.06 ± 0.01	17.1 ± 0.4	238.3 ± 0.0	22.8 ± 0.2	7.9 ± 0.1	15.1 ± 0.2	0.2 ± 0.0	54.4 ± 0.9
6.2	0.06 ± 0.01	0.11 ± 0.01	0.096 ± 0.006	0.63 ± 0.02	0.03 ± 0.00	4.6 ± 0.1	182.0 ± 0.8	6.9 ± 0.2	1.54 ± 0.04	10.1 ± 0.2	0.26 ± 0.01	24.7 ± 0.6
7.1	0.04 ± 0.01	0.14 ± 0.01	0.062 ± 0.002	1.59 ± 0.05	0.016 ± 0.001	0.1 ± 0.1	157.9 ± 0.0	6.4 ± 0.2	0.73 ± 0.03	5.0 ± 0.1	0.16 ± 0.01	8.8 ± 0.1
7.2	0.09 ± 0.01	0.16 ± 0.01	0.031 ± 0.004	0.65 ± 0.03	0.026 ± 0.001	5.0 ± 0.2	154.9 ± 0.3	6.9 ± 0.2	0.74 ± 0.03	11.2 ± 0.1	0.34 ± 0.01	9.5 ± 0.4
8.1	0.06 ± 0.01	0.18 ± 0.01	0.014 ± 0.001	0.165 ± 0.002	0.025 ± 0.003	1.65 ± 0.04	118.8 ± 0.0	7.0 ± 0.2	0.28 ± 0.00	4.8 ± 0.1	0.21 ± 0.01	3.03 ± 0.03
9.1	0.03 ± 0.01	0.11 ± 0.01	0.018 ± 0.004	0.11 ± 0.01	0.002 ± 0.002	1.01 ± 0.03	83.19 ± 0.02	3.9 ± 0.3	0.35 ± 0.01	1.39 ± 0.02	0.1 ± 0.0	1.84 ± 0.03
9.2	0.04 ± 0.01	0.27 ± 0.02	0.018 ± 0.001	0.12 ± 0.01	0.07 ± 0.00	1.5 ± 0.1	79.3 ± 0.1	6.7 ± 0.1	0.42 ± 0.03	2.04 ± 0.03	0.19 ± 0.02	2.4 ± 0.1
10.1	0.08 ± 0.01	0.28 ± 0.01	0.005 ± 0.001	0.12 ± 0.01	0.021 ± 0.004	0.30 ± 0.01	59.2 ± 0.1	14.1 ± 0.1	0.06 ± 0.00	3.18 ± 0.05	0.18 ± 0.01	2.2 ± 0.1

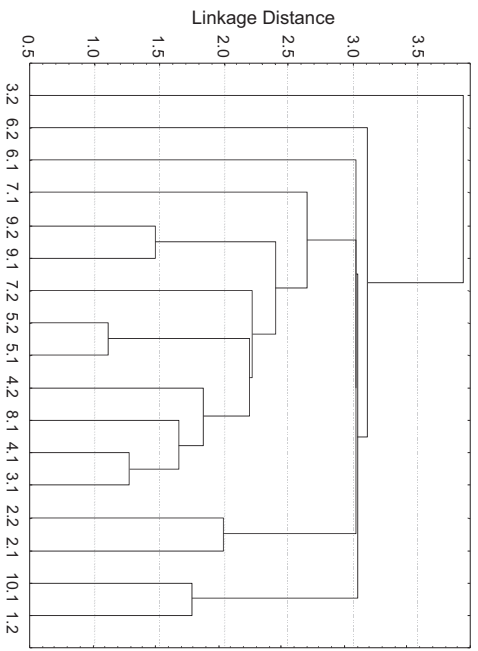
<sup>a</sup> The underlined concentration highly exceeded the LAL values.



**Fig. 6.** Scores of sampling sites on the plane defined by the principal components of PCA analysis for total metal concentrations and %OM in sediments from Algictras Bay.



**Fig. 7.** Dendrogram of hierarchical cluster analysis for total metal concentrations and %OM in sediments from Algictras Bay.



**Fig. 8.** Dendrogram showing clustering of the studied sampling sites in Algictras Bay for total metal concentrations and %OM in sediments.

**Table 9**  
ANOVA test results for the significant values ( $p < 0.05$ ) of total metals among sampling sites for five selected points used for speciation studies.

Variable	Square sum	Mean square	F	p
Co	510	128	5.44	0.0064
Cr	159351	39838	10.07	0.0004
Cu	669	167	6.01	0.0043
Fe	$6 \times 10^8$	$1 \times 10^8$	7.29	0.0018
Mn	$8 \times 10^{-6}$	$2 \times 10^{-6}$	6.06	0.0041
Ni	29892	7473	5.31	0.0072
V	12382	3096	4.78	0.0109
Zn	6476	1619	10.60	0.0003

between 2.1–2.2 (Isla Verde) and 1.2–10.1 (the most external sites). Sites 6.1, 6.2 and 7.1 were distinguished from the rest with high influence of industrial pollution. Site 3.2 was clearly not grouped probably due to its position near the Algeciras Port with a high As content.

Significant differences among metal concentrations obtained from the control site and the pollution hotspots were investigated by applying ANOVA and Tukey tests, studying sampling sites (sites 1.2, 2.1, 6.1, 7.1 and 8.1), sampling campaigns (1st, 2nd, 3rd and 4th), seasons (autumn–spring) and years (2007, 2008 and 2009). The concentrations of As and Mn were not normally distributed (showed by Shapiro–Wilk test) and (logAs) and (1/Mn) conversions were made to transform the data into a normal distribution. According to one-way ANOVA test Co, Cr, Cu, Fe, Mn, Ni, V and Zn showed significant differences ( $p < 0.05$ ) in concentrations among sampling sites (Table 9). Cd showed significant differences among sampling campaigns ( $p = 0.023$ ) and years ( $p = 0.039$ ). Significant seasonal differences were not observed for any metal. The post hoc Tukey test (or multiple comparison test) was applied to determine the found significant differences among groups. Co, Cr, Cu, Fe, Ni, V and Zn showed significant differences between the sampling site 1.2 (Getares beach) and 6.1 (Palmones) and Cr, Ni and Zn did it between 2.1 (Isla Verde) and 6.1 (Palmones). It is outstanding that Fe concentrations of site 6.1 showed differences with all sampling sites. Co, Cr and Zn also showed significant differences between 1.2 and 7.1 (Guadarranque). These metals presented higher concentrations at Palmones and Guadarranque sites than at Getares beach, probably due to their proximity to the steel manufacturing plant, thermal power plants and the petroleum refinery. It is noteworthy that Co at Guadarranque recorded lowest contents in the residual fraction and demonstrated to be mainly extractable by weak acids, being quite bioavailable metal. The rest of the above metals at Palmones and Guadarranque were found mainly in the residual fraction, concluding that they were not bioavailable at those areas. Regarding Mn, it was found that the sampling site 1.2 was significantly different from the sites 7.1 and 8.1 (Puente Mayorga). At Getares beach, higher concentrations of this metal were observed, maybe because of atmospheric deposition from the industrialized regions. Mn presented a high percentage in the acid extractable and reducible fraction at Getares beach, meaning its availability to aquatic organisms although is low toxic. Significant differences were found for Cd between the first sampling and the last one (October 2007 and May 2009), as well as between the years 2007 and 2009, recording the highest contents in May 2009.

Non-parametric analyses (Kruskal–Wallis ANOVA) (data set could not be normalized) were applied to speciation data (in percentage) in order to understand the significant differences among sampling sites for each fraction of speciation scheme (Table 10). It was found that all metals in the acid extractable and reducible fractions showed significant differences among sampling sites except for Ba and V in the first fraction and Ba in both ones. However the oxidizable and residual fractions showed less significant differences, being only significant for Co, Fe, Ni, and Zn. As and V in

**Table 10**  
Kruskal–Wallis ANOVA for the fractions of speciation scheme using sampling point as independent variable.

Metal	p-Value <sup>a,b</sup>			
	Exchangeable fraction	Reducible fraction	Oxidizable fraction	Residual fraction
As	0.0277	0.0039	<u>0.1056</u>	0.0063
Ba	<u>0.1307</u>	<u>0.0607</u>	0.0051	<u>0.2323</u>
Cd	0.0050	0.0388	<u>0.2674</u>	<u>0.8190</u>
Co	0.0019	0.0059	<u>0.0334</u>	<u>0.0044</u>
Cr	<u>0.1120</u>	0.0123	<u>0.1857</u>	<u>0.0566</u>
Cu	0.0226	0.0144	<u>0.0522</u>	<u>0.0553</u>
Fe	0.0020	0.0033	0.0064	0.0024
Mn	0.0134	0.0140	0.0429	<u>0.1401</u>
Ni	0.0089	0.0146	0.0188	0.0078
Pb	0.0053	0.0070	0.0238	<u>0.0745</u>
V	<u>0.0932</u>	0.0094	<u>0.2079</u>	<u>0.0037</u>
Zn	0.0034	0.0126	0.0136	0.0404

<sup>a</sup> The underlined p values showed no significant differences.

<sup>b</sup> Significant differences with  $p < 0.05$ ;  $n = 20$ .

the residual fraction and Ba and Mn in the oxidizable fractions also presented significant differences among sampling sites. From this statistical analysis can be concluded that depending on the influence of pollution hotspot the availability of the metals studied may be different. The significant differences found among metals for more available fractions mean that the pollution reaching the Algeciras Bay is potentially toxic. Although they may transfer to the less available fraction after geochemical distribution, all metals do not, as for As, Cd, Pb and Zn above mentioned in the speciation study.

#### 4. Conclusions

The metallic contamination in sediments from Algeciras Bay was investigated in this study. Fe, Mn, Ba and Cr showed the highest contents. Quality guidelines values for metal concentrations were exceeded for Ni and Cr. Potential toxicity of metals was studied and low alert levels of Co, Cu, Zn, Cd, Ni and Pb were exceeded at most sampling sites, mainly at Palmones. In addition, speciation analysis of sediment was performed providing information of availability of metal. Cd, Zn, Pb and As were found as the most available metals. EF, CF and  $I_{geo}$  showed that Algeciras Bay is still in its pristine state regarding metal pollution except for As, Ni, Cr, Pb and Cd. Principal component, cluster and ANOVA analyses were applied to assess the sources of metals and to study the influence of seasonality and anthropogenic activities on the quality of the sediments. The effectiveness of these analyses for environmental studies was demonstrated; important and reliable information was obtained describing the quality of sediments from Algeciras Bay. It has been shown that pollution from industrial activities, sewage from the city of Algeciras and maritime activities from the Algeciras Port are affecting this ecosystem.

#### Acknowledgements

The research was supported by Andalusia Government (Project PAI-EXCEL-06-FQM-02075) and the grants: Formación Profesorado Universitario (MEC) for M. Díaz, Erasmus Mundus External Window Cooperation for C. Mitto and Formación Personal Investigador (UCA) for M.J. Casanueva.

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