Fractionation of metals and As in sediments from a biosphere reserve (Odiel salt marshes) affected by acidic mine drainage

José Morillo · José Usero · Raquel Rojas

Abstract The Odiel salt marshes (Marismas del Odiel) are an important nature area declared a Biosphere Reserve, but they are greatly affected by pollution from the Odiel River. Surface sediments from this area were analysed using the latest version of the BCR sequential extraction procedure to determine the fractionation of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn among four geochemical phases (acid-soluble, reducible, oxidisable and residual). The total content of each of the metals and As was also determined. The results showed high concentrations of As, Cd, Cu, Pb and Zn, with maximums of 791 mg kg^{-1} of As, 8.5 mg kg^{-1} of Cd, 2,740 mg kg^{-1} of Cu, 1,580 mg kg^{-1} of Pb and 3,920 mg kg^{-1} of Zn. The concentrations of Cr, Mn and Ni were low since there are no sources of pollution by them in the area. A comparison of the metal and As levels with the sediment quality guidelines showed that the pollution is sufficient to produce noxious effects in aquatic organisms in most of the Odiel salt marshes. Based on the chemical distribution of the elements, it was found that Cd and Zn were the most mobile (i.e., elements that can pass easily into the water under changing environmental conditions). However, Cr, Fe, Ni and As were present in the greatest percentages in the residual fraction, which implies that these elements are strongly linked to the sediments.

Keywords Sediment · Metal pollution · Metal fractionation · Sequential extraction

Introduction

The Odiel salt marshes (Marismas del Odiel) are a nature area of 7,158 ha located near the city of Huelva in southwestern Spain. Salt marshes are a valuable type of habitat for ecosystems in terms of nutrient regeneration, primary production, habitats for fish and birds and as shoreline stabilisers (França et al. 2005). The Odiel salt marshes were declared a Biosphere Reserve by UNESCO in 1983 and include a Special Protection Area (SPA) for birds, as designated by the European Union (Luque et al. 1999). They have been included in the European Ecological Network "Natura 2000" since 1994.

However, these marshes have a serious problem. The area is located in the joint estuary of the Odiel and Tinto Rivers, which drain an important mining field located to the north. Spoil and sulphide ores exposed to atmospheric oxygen and moisture, aided by bacteria, can undergo oxidation and hydrolysis reactions

J. Morillo (✉) · J. Usero · R. Rojas
Department of Chemical and Environmental Engineering,
University of Seville,
Camino de los Descubrimientos s/n,
41092 Seville, Spain
e-mail: jmorillo@esi.us.es

producing sulphuric acid and metals (Cabrera et al. 1999) which are carried downstream by the rivers. Furthermore, in the 1960s an industrial estate (the Huelva Industrial Centre) was constructed in the surroundings of the Odiel salt marshes. As a result of all this, both the water and sediment quality of the Odiel River have been seriously affected (Luque et al. 1999; Morillo et al. 2002).

Pollution by heavy metals and As is a serious problem due to their toxicity and their ability to accumulate in the biota. Furthermore one of the most crucial properties of heavy metals, which differentiate them from other toxic pollutants, is that they are not biodegradable in the environment. In aquatic ecosystems, sediments are the main sink for heavy metals (Ridgway and Shimmield 2002) and other pollutants (Pandey et al. 2004), but when environmental conditions change (pH, sediment redox potential, etc.), some of the sediment-bound contaminants can remobilise and be released back into the water, acting as a source of pollutants (Zoumis et al. 2001; Horsfall and Spiff 2002) that can have adverse effects on living organisms (Izquierdo et al. 1997; Li et al. 2001; Patra et al. 2004). The mobility of these elements facilitates their being taken up by benthic invertebrates living in sediments. Benthic invertebrates are an important link in the transfer of substances to higher trophic levels because of their close association with sediments and their ability to accumulate metals (Burgos and Rainbow 2001). Furthermore, they are often a major component in the diet of many fish (Summers 1980).

Information on the total metal and As concentration alone is not sufficient to assess the environmental impact of polluted sediments because they can be present in different chemical forms in sediments (easily exchangeable ions, metal carbonates, oxides, sulphides, organometallic compounds, ions in crystal mineral lattices, etc.), which determine their mobilisation capacity and bioavailability (Weisz et al. 2000; Kuang-Chung et al. 2001). Several methods for determining the different forms of metals in sediments are described in the scientific literature (López-Sánchez et al. 1993; Das et al. 1995). The most widely used methods are based on sequential extraction procedures, whereby several reagents are used consecutively to extract operationally defined phases from the sediment in a sequence. In view of the diversity of existing procedures and the lack of uniformity in the different protocols used by the various authors, the European Union's Standards, Measurements and Testing programme (SMT, formerly BCR) launched a project to harmonise the measurement of the extractable trace metal content in soils and sediments (Sahuquillo et al. 1999; Rauret et al. 2000; Gleyzes et al. 2002). This scheme was then applied to the certification of a sediment reference material (CRM 601), which enabled it to be validated. In our study we used the modified BCR procedure (Rauret et al. 1999) which consists of three successive extractions to associate the metals with one of the different phases that can be present: acid-soluble phase (fraction 1), reducible phase (fraction 2), oxidisable phase (fraction 3).

The purposes of this study of sediments from the Odiel salt marshes are: (a) to determine the pollution levels in sediments in this area and (b) to determine the forms or phases in which heavy metals and As are associated with the sediments, which will tell us about the bioavailability and mobility of these contaminants in the sediments – i.e., their ability to pass into water in the appropriate environmental conditions (pH, sediment redox potential, etc.). In the scientific literature consulted, we found no prior study that looked at the mobility of all these pollutants in the sediments of this important nature area.

Materials and methods

Sampling and analysis

Undisturbed surface sediment samples were collected in March 2006 using an impact grab from 12 sampling sites distributed throughout the Odiel marshes, as shown in Fig. 1. Only grabs that showed no evidence of leakage or surface disturbance were retained; the top 3 cm were then placed in plastic bottles (PVC) and stored at 4°C until arrival at the laboratory.

In the laboratory the samples were kept in darkness at 4°C until processing and analysis. The samples were dried at 45°C in an inert nitrogen atmosphere. The analyses were performed in the <63 μm fraction because metals and As are usually associated with small grains (Dassenakis et al. 2003). This fraction is also easily homogenised for better reproducibility in metal measurements (Förstner and Salomons 1988).
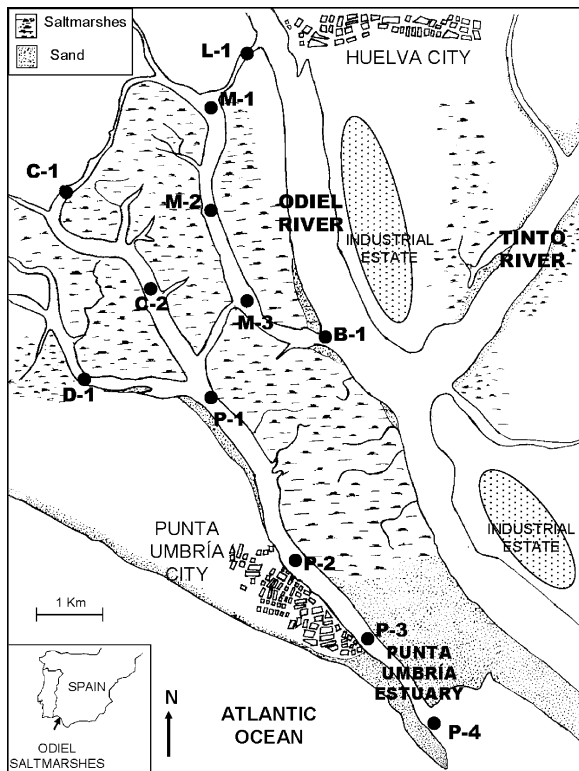
Fig. 1 Map of the study area showing the location of the sampling points in the Odiel salt marshes (Huelva, SW Spain)

The sieving of dried sediment samples was performed with an Electromagnetic sieve shaker (CISA, RP15 model). The <63 μm fraction was homogenized and stored in polyethylene vessels at room temperature in desiccators until digestion or sequential extraction. All the sieving and sequential extraction procedures were performed in a chamber purged with nitrogen to avoid possible changes in species distribution (Batley 1991).

Metal and As contents were determined with aqua regia at high temperature. The method is based on the extraction protocol proposed as the standard method in ISO 11466 (1995). Samples were digested with a mixture of HCl (37%) and HNO_3 (70%), in a ratio of 3:1 (v/v), at room temperature for 16 h and, after, at 130°C for 2 h, under reflux conditions. Each suspension was then filtered, diluted to 100 ml with 0.5 mol l^{-1} HNO_3, and stored at 4°C until analysis.

The chemical distribution of metals and As was determined by means of the sequential extraction scheme proposed by the European Union's Standards, Measurements and Testing program (SM&T, formerly

BCR). A detailed description of the protocol can be found elsewhere (Rauret et al. 1999). This procedure, which has been improved as a result of extensive collaborative research studies (Sahuquillo et al. 1999; Rauret et al. 2000) consists of three successive extractions that allow us to associate the metals with one of the following phases:

Acid-soluble phase (fraction 1) This was obtained by adding 40 ml of acetic acid (0.11 mol l^{-1}) to a 1-g sample in a 100-ml centrifuge tube. After 16 h of shaking, the supernatant was separated from the solid residue by centrifugation. The residue was washed with 20 ml of distilled water, shaken for 15 min and centrifuged for 20 min. This phase is made up of exchangeable metals and others bound to carbonates that are able to pass easily into the water column, for example, when the pH drops. It is the fraction with the most labile bond to the sediment and, therefore, the most dangerous for the environment.

Reducible phase (fraction 2) This phase was removed by adding 40 ml of hydroxylamine hydrochloride (0.5 mol l^{-1}, acidified with HNO_3 0.05 mol l^{-1}) to the residue of the first step and proceeding in a similar fashion to the first step. This phase consists of metals bound to iron and manganese oxides that may be released if the sediment changes from the oxic to the anoxic state, which may be caused, for example, by the activity of microorganisms present in the sediments.

Oxidisable phase (fraction 3) This phase was removed with 10 ml of H_2O_2 (8.8 mol l^{-1}, acidified to pH 2–3 with HNO_3) added to the residue of the second step. After hot digestion for 1 h at 85°C and reduction of the liquid to a small volume, another 10 ml of H_2O_2 were added and the step repeated (1 h of digestion at 85°C and reducing the liquid to a small amount). The cool, moist residue was topped up with 50 ml of ammonium acetate (1.0 mol l^{-1}, acidified to pH 2 with HNO_3) and the procedures described for the previous steps were repeated. This phase shows the amount of metal bound to organic matter and sulphides that can be released under oxidising conditions. Such conditions can occur, for example, if the sediment is resuspended (by dredging, currents, flooding, tides, etc.) and the sediment particles come into contact with oxygen-rich water.

Furthermore, a fourth phase – *residual or inert (fraction 4)* – was determined; this is the difference between the total metal content and the sum of the contents in the three previous phases. The metals found primarily in this fraction are those that are associated with minerals, forming part of their crystalline structure and which, as a result, are unlikely to be released from sediments.

The analysis of metals in the solutions obtained following sample digestion and sequential extraction was carried out by atomic absorption spectrophotometry (AAS) using a double-beam Perkin–Elmer 2380 with deuterium background correction and, in some cases (low concentrations), by graphite furnace atomic absorption spectrometry (GFAAS) using a Perkin–Elmer 4110 ZL with Zeeman background correction. The hydride-generation technique was used to determine As, employing a Perkin–Elmer MSH-10 connected to a Perkin–Elmer 2380 spectrophotometer.

Reagents and quality assurance

All reagents were Merck analytical grade or extra-pure quality. Stock solutions (Merck) of 1,000 mg l^{-1} with certificates of analysis traceable to NIST of the different elements analysed were used to prepare the calibration standards. The water used was purified using a Milli-Q water-purification system (Millipore, Bedford, MA, USA).

All the analyses were performed within the laboratory's updated rigorous quality control system (International Standard Organization ISO/IEC 17025 1999), which guarantees the reliability of all the results. In order to check the accuracy of the analytical procedures for total metal and As determinations it was used the sediment reference material CRM 320 t, and to verify the accuracy of the sequential extraction method, the standard reference material (CRM 701). Replicate analysis of BCR 320 t showed good accuracy, with recovery rates between 89 and 102% (Table 1). The recovery rates for heavy metals and As in the standard reference material CRM 701 were between 85 and 105% (Table 2). In order to determine the precision of the analytical processes, one sample was analysed in quadruplicate. The average values of the relative standard deviation obtained (in general, less than 10%) can be considered satisfactory for environmental analysis.

Table 1 Recovery rates (%) for metals and As in BCR–CRM 320 t

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
Recovery (%)	89	102	95	98	92	91	97	97	101

Results and discussion

Total metal and As content

A comparison of the metal and As concentrations (Table 3) with background values revealed that most of the samples from the Odiel salt marshes were polluted with As, Cd, Cu, Pb and Zn. These results are not surprising since the Odiel River is affected by the mining that has taken place in the river basin for centuries and because the minerals from the mines contain high concentrations of these metals (Morillo et al. 2002) and As (Jemiola-Rzeminska et al. 2007).

The most hazardous metals for the area under study are Ag, As, Cu, Pb and Zn, since their concentrations exceed the ERM values at almost all the sampling points (Table 4). The least problematic elements for the induction of toxic effects are Cd, Cr and Ni, since they do not exceed ERM values in the area studied (Table 4).

Fractionation of metals and As in sediments

Cd and Zn are the most mobile of the elements studied. They show the highest content in fraction 1 (around 50%) and the lowest in the residual fraction (usually not more than 20%) (Fig. 2). The Cd and Zn fractionation is not unusual; high percentages of total Cd and Zn have been found associated with more labile fractions in other studies of polluted sediments in different areas of the world (Guhathakurta and Kaviraj 2000; Bird et al. 2003). The behaviour of Zn and Cd is perhaps not surprising given that the chemistries of Cd and Zn are similar, particularly in their ionic structures, electro-negativities and ionisation energies (Bird et al. 2003).

Table 2 Recovery rates (%) for metals in BCR–CRM 701

	Cd	Cr	Cu	Ni	Pb	Zn
First step	102	98	105	102	85	93
Second step	98	97	102	92	97	96
Third step	96	105	101	88	94	92

Table 3 Metal content (mg kg^{-1} dry weight, mean ± uncertainty[a]) of samples analysed

Samples	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
L-1	271±6.5	2.8±0.6	48±8	1,470±130	57,200±4,100	535±59	21±4	382±71	1,930±90
M-1	436±10	4.2±0.9	98±16	2,610±240	66,900±4,800	626±69	31±5	475±88	3,180±150
M-2	791±19	4.6±0.9	99±18	1,910±180	88,400±6,400	800±88	38±7	906±169	3,250±160
M-3	325±8	2.4±0.5	89±15	1,070±100	79,000±5,700	751±83	35±6	302±56	2,580±120
C-1	480±12	2.7±0.5	43±7	1,490±140	65,900±4,700	770±85	33±6	420±78	3,920±190
C-2	324±8	8.5±1.7	98±16	1,690±160	62,700±4,500	800±88	39±7	725±135	3,310±160
D-1	117±4	0.94±0.2	82±14	909±84	56,900±4,000	731±80	48±8	213±40	1,520±70
B-1	778±19	5.7±1.1	119±20	2,740±250	92,800±6,700	604±66	32±5	1,580±290	3,110±150
P-1	115±3	0.78±0.16	74±12	783±72	47,900±3,400	608±67	29±5	204±38	1,310±60
P-2	210±5.0	1.9±0.4	74±12	609±56	71,400±5,100	735±81	28±5	140±26	3,660±180
P-3	48±2	0.23±0.05	44±7	311±29	32,200±2,300	594±65	20±3	91±17	516±25
P-4	22±1	0.15±0.03	13±2	190±17	26,800±1,900	417±46	21±4	52±10	230±10
Background values	13[c]	0.35[b]	92[b]	54[b]	42,900[b]	850[c]	24[b]	46[b]	143[b]

[a] The expanded uncertainty was estimated by multiplying the relative standard deviation by 2 (Usero et al. 2005).

[b] Regional background values of samples taken from the mouth of the Odiel River, which can be considered a reference, or unpolluted, point (Morillo et al. 2002).

[c] Turekian and Wedepohl 1961.

The least mobile elements are Cr, Fe, Ni and As, since they are present in a high proportion in the residual fraction (over 70% for Cr and around 65% for Fe, Ni and As), and in a low percentage in the acid-soluble fraction (less than 1% for Cr, and under 15% for Fe, Ni and As). In other sediment studies (Jones and Turki 1997; Sahuquillo et al. 2003; Cuong and Obbard 2006) large amounts of Ni and Cr were also found in the residual fraction. Thus, given that these elements have low mobility and were found in small concentrations in the sediments we studied, it is

unlikely that the sediments in this salt marsh are an important source of Ni and Cr in the water. The high accumulation of Fe in the residual fraction is probably due to its being an element fundamentally of natural origin (it is one of the most common elements in the earth's crust). The results for As are in agreement with other sediments studies (Bird et al. 2003; Nikolaidis et al. 2004), in which a large proportion of As is in the residual phase, suggesting that it is strongly bound to, or incorporated within, the lattices of silicates and other resistant minerals. Although the total content of As is high, its partitioning indicates that this metal is a relatively inactive chemical form, and at present it may be considered environmentally insignificant with respect to ecotoxicity and chemical mobilisation in the Odiel salt marsh sediments.

Table 4 ERL[a] and ERM[b] guideline values for metals (mg kg^{-1}, dry weight) and incidence of biological effects (Long et al. 1995)

Metal	ERL[1]	ERM[2]
As	8.2	70
Cd	1.2	9.6
Cr	81	370
Cu	34	270
Pb	46.7	218
Ni	20.9	51.6
Ag	1.0	3.7
Zn	150	410

[a] ERL (effects range-low) indicate concentrations below which adverse effects rarely occur.

[b] ERM (effects range-median) represent concentrations above which effects frequently occur.

Mn mobility is intermediate since it accumulates mainly in the residual phase, but it is also in the most mobile fractions. This Mn distribution is similar to that found by Marin and Giresse (2001) in sediments from the Gulf of Lions in the Mediterranean Sea (France). The high percentage of Mn in the acid-soluble fraction is probably because of the known close association of Mn with carbonates (Dassenakis et al. 2003). This agrees with the results of other sediment studies (Yuang et al. 2004; Caplat et al. 2005) in which a high proportion of Mn was found in the acid-soluble fraction.
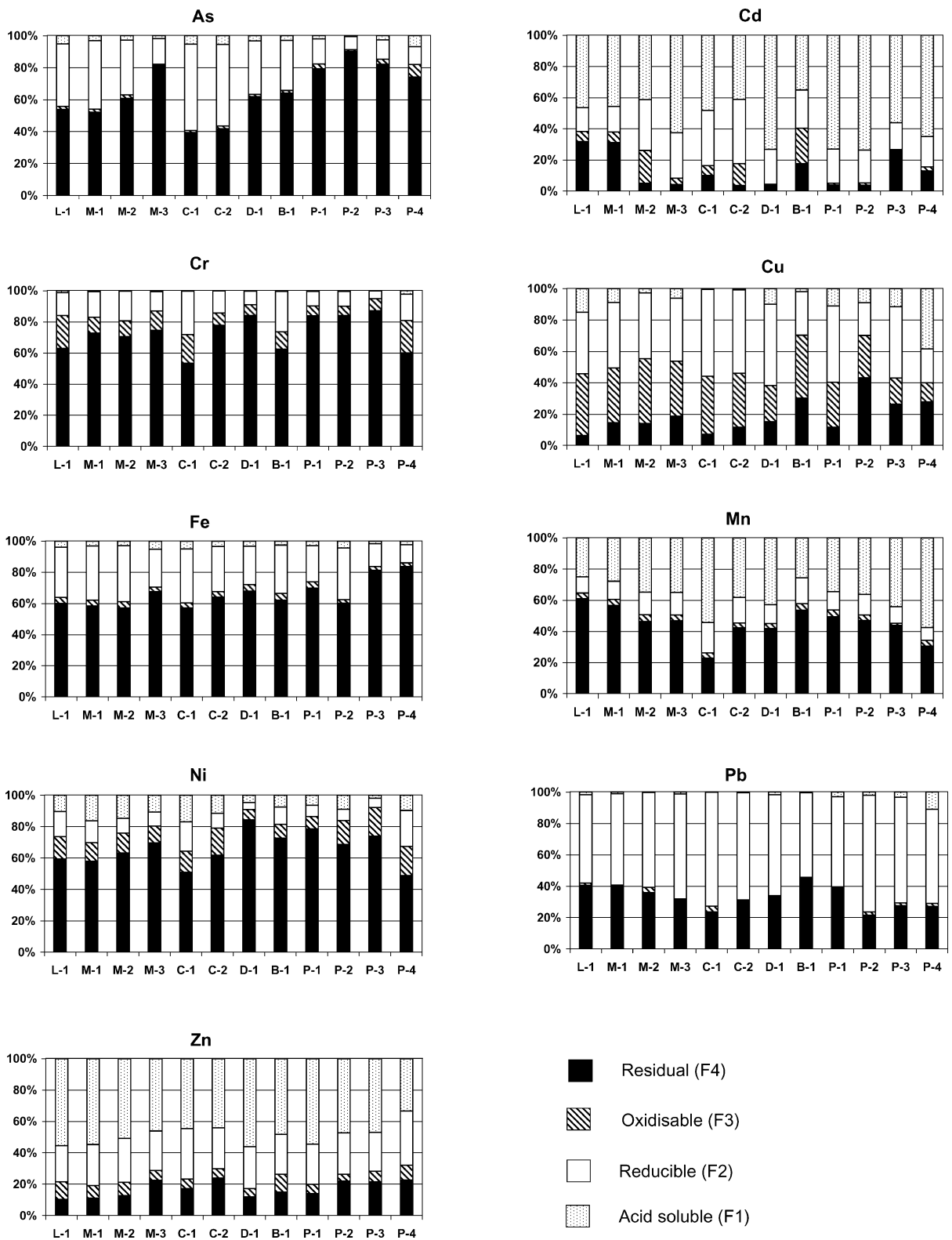
Fig. 2 Metal distribution in the different phases. The percentage of metal removed in each step of the extraction procedure is represented as a *bar chart*

Among the elements studied, Cu shows the highest proportion in the oxidisable fraction (coinciding with organic and sulphur compounds). Chemical discrimination between organic and sulphide phases is difficult (Jones and Turki 1997), but the affinity of Cu for organic particles is well known. This metal can easily form complexes with organic matter due to the high stability constant of organic Cu compounds (Xiangdong et al. 2001; Ramirez et al. 2005). This agrees with the results of different studies (Morillo et al. 2004; Roychoudhury and Starke 2006), which state that a large proportion of Cu in sediments may form part of organic matter. Cu mobility in the salt marsh is less than that of Cd and Zn. The organic fraction released in the oxidisable step cannot be considered very mobile or available because it is thought to be associated with stable high-molecular-weight humic substances that release small amounts of metals slowly (Sing et al. 1998).

Most of the Pb accumulates in the reducible fraction, which showed values of around 60% in most of the samples. This is consistent with results obtained by several authors (Ramos et al. 1999; Guhathakurta and Kavirai 2000; Neff 2002) who found that hydrous Fe and Mn oxides are major scavengers of Pb in sediments. In sediments from this marsh it is mainly the Fe oxides that scavenge Pb in the reducible fraction. A significant correlation (0.741, $p<0.05$) was found between the Fe and Pb contents in the reducible fraction, whereas it was not so for Mn and Pb (reducible Mn showed a low correlation with reducible Pb). These high percentages in the reducible fraction may be a hazard for the aquatic environment because Fe and Mn species can be reduced into the porewater during early diagenesis and microbially mediated redox reactions (Canfield 1989). Dissolution will also release the Pb associated with oxide phases to the porewater, possibly to the overlying water column (Petersen et al. 1995) and to benthic biota (Jones and Turki 1997).

Conclusions

The content of As, Cd, Cu, Pb and Zn in Odiel salt marsh sediments is high. The highest values were found at the area nearest the Odiel River, whereas the lowest levels were found at the points closest to the coast. Toxic effects may commonly occur in aquatic organisms living in the Odiel salt marshes since concentrations of As, Cu, Pb and Zn exceed the ERM values defined by Long et al. (1995), with the exception of the area nearest the coast, where these elements were present at levels under the ERL values.

This study provides valuable information on the potential mobility of heavy metals and As in sediments. The elements studied can be ranked as follows according to the percentage of each in the acid-soluble fraction (the most mobile and bioavailable): Cd>Zn>Mn>Ni>Cu>As>Fe>Pb>Cr.

To sum up, it can be concluded that the most problematic elements are Cd, Pb, Zn and Cu since they are highly mobile and are present in high concentrations and, on the basis of the toxicity guidelines, may commonly produce toxic effects. On the contrary, Cr, Fe, Ni and Mn are the least likely to cause alterations in the ecosystem since their mobility is low, in the case of Cr, or intermediate but their concentrations are low, which is the case of Fe, Ni and Mn.

References

Batley, G. (1991). Collection, preparation, and storage of samples for speciation analysis. In G. Batley (Ed.), *Trace element speciation: Analytical methods and problems* (pp. 1–24). Boca Raton, FL: CRC Press.

Bird, G., Brewer, P., Macklin, M., Balteanu, D., Driga, B., Serban, M., et al. (2003). The solid state partitioning of contaminant metals and As in river channel sediments of the mining affected Tisa drainage basin, northwestern Romania and eastern Hungary. *Applied Geochemistry, 18*, 1583–1595.

Burgos, M., & Rainbow, P. (2001). Availability of cadmium and zinc from sewage sludge to the flounder, *Platichthys flesus*, via a marine food chain. *Marine Environmental Research, 51*, 417–439.

Cabrera, F., Clemente, L., Díaz Barrientos, E., López, R., & Murillo, J. (1999). Heavy metal pollution of soil affected by the Guadiamar toxic flood. *Science of the Total Environment, 242*, 117–129.

Canfield, D. (1989). Reactive iron in marine sediments. *Geochimica et Cosmochimica Acta, 53*, 619–632.

Caplat, C., Texier, H., Barillier, D., & Lelievre, C. (2005). Heavy metals mobility in harbour contaminated sediments: The case of Port-en-Bessin. *Marine Pollution Bulletin, 50*(5), 504–511.

Cuong, D., & Obbard, J. (2006). Metal speciation in coastal marine sediments from Singapore using a modified BCR-

sequential extraction procedure. *Applied Geochemistry, 21* (8), 1335–1346.

Das, A., Chakraborty, R., Cervera, M., & de la Guardia, M. (1995). Metal speciation in solid matrices. *Talanta, 42,* 1007–1030.

Dassenakis, M., Andrianos, H., Depiazi, G., Konstantas, A., Karabela, M., Sakellari, A., et al. (2003). The use of various methods for the study of metal pollution in marine sediments, the case of Euvoikos Gulf, Greece. *Applied Geochemistry, 18,* 781–794.

Förstner, U., & Salomons, W. (1988). *Environmental management of solid waste.* Heidelberg: Springer.

França, S., Vinagre, C., Caçador, I., & Cabral, H. (2005). Heavy metal concentrations in sediment, benthic invertebrates and fish in three salt marsh areas subjected to different pollution loads in the Tagus Estuary (Portugal). *Marine Pollution Bulletin, 50*(9), 998–1003.

Gleyzes, C., Tellier, S., & Astruc, M. (2002). Fractionation studies of trace elements in contaminated soils and sediments: A review of sequential extraction procedures. *TrAC. Trends in Analytical Chemistry, 21*(6–7), 451–467.

Guhathakurta, H., & Kaviraj, A. (2000). Heavy metal concentration in water, sediment, shrimp (*Penaeus monodon*) and mullet (*Liza parnia*) in some brackish water ponds of Sunderban, India. *Marine Pollution Bulletin, 40*(11), 914–920.

Horsfall, M., & Spiff, A. (2002). Distribution and Partitioning of trace metals in sediments of the lower reaches of the New Calabar River, Port Harcourt, Nigeria. *Environmental Monitoring and Assessment, 78,* 309–326.

ISO 11466 (1995). *Soil quality: Extraction of trace elements soluble in aqua regia.* Geneva: International Organization for Standardization.

ISO/IEC 17025 (1999). *General requirements for the competence of testing and calibration laboratories.* Geneva: International Organization for Standardization.

Izquierdo, C., Usero, J., & Gracia, I. (1997). Speciation of heavy metals in sediments from salt marshes on the southern Atlantic coast of Spain. *Marine Pollution Bulletin, 34*(2), 123–128.

Jemiola-Rzeminska, M., Rivera, C., Suwalsky, M., & Strzalka, K. (2007). Interaction of arsenic compounds with model phospholipid membranes. *Thermochimica Acta, 458,* 132–137.

Jones, B., & Turki, A. (1997). Distribution and speciation of heavy metals in superficial sediments from the Tees estuary, North-east England. *Marine Pollution Bulletin, 34*(10), 768–779.

Kuang-Chung, Y., Li-Jyur, T., Shih-Hsiuns, C., & Shien-Tsong, H. (2001). Correlation analyses binding behavior of heavy metals with sediment matrices. *Water Research, 35*(10), 2417–2428.

Li, X., Shen, Z., Wai, O., & Li, Y. (2001). Chemical forms of Pb, Zn and Cu in the sediment profiles of the Pearl River estuary. *Marine Pollution Bulletin, 42*(3), 215–223.

Long, E., Macdonald, D., Smith, S., & Calder, F. (1995). Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. *Environmental Management, 19*(1), 81–97.

López-Sánchez, J., Rubio, R., & Rauret, G. (1993). Comparison of two sequential extraction procedures for trace metal partitioning in sediments. *International Journal of Environmental Analytical Chemistry, 51,* 113–121.

Luque, C., Castellanos, E., Castillo, J., Gonzalez, M., Gonzalez-Vilches, M., & Figueroa, M. (1999). Metals in halophytes of a contaminated estuary (Odiel Salt Marshes, SW Spain). *Marine Pollution Bulletin, 38,* 49–51.

Marin, B., & Giresse, P. (2001). Particulate manganese and iron in recent sediments if the Gulf of Lions continental margin (north-western Mediterranean Sea): Deposition and diagenetic process. *Marine Geology, 172*(1), 147–165.

Morillo, J., Usero, J., & Gracia, I. (2002). Partitioning of metals in sediments from the Odiel River (Spain). *Environment International, 28,* 263–271.

Morillo, J., Usero, J. & Gracia, I. (2004). Heavy metal distribution in marine sediments from the southwest coast of Spain. *Chemosphere, 55,* 431–442.

Neff, J. (2002). *Bioacumulation in marine organism.* Oxford: Elservier.

Nikolaidis, N., Dobbs, G., Chen, J., & Lackovic, J. (2004). Arsenic mobility in contaminated lake sediments. *Environmental Pollution, 129*(3), 479–487.

Pandey, P., Nair, S., Bhui, A., & Pandey, M. (2004). Sediment contamination by arsenic in parts of central–east India and analytical studies on its mobilization. *Current Science, 86* (1), 190–197.

Patra, M., Bhowmik, N., Bandopadhyay, B., & Sharma, A. (2004). Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. *Environmental and Experimental Botany, 52,* 199–223.

Petersen, W., Wallman, K., Li, P., Schroeder, F., & Knauth, H. (1995). Exchange of trace elements at the sediment-water interface during early diagenesis processes. *Marine and Freshwater Research, 46,* 19–26.

Ramirez, M., Massolo, S., Frache, R., & Correa, J. (2005). Metal speciation and environmental impact on sandy beaches due to El Salvador copper mine, Chile. *Marine Pollution Bulletin, 50*(1), 62–72.

Ramos, L., González, M., & Hernández, L. (1999). Sequential extraction of copper, lead, cadmium, and zinc in sediments from Ebro River (Spain): Relationship with levels detected in earthworms. *Bulletin of Environmental Contamination and Toxicology, 62,* 301–308.

Rauret, G., López-Sánchez, J., Sauquillo, A., Barahona, E., Lachica, M., Ure, A., et al. (2000). Application of a modified BCR sequential extraction (three-step) procedure for the determination of extractable trace metal contents in a sewage sludge amended soil reference material (CRM 483), complemented by a three-year stability study of acetic acid and EDTA extractable metal content. *Journal of Environmental Monitoring, 2,* 228–233.

Rauret, G., López-Sánchez, J., Sauquillo, A., Rubio, R., Davidson, C., Ore, A., et al. (1999). Improvement of the BCR three step sequential extraction procedure prior to the certification of new sediment and soil reference materials. *Journal of Environmental Monitoring, 1,* 57–61.

Ridgway, J., & Shimmield, G. (2002). Estuaries as repositories of historical contamination and their impact on shelf seas. *Estuarine Coastal and Shelf Science, 55,* 903–928.

Roychoudhury, A., & Starke, M. (2006). Partitioning and mobility of trace metals in the Bleskbokspruit: Impact assessment of dewatering of mine waters in the East Rand, South Africa. *Applied Geochemistry, 21,* 1044–1063.

Sahuquillo, A., López-Sánchez, J., Rubio, R., Rauret, G., Thomas, R., Davidson, C., et al. (1999). Use of a certified reference material for extractable trace metals to assess sources of uncertainty in the BCR three-stage sequential extraction procedure. *Analytica Chimica Acta, 382*, 317–327.

Sahuquillo, A., Rigol, A. & Rauret, G. (2003). Overview of the use of leaching/extraction test for risk assessment of trace metals in contaminated soils and sediments. *TrAC. Trends in Analytical Chemistry, 2*(3), 152–159.

Sing, S., Tack, F., & Verloo, M. (1998). Heavy metals fractionations and extractability in dredged sediment derived surface soils. *Water, Air and Soil Pollution, 102*, 313–328.

Summers, R. (1980). The diet and feeding behaviour of the flounder *Platichthys flesus* (L.) in the Ythan Estuary, Aberdeenshire, Scotland. *Estuarine, Coastal and Shelf Science, 2*, 217–232.

Turekian, K., & Wedepohl, K. (1961). Distribution of the elements in some major units of Earth's crust. *Geological Society of America Bulletin, 72*, 175–192.

Usero, J., Morillo, J., & Gracia, I. (2005). Heavy metal concentrations in molluscs from the Atlantic coast of southern Spain. *Chemosphere, 59*, 1175–1181.

Weisz, M., Polyák, K., & Hlavay, J. (2000). Fractionation of elements in sediment samples collected in rivers and harbors at Lake Balaton and its catchment area. *Microchemical Journal, 67*, 207–217.

Xiangdong, L., Zhenguo, S., Onyx, W., & Yok-Sheung, L. (2001). Chemical forms of Pb, Zn and Cu in the sediments profiles of the Pearl River Estuary. *Marine Pollution Bulletin, 42*(3), 215–223.

Yuang, C., Shi, J., He, B., Liu, J., Liang, L., & Jiang, G. (2004). Speciation of heavy metals in marine sediments from the East China Sea by ICP-MS with sequential extraction. *Environment International, 30*(6), 769–783.

Zoumis, T., Schmidt, A., Grigorova, L., & Calmano, W. (2001). Contaminants in sediments: Remobilisation and demobilisation. *The Science of the Total Environment, 266*, 195–202.