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Acid mine drainage pollution in the Tinto and Odiel rivers (Iberian Pyrite Belt, SW Spain) and bioavailability of the transported metals to the Huelva Estuary

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Abstract

The Tinto and Odiel rivers are seriously affected by acid mine drainage (AMD) from the long-term mining activities in Iberian Pyrite Belt (IPB). As a consequence, the Huelva estuary is heavily contaminated by metals and metalloids. This study presents an estimation of the seasonal variation, and the dissolved contaminant load transported by both rivers from February 2002 to September 2004. Besides, toxicity and bioaccumulation tests with the sediments of the estuary have been conducted in order to measure the mobility of the toxic metals. Results show that the Tinto and Odiel rivers transport enormous quantities of dissolved metals to the estuary: 7900 t yr^{-1} of Iron (Fe), 5800 t yr^{-1} Aluminium (Al), 3500 t yr^{-1} Zinc (Zn), 1700 t yr^{-1} Copper (Cu), 1600 t yr^{-1} Manganese (Mn) and minor quantities of other metals and metalloids. These values represent 37% of the global gross flux of dissolved Zn transported by rivers in to the ocean, and 15% of the global gross flux of dissolved Cu. These metals and metalloids usually sink in the estuarine sediments due to pH and salinity changes. The increase of salinity in the estuary favours the adsorption and trapping of metals. For this reason, the mobility and bioavailability of metals such as Zn, Cd and Cu is higher in sediments located in the area of fresh water influence that in sediments located in the marine influenced area of the estuary, showing a higher percentage of fractionation and bioaccumulation of these metals in the station influenced by the fresh water environment.

Keywords: Tinto and Odiel rivers; Acid mine drainage; Water and sediment pollution; Huelva estuary; Iberian Pyrite Belt

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1. Introduction

Acid Mine Drainage (AMD) is one of the biggest environmental problems caused by sulfide deposits mining (Akcil and Koldas, 2006). AMD can be responsible for the pollution and degradation of groundwater, streams, rivers, and complete river basins, as is the case of the Tinto and Odiel river basins in Huelva (SW Spain). The Iberian Pyrite Belt (IPB) is one of the most famous sulfide mining regions in the world; it contains original reserves in the order of 1700 Mt (Sáez et al., 1999). Mining activity in the IPB dates back to prehistoric times (Nocete et al., 2005), and though today there is no active mining, the pollution continues to generate. This is due to the oxidation of mining wastes such as those generated in the mines of Tharsis, Riotinto, Cueva de la Mora, San Miguel, Concepción, San Telmo, etc. (Fig. 1).

A series of chemical and biochemical reactions take place when pyrite and other sulfide minerals associated are exposed to water and oxygen. These reactions can be generalized by the following equations (Singer and Stumm, 1970):

Oxidation of pyrite by oxygen in the presence of water:

$$FeS_{2(s)} + 7/2O_{2(aq)} + H_2O \rightarrow Fe^{2+} + 2SO_4^{=} + 2H^{+} \qquad (1)$$

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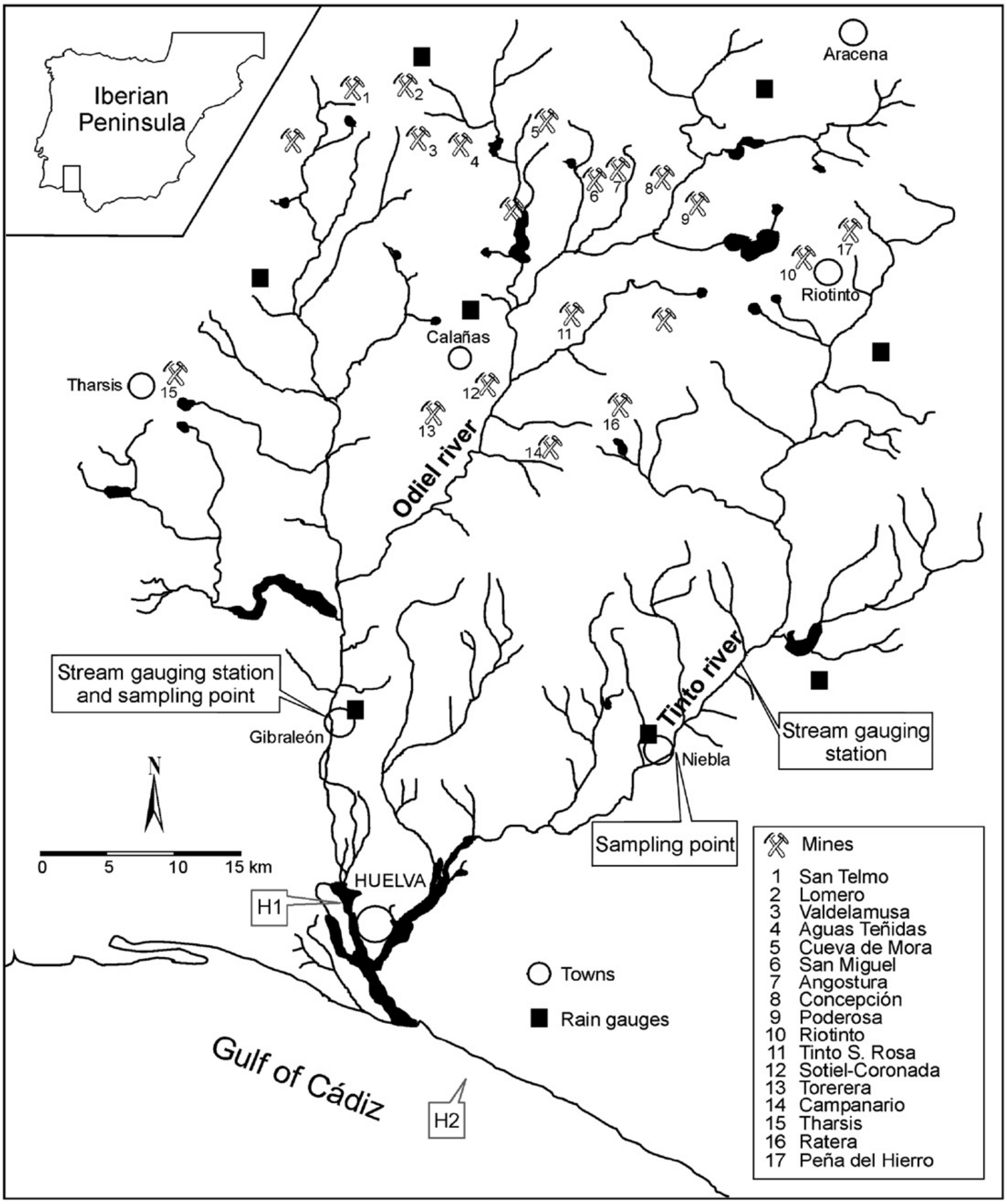

Fig. 1. Sketch map of the Tinto and Odiel rivers, showing the location of the sampling points, stream gauging stations, rain gauges, and main mine localities.

Oxidation of pyrite by ferric iron:

$$FeS_{2(s)} + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{=} + 16H^{+} \qquad (2)$$

Oxidation of ferrous iron by oxygen:

$$Fe^{2+} + 1/4O_{2(aq)} + H^{+} \rightarrow Fe^{3+} + 1/2H_2O \qquad (3)$$

Precipitation of ferric iron:

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_{3(s)} + 3H^{+} \qquad (4)$$

The metal sulfides oxidation is accelerated by the presence of oxidizing bacteria (Gónzalez-Toril et al., 2003). Eq. (3) determines the rate of the overall acidification process, and bacteria can accelerate the overall process six fold by catalyzing reactions 1 and 3 above (Singer and Stumm, 1970). Thus, the oxidation of mine tailings by biologically-catalyzed processes is quite rapid and leads to an overall decrease in pH. With decreasing pH, the mobility of trace elements tends to increase. This produces a mine water discharge characterized by elevated acidity and high concentration of sulfates and metals such as Fe, Cu, Zn, Co, Cr, Mn, Pb, Cd, etc.

The Tinto and Odiel rivers flow into a coastal wetland known as the Ría of Huelva estuary, which forms part of an important Natural Reserve. These two rivers release a significant contaminant load to the estuarine area due to the high concentration of dissolved metals and sulfates that they transport. Studies at the Odiel River next to the estuary show concentration averages of 1200 mg/L of sulfates, 23.5 mg/L of Fe, 7.6 mg/L of Cu, etc., (Olías et al., 2004), and the calculated mean contaminant load transported by the Odiel River to the Huelva estuary and Gulf of Cádiz is 820.4 t/day of sulfate and 45 t/day of metals (Fe+Zn+Mn+Cu+Pb+Cd) (Sarmiento et al., 2004). Therefore, the Huelva estuary is heavily contaminated by metals and metalloids (Nelson and Lamothe, 1993; Ruiz, 2001; Grande et al., 2003). During estuarine mixing, metals are removed from solution in relation to pH and salinity increase (Elbaz-Poulichet et al., 2001a; Braungardt et al., 2003; Achterberg et al., 2003). Nevertheless, the inputs from the Ría de Huelva produce a plume of contaminants in the Gulf of Cadiz which even enters into the Mediterranean Sea (Van Geen et al., 1997; Elbaz-Poulichet et al., 2001b).

The high mining-related contamination existing in the area for years has generated numerous publications dealing with contaminants within the Tinto and Odiel estuaries (Grande et al., 1999; Elbaz-Poulichet et al., 2001a; Borrego et al., 2002; Sainz et al., 2002) but none of these studies have connected the water and sediment contamination with the toxicity for the biota.

Metals and metalloids reaching the estuary usually sink in the sediments of these areas, which act as both a sink and a source of metal in the estuary. Recent studies have shown that pH and especially salinity plays an important role in the bioavailability of these metals to different organisms, including toxicity (Riba et al., 2003, 2004; Baldó et al., 2005). These studies have pointed out the influence of the metal contamination in the toxicity of metals in sediments.

The main objectives of this work are to provide a reassessment of the dissolved contaminant load discharged by the Tinto and Odiel rivers to the Huelva estuary, and to increase our understanding of the processes which can affect the biota from these estuarine ecosystems. For this purpose, toxicity and bioaccumulation tests are conducted under laboratory controlled conditions exposing organisms to field-collected samples. In this study, a general assessment of the adverse effects measured in these tests in samples collected in the area of the Ría of Huelva is described. Furthermore, the effects measured are related to the different inputs from the contaminated waters flowing from the areas affected by the mining activities in the Iberian Pyrite Belt using as a case study the Ría of Huelva.

2. Experimental

2.1. Sampling and analytical determination of water samples in the rivers

In February 2002, samplings in both rivers were started, with approximately a weekly periodicity. Sampling points are located before the rivers enter in the estuary (Fig. 1). In this study, results obtained until September 2004 are discussed.

The main physico-chemical parameters were measured *in situ*. Temperature, pH and electrical conductivity were measured using a portable MX 300 meter (Mettler Toledo). The redox potential was also measured in the field using Hanna meter with Pt and Ag/AgCl electrodes (Crison). Redox potential and pH were properly calibrated on site against supplied calibration standards Hanna standard solutions (pH 4.01 and pH 7.01) for pH and Hanna standard solution (240 mV and 470 mV) for Eh.

Water samples were filtered immediately after collection through 0.45 μm Millipore filters on Sartorius polycarbonate filter holders and were acidified in the field to pH <2 with HNO_3 (2%) suprapur and stored at 4 °C in polyethylene bottles until analysis.

Analyses were carried out in the Central Research Services of the University of Huelva. Dissolved concentrations of Al, As, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Li, Pb, S, Si, Sr and Zn were determined by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES Yobin-Ybon Ultima2) using a protocol especially designed for AMD samples (Tyler et al., 2004). Certified standards from SCP Science ICP Standard Solutions were employed for the preparation of multielemental calibration standards. Certified Reference Material SRM-1640 NIST (fresh-water-type) and inter-laboratory standard IRMM-N3 (wastewater test material, European Commission Institute for Reference Materials and Measurements), were also used to check the precision and accuracy of the measurements. Multielemental Reference Standards and blanks were employed at the beginning and at the end of each sequence. Detection limits were calculated by average, and the standard deviations from the measurement of ten blanks. Detection limits for cations were less than 150 μg/L for Zn, less than 60 μg/L for Al and K, and less than 20 μg/L for Na and Ca. For the rest of elements were less than 3 μg/L.

Flow river data were obtained from two stream-gauging stations belonging to the Hydrographical Confederation of the Guadiana River. The Odiel gauging station is placed in the sampling point, whereas the Tinto gauging station is located 9 km upstream from the sampling point. These stations have a poor operation and report frequent periods with no data.

2.2. Sampling and analytical determination for sediments samples in the estuary

Two different stations located in the Huelva area H1 and H2 (Fig. 1) were selected for chemical and geochemical metal characterization. The sediments from station H1 were located in the fresh water area of influence in the estuary with salinity ranges in the overlaying water ranging from 10 to 25, whereas the station H2 was located in the marine influenced area of the estuary and the values of salinity ranged between 20 and 33 in this area.

Chemical analyses in sediments were carried out to determine the geochemical fractionation of the six heavy metals (Fe, Mn, Zn, Cd, Pb and Cu) typically associated with the metal contamination in the area of Huelva (Riba et al., 2003, 2004). This set of chemical analysis also implies a total digestion of the sample using those methods outlined by Loring and Rantala (1992) and to determine the quality assurance of the analysis and the sequential extraction conducted on the samples. Replicates from the selected sites were homogenized to perform the sequential extraction schema and to establish the heavy metal geochemical associations. The homogenized sediment samples were dried at 60 °C prior to chemical analysis. Dried sediments were gently homogenized. The speciation of the metals in the homogenized sediments was determined following the method proposed by Tessier et al. (1979) modifying the different extractions as previously described in studies in the area by Riba et al. (2002a,b,c). Also, and separately for the same station, the homogenized sediments were totally digested such as described by Loring and Rantala (1992) to calculate the percent of recovery for each metal and sample (98±17%). Heavy metals bound to the first fractions (F1, F2, F3 and F4) are considered as much more susceptible to remobilization than metals which reside in the lattices of sediment minerals (F5) (Luoma and Davis, 1983; Förstner et al., 1989). Metals associated with the lithogenic fraction (residual, F5) are considered as originating from natural sources (Coetzee, 1993).

The concentrations of the heavy metals Fe and Mn in the extracts were determined with a Perkin Elmer 2100 Flame Atomic Absorption Spectrophotometer. The other trace metals were measured by graphite furnace atomic absorption spectrophotometry (Perkin Elmer, 4100 ZL). Results are expressed as mg kg^{-1} dry sediment. The analytical procedures were checked using reference material (MESS-1 NRC and CRM 277 BCR) and allow agreement with the certified values higher than 90%.

2.3. Bioaccumulation of metals in clams exposed to sediments from the Huelva estuary

Sediments were collected with a 0.025 m^2 Van Veen grab and transferred to the cooler. When sufficient sediment had been collected from the sediment station located in the Huelva estuary, the cooler was transported to the laboratory. The content of the cooler was homogenized with a Teflon spoon until no colour or textural differences could be detected. The sediments were subsampled for chemical quantification (1.5 L aliquots). Then the sediment samples were kept in the cooler at 4 °C in the dark until they were used for sediment toxicity assays, but in no case for longer than 2 weeks.

The clams (*Ruditapes philippinarum*; shell length about 1 cm, average weight about 0.35 g) were obtained from an aquaculture farm (Amalthea, S.L., Cádiz, Spain) and kept in our laboratory for 1 month before being acclimated to the three salinity values selected for the assay (10, 20 and 35). During this period, clams were fed on a mixture of micro algae (*Tetraselmis chuii*, *Isocrhysis galbana*, *Chaetoceros gracilis*).

Different values of salinity (10, 20 and 35) were spiked in overlying water prior to the exposure of the organisms to the sediment samples. The salinity of this clean overlying water, used in the toxicity assays and in the controls, was adjusted by diluting natural sea water (S=36) with distilled water (Milli-Ro). Once the salinity values selected had been separately fixed, this sea water was used during the acclimation period of the organisms prior to performing the assays. During this period the animals were maintained in tanks of about 20 L capacity, fed on a mixture of micro algae as described above, and the water was continuously aerated and replaced (80% v/v) every 3 days with fresh sea water of salinity values adjusted to those required. The pH, salinity, temperature (20 °C) and the concentration of dissolved oxygen (>5 mg L^{-1}, 60% saturation) were measured and controlled (salinity) every day.

After the acclimation period the bioassays were performed in replicate using different values of salinity. The assay was performed in whole sediment (2 L per assay) using a water to sediment ratio of 1:4 v/v, at constant temperature (20 °C), as reported by DelValls et al. (2002) using vessels of 15 L capacity. Briefly, 40 organisms previously acclimated to each particular set of salinity values were added to each vessel and exposed for

Table 1
Results obtained in the Tinto and Odiel rivers from the weekly sampling (February 2002 to September 2004)

	Tinto River				Odiel River			
	Mean	Minimum	Maximum	S.D.	Mean	Minimum	Maximum	S.D.
pH	2.89	2.22	5.01	0.60	3.76	2.95	5.05	0.50
EC (mS/cm)	2.26	0.43	8.22	1.20	1.00	0.23	3.88	0.50
SO_4 (mg/L)	1221	150	5547	894	643	110	2379	395
Ca (mg/L)	73.9	11.1	225.5	50.9	45.7	11.9	161.0	26.5
Mg (mg/L)	64.1	8.9	363	47.4	70.5	10.1	224.1	45.2
Na (mg/L)	38.2	6.7	97.2	23.3	17.3	7.9	32.7	5.7
K (mg/L)	3.6	1.0	23.6	2.7	2.4	0.2	37.3	3.7
SiO_2 (mg/L)	19.3	2.8	111.5	22.8	20.4	10.4	83.6	18.7
Al (mg/L)	66.5	0.03	434.4	60.4	32.8	0.58	175.8	26.1
Fe (mg/L)	123	0.07	2804	307	4.9	0.31	23.5	4.9
Cu (mg/L)	15.7	0.2	84.3	11.9	5.4	0.5	17.1	3.2
Mn (mg/L)	6.8	0.7	39.2	5.4	8.1	0.9	32.1	6.1
Zn (mg/L)	24.1	2.2	152.3	22.2	11.5	1.3	36.4	7.1
As (μg/L)	147	<3	2290	416	4	<3	22	5
Ba (μg/L)	15	5	57	9	21	<1	42	9
Cd (μg/L)	107	11	532	82	52	5	176	37
Co (μg/L)	476	52	3754	456	269	33	938	178
Cr (μg/L)	11	<2	86	13	5	<2	16	4
Li (μg/L)	113	<1	403	83	58	<1	217	37
Ni (μg/L)	135	16	742	105	145	19	500	113
Pb (μg/L)	121	<7	698	103	45	<7	267	42
Sr (μg/L)	257	60	673	149	114	30	237	47

S.D.: standard deviation.

10 days. Clean sediment was used as negative control (C). Water replacement (80%) was performed on day 5 of the experiment.

The sediment bioaccumulation test was used to measure tissue residues in clams in the selected station at Huelva area. Organisms from the tests were analyzed for chemical concentration in their tissues at the end of the experimental exposure period (10 days). Organisms were depurated for 48 h before processing to avoid any sediment contamination. Then, organisms were dissected and damped out. Soft body samples were divided, in general, into three pools of 5–10 specimens and then lyophilized in a VIR-TIS lyophilizer. Lyophilized samples were crushed and homogenized to a fine powder in an agate bowl with a Planetary Mono mill (Pulverisette 6, Fritsch). Samples were digested according to the procedure of Amiard et al. (1987). Heavy metal concentrations (Cu and Zn) were analyzed by FAAS (Flame Atomic Absorption Spectrophotometry, using a Perkin Elmer, mod. 3110) and heavy metals Cd and Pb by GFAAS (Graphite Furnace Atomic Absorption Spectrophotometry, using a Perkin Elmer, mod 4100 ZL). The results are expressed as μg g^{-1} dry or wet weight. The analytical procedures were checked using reference material (TORT1 of NRC Canada) and allow agreement with the certified values higher than 90% for all the metals.

Quality assurance/quality control (QA/QC) procedures used in all the processes from the field to the final data were followed those outlined by Kratochvil and Taylor (1981) and Taylor (1981); and for ecotoxicity tests those outlined by DelValls et al. (2003).

3. Results and discussion

3.1. Hydrogeochemistry characteristics

Results obtained are shown in Table 1 and Fig. 2. In the Tinto River, pH mean value was 2.89, although it was close to pH 5.0 during high flood events. Electrical conductivity mean value was 2.49 mS/cm, with a range between 0.43 and 8.22 mS/cm. Sulfate mean concentration was 1221 mg/L, with a maximum value above 5500 mg/L. Regarding metals, Fe concentrations are the highest with a mean value of 123 mg/L and a maximum of 2804 mg/L, followed by Al (67 mg/L), Zn (24 mg/L), Cu (16 mg/L) and Mn (7 mg/L). Mean values of the rest of toxic elements are below 1 mg/L.

The Odiel River shows a pH value slightly higher, with a mean value of 3.76. Electrical conductivity and sulfate content are around half of the values recorded in the Tinto River (mean of 1.00 mS/cm and 643 mg/L, respectively). Low Fe concentration (mean values of 5 mg/L) is remarkable compared to the values recorded in the Tinto River (Table 1). Other toxic elements with mean concentrations over 1 mg/L are in decreasing order: Al (33 mg/L), Zn (11.5 mg/L), Mn (8.1 mg/L) and Cu (6 mg/L).

Al, Cd, Co, Li, Na, SO_4, Sr and Zn concentrations in the Tinto River are approximately twice as much as the concentrations in the Odiel River. On the contrary, Ba concentration is higher in the Odiel River, whereas Mg, Mn, Ni, and SiO_2 values are similar in both rivers. Fe and As concentrations are approximately 25 and 36 times higher in the

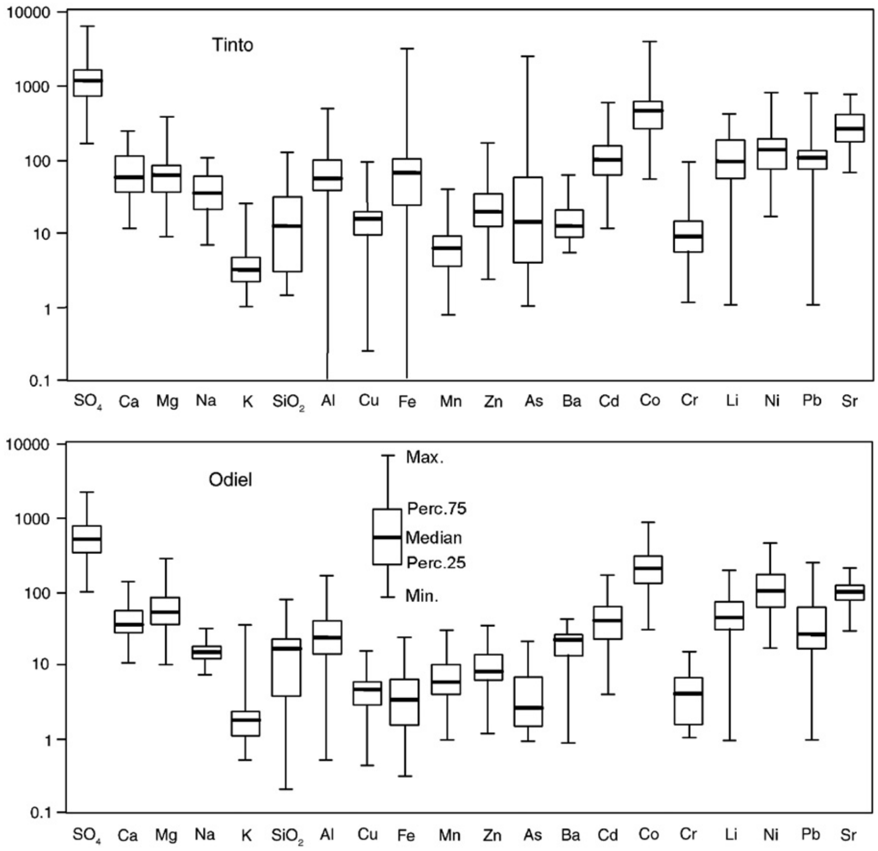

Fig. 2. Box and whisker plot of the dissolved elements in the Tinto and Odiel rivers (values in mg/L except for As, Ba, Cd, Co, Cr, Li, Ni, Pb and Sr which are in μg/L).

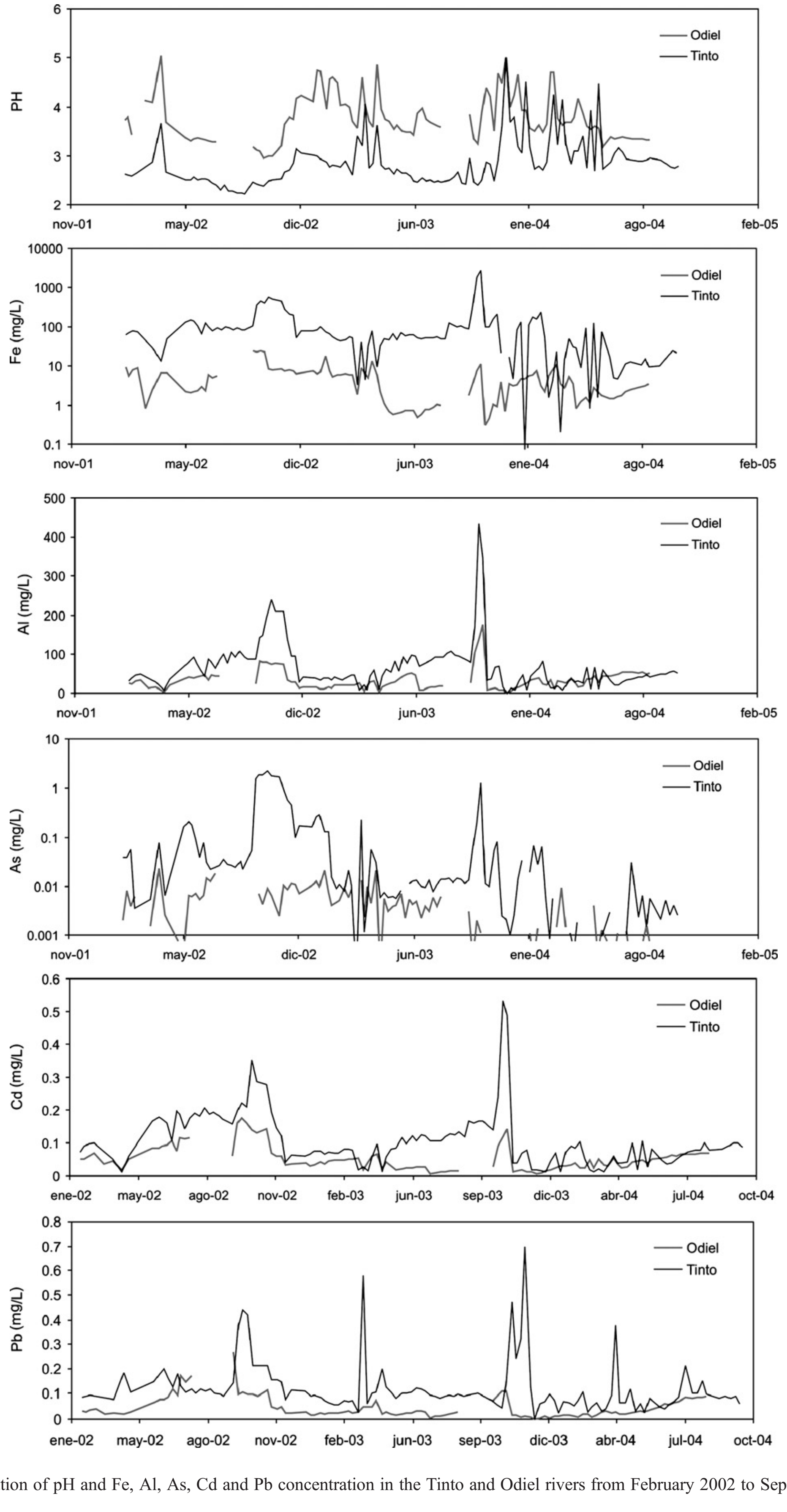

Fig. 3. Evolution of pH and Fe, Al, As, Cd and Pb concentration in the Tinto and Odiel rivers from February 2002 to September 2004.

Tinto River, respectively. Cr, Cu and Pb contents in the Tinto River are more than double than those in the Odiel River.

3.2. Time evolution

Seasonal variation of the Tinto and Odiel River concentrations has been studied by Olías et al. (2004). During spring and summer, salinity increases progressively due to the strong evaporation, producing soluble Fe salts precipitation in the river bed and mining areas. In autumn the highest electrical conductivity and toxic metal contents are recorded, due to the first rainfalls that provoke a redissolution of salts that have precipitated during summer. During the winter, dilution by surficial runoff water causes a slight increase in pH, and at the same time the concentrations of sulfates and metals decrease.

Fig. 3 shows the evolution of some representative variables in both rivers. In the Odiel River there are no analytical data during the summer, as the water frequently stops flowing and gets stagnant. pH evolution shows a minimum during the summer and higher values during the winter. Toxic metals show a maximum during the first autumn rainfalls, due to the evaporitic salts redissolution. This process, called ‘rinse out’ or ‘flush out’, has been observed in the Tinto and Odiel rivers (Hudson-Edwards et al., 1999; Olías et al., 2004) and in others rivers affected by AMD (e.g. Wirt et al., 1999; Keith et al., 2001). During the autumn of 2002 there is a quite wide period with high concentrations, whereas in 2003 this period is narrower, reaching higher concentrations. This is because in 2003 the autumn rainfall was more intense and therefore the “rinse out” process was faster.

As regards to Fe evolution, the wide variation range is remarkable (see log scale); the highest values are recorded during the autumn and the lowest during the winter, where there are frequent peaks depending on the river flow regime. During the summer, Fe concentration remains stable and does not increase like sulfates (not shown), Al, Cd and others metals. Thus, the ratio between Fe and SO_4, Al, Cd, Mn, Zn, etc. decreases during the summer due to a higher Fe precipitation (Olías et al., 2004).

Arsenic evolution has a different behaviour from the rest of elements and shows lower values during the summer – especially in the Odiel River, where values are often under the detection limit during that period – because As has a strong affinity to be sorbed or coprecipitated with the Fe oxyhydroxides (Smedley and Kinniburgh, 2002; Olías et al., 2004). In the Tinto River there is a maximum value, which coincides with the autumn washing, that does not occur in the Odiel River probably because its dissolved concentrations are much lower and, thus, the evaporitic salts must have less As.

Table 2
Average values of the contaminant load transported by the Tinto and Odiel rivers

	Tinto River		Odiel River		Total
	t/yr	%	t/yr	%	t/yr
SO_4	36,589	20	147,213	80	183,803
As	12	35	23	65	36
Cd	4	36	7	64	11
Cu	469	27	1252	73	1721
Fe	5075	64	2847	36	7922
Mn	163	10	1452	90	1615
Pb	15	56	12	44	27
Zn	863	25	2612	75	3475
Al	1224	21	4557	79	5781
Co	9	12	62	88	71
Ni	2	6	34	94	36

In the Tinto River Pb shows a slightly different behaviour to the rest of elements; it presents sudden peaks in spring, which are not observed in the rest of the analyzed parameters. In the autumn of 2003, the highest peak does not occur simultaneously with the rest of metals, but a bit later on (Fig. 3). Cánovas et al. (2005) have found that Pb concentration increase with the flow during floods, contrary to the other elements. The hypotheses we are analyzing to explain this behaviour are: (1) its concentration could be controlled by the anglesite solubility or other Pb rich minerals of the jarosite group (Nieto et al., 2003) and during floods, when the sulfate concentration decrease, Pb content can increase, and (2) its affinity to be sorbed on the particulate material, that increase strongly during floods.

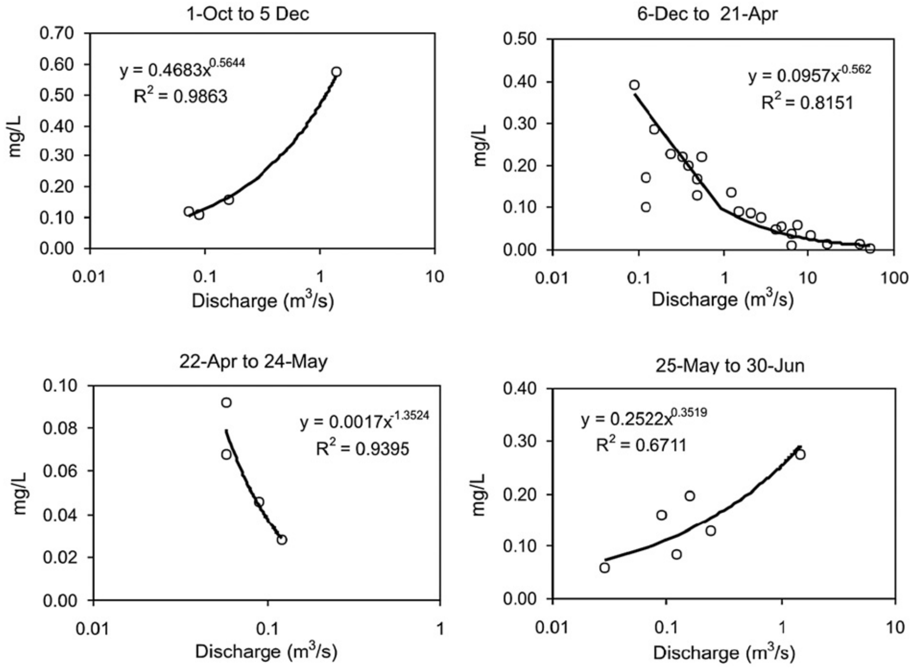

Fig. 4. Relationships Cd-discharge in the Tinto river for the different periods used for the contaminant load calculation (hydrological year 1996/1997).

Table 3
Comparison of the contribution of the Tinto and Odiel rivers with global river flux estimations of GESAMP (1987)

	Tinto and Odiel Rivers flux	Global gross flux (GESAMP, 1987)	Total	Fraction
	t/yr	t/yr	t/yr	%
As	36	10,000	10,036	0.4
Cd	11	340	351	3.2
Cu	1721	10,000	11,721	14.7
Fe	7922	1,400,000	1,407,922	0.6
Mn	1615	280,000	281,615	0.6
Pb	27	2000	2027	1.3
Zn	3475	5800	9275	37.5
Co	71	1700	1771	4.0
Ni	36	11,000	11,036	0.3

3.3. Contaminant load estimation

Olías et al. (2006) have carried out a precise estimation of the contaminant load transported by the Tinto and Odiel rivers, from 1995 to 2003, using analytical data from several sources (mean of 52 samples per year in each river).

With the available data the average flow is 1.6 and 29 m^3/s for the Tinto and Odiel Rivers, respectively. The river discharge is highly irregular depending on the rainfall regime. In the wettest years these values increase to 2.5 and 67 m^3/s. In the driest year the average flow rate of the Tinto River was reduced to just 0.2 m^3/s (during this year there are numerous missing flow data for the Odiel). During the floods, the discharge of both rivers can change from less 1 m^3/s to more than 100 m^3/s in a few hours.

The methodology used to estimate the contaminant load is based on establishing relationships between flow and the concentration of the dissolved elements. When the flow increases in a river generally a decrease in concentration of dissolved substances is observed due to the dilution effect of the less concentrated surface runoff (Langmuir, 1997). This effect has been verified in the Odiel and Tinto Rivers (Braungardt et al., 2003; Olías et al., 2004; Cánovas et al., 2005). If it is possible to establish a relationship between elemental concentrations and flow rates using the daily average flow rates we can calculate the contaminant concentration of this day and thus the load transported by the river.

However, these correlations are not always simple to establish. For example at the start of the hydrological year, in autumn, the first rains produce the dissolution of the weathering products of pyrite accumulated during the summer (Fig. 3), such that the contaminant concentration increases with flow. Thus the relationship between flow and contaminant concentration is not constant over the entire year, but is dependant on the time of year, the previous precipitations, temperature, possible reservoir releases in the drainage basin, etc. Fig. 4 is an example of the relationship between Cd and discharge for the hydrological year 1996/1997 in the Tinto River and shows the different periods used to obtain the regression lines.

There also exist a number of elements which do not show any significant correlation with flow; where this is the case this methodology cannot be applied. In these cases the monthly load has been calculated taking into account the daily flow for the sample in question to balance the importance of this sample in to the context of monthly contribution of the river. For a number of samples (n) in a month the weighted monthly average (C_{mw}) would be:

$$C_{\mathrm{mw}} = \frac{C_1Q_1 + C_2Q_2 + \dots\dots\dots\dots + C_nQ_n}{Q_1 + Q_2\dots\dots + Q_n} \tag{5}$$

This weighted average concentration is multiplied by the monthly discharge to calculate the monthly load for each element. Despite this, the method is also not exact when there are peaks in flow rates that have not been sampled, because storm activity produces a strong dilution effect on the contaminant concentration (Olías et al., 2004; Cánovas

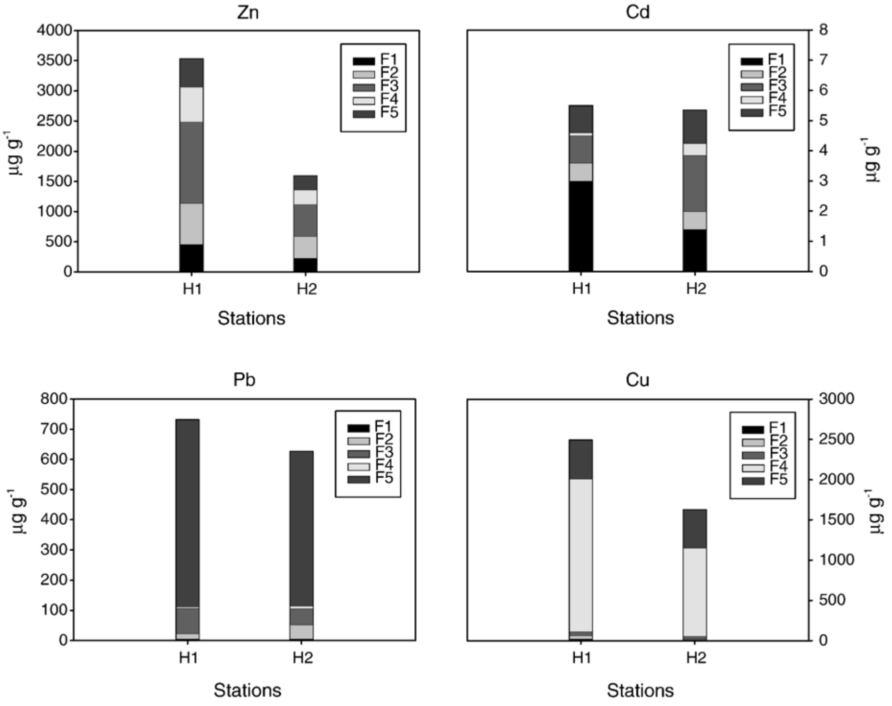

Fig. 5. Trace metal distribution in the five geochemical fractions of the sediment collected from the Huelva estuary. Results are expressed as ìg/g dry sediment. Sediments collected in station H1 are in the fresh water area of influence of the estuary, whereas sediments located in H2 are in the sea water area of influence of the estuary.

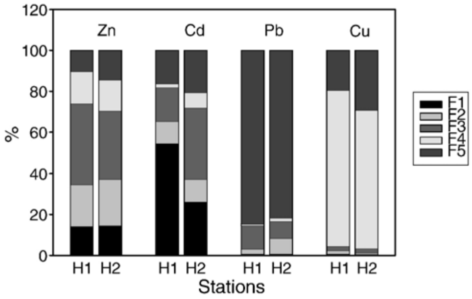

Fig. 6. Trace metal distribution in the five geochemical fractions of the sediment collected from the Huelva estuary. Sediments at H1 were located in the area of fresh water influence, whereas H2 was at the sea water influence area of the estuary. Metals concentration in each fraction is expressed as percentage of total concentration of the same metal for each station.

et al., 2005). Statistically there is little possibility of sampling these storm effects that occur during only a few days.

Another frequently encountered problem was missing flow data; in the 8 years of our selected time frame there are 12 months of missing data for the Tinto River and 23 months for the Odiel River. To solve these problems, monthly contaminant load results obtained from the previous methods were correlated with monthly precipitation rates recorded at 9 different rain gauge stations distributed throughout the Tinto and Odiel drainage basins, as a way to obtain the contaminant load from its relation with rainfall.

Results for both rivers are shown in Table 2. The Al, Ni and Co loads have only been estimated for two years (2001/2002 and 2002/2003) in which analytical data exist. Estimations for As and Pb have a greater degree of uncertainty as they do not normally show any relationship with flow, and have been estimated using the weighted monthly average method or using their relationship with precipitation. The average quantities transported by both rivers annually are: 183,000 t of sulfates, 7900 t Fe, 5800 t Al, 3500 t Zn, 1700 t Cu and 1.600 t Mn. The amount of As, Cd, Co, Ni and Pb are much lower, with respective values of 36, 11, 71, 36 and 27 t.

The amount of contaminants transported annually depends on the precipitation regime. The time period considered in this study (1995/1996 to 2002/2003) was somewhat more humid than normal, thus quantities calculated maybe higher than if a longer time period were selected.

The Tinto River, although with a much lower water discharge, supplies more Fe (64% of total) because its concentrations are much higher than those of the Odiel River (Table 1). Also the Tinto supplies more Pb (56%) due to its values increase during floods, as mentioned before. For the other elements the greatest contribution comes from the Odiel, with maximum values for Mn and Ni (90 and 94% of the total respectively) because of the greater abundance of Mn and Ni mineralizations in the Odiel basin.

Comparing these values with the estimations published by GESAMP (1987) of the global gross flux of dissolved metals transported by rivers in to the oceans, we obtain some very surprising results (Table 3): the load transported by the Tinto and the Odiel Rivers represents 37% of the global gross flux of Zn and 15% of the Cu (Table 3). These results point out the extreme degree of contamination that the Tinto and Odiel rivers suffer.

3.4. Chemistry of sediments in the estuary

In Fig. 5 is shown the chemical concentrations (total and fractionated in the different geochemical fraction of sediments) of metals (Zn, Cd, Cu and Pb) in the sediments selected in the two areas of fresh water and sea water influence in the estuary of Huelva, respectively H1 and H2.

The concentration of the four metals analyzed is highest in the sediments located in the fresh water area of influence, which is related to the source of inputs of these metals from the rivers Tinto and Odiel (Machado et al., 2005). The increase of salinity in the estuary produces the adsorption and trapping of the metals in the sediments of station H1 and most of the metal originated in the area of the mining influence in

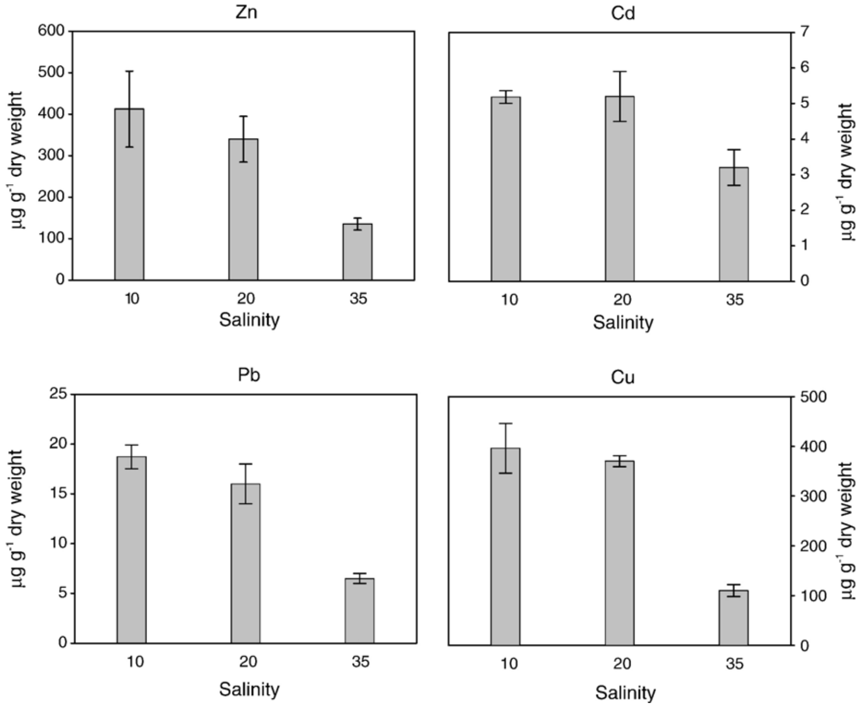

Fig. 7. Histogram of summarized results of trace metal concentration analyzed in tissues of clams *Ruditapes philippinarum* exposed to sediments collected from the Huelva estuary at three salinity values (10, 20 and 35). Results are expressed as microgram per gram of dry weight.

the rivers Tinto and Odiel sank in these sediments. It can explain the higher concentration of all the metals in sediments at station H1 than at station H2. This is clearer for the metals Zn and Cu.

In Figs. 5 and 6 are shown the geochemical fractionation of metals for sediments located in both stations H1 and H2 in the area of the Huelva estuary. The highest mobility of metals is associated with those concentrations associated with the first four fractions (F1 to F4) of the geochemical matrix of sediments (being highest at F1) which are considered the bioavailable concentration of metals in the sediments. The results show that metal mobility significantly increases for Zn, Cd and Cu in sediments located in the area of fresh water influence in which salinity values ranged between 10 and 25, whereas the mobility of metals Cd and Pb present similar behaviour for both stations. The sums of the trace metal partitioning (expressed as percentage, Fig. 6) in the first four fractions (F1 to F4) account for 92% of the total Zn in the sediment from H1, and 85% in H2; in the case of Cd the sums are 85% in H1 and 79% in H2, for Pb 16% in H1 and 18% in H2, and for Cu 80% in H1 and 70% in H2. The results confirm the influence of the salinity values in the mobility of metals trapped in sediments in an estuarine area such as those results previously reported by Riba et al. (2004), Abrantes et al. (2005) and Cobelo-Garcia et al. (2005).

Fig. 7 shows the concentration of metals in the total soft body of the clam exposed to the field-collected sediments in the area of Huelva. The results show that the concentration of metals in the total body of the clams exposed to the sediments increase for all the metals when salinity is decreased. The concentration of metals in the soft body of the clams exposed to sediments at salinity 10 and 20 were significantly different ($p<0.05$) to those concentrations of metals measured in the soft body of clams in organisms exposed to sediments at salinity of 35.

The concentration of the four metals in the body of clams exposed to the same sediment at different values of salinity is related to the differences in their chemical speciation. Thus, metals mobilized from sediments are incorporated and accumulated by the organisms (in this case clams) as a function of the quantity and nature of the ligands available in the water at the different salinity values. The increase of the concentration of ligands such as chlorides and other ligands in the water decrease the bioavailability and accumulation of the metals by the organisms. Similar behaviours of bioaccumulation of these four metals have been previously reported in other studies (Riba et al., 2004; Campana et al., 2005; Morgado and Bebianno, 2005; Acosta and Lodeiros, 2005) and in different field collected organisms around the world (Luoma and Rainbow, 2005).

In conclusion, it can be shown that the continuous influx of metals from the rivers affected by mining activities produce a slow but constant increase of metal in the sediments of the estuary. The bioavailability of these metals varies depending on the pH and salinity values although salinity is the key variable to determine the physicochemical conditions of metals in the estuary. The results show how the salinity also affects the mobility of metals sink in sediments to water and can increase the bioavailability and bioaccumulation of them, if the salinity decrease is equal or lower than 20, as previously proposed by different studies in the area (Riba et al., 2004).

These results show a first and general approach to point out the potential influence of the salinity in the geochemical fractionation and bioavailability of metals presented in the sediments and do not intend to demonstrate this influence, but to show the potential interest of the salinity role regarding bioavailability and distribution of metals in a metallic contaminated area such as that studied in this work. Future studies in the area and in other estuaries can address specifically the mechanisms and characteristics of the bioavailability and bioaccumulation of the metals in high hydrodynamic areas such as the estuaries. Besides, the influence of the salinity and other physicochemical conditions with high variability in estuaries, including pH, organic matter content and nature etc., should be addressed to establish their influence in the geochemical fractionation, bioavailability and adverse effects associated with, such as the toxicity of contaminants in the water and sediments in these areas.

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