ELSEVIER

Chemosphere 58 (2005) 659–669

CHEMOSPHERE

www.elsevier.com/locate/chemosphere

Heavy metal bioavailability and effects: I. Bioaccumulation caused by mining activities in the Gulf of Cádiz (SW, Spain)

I. Riba [a,b], J. Blasco [b], N. Jiménez-Tenorio [a], T. Ángel DelValls [a,*]

[a] Dpto. de Química Física, Facultad de Ciencias del Mar., Universidad de Cádiz, Apartado 40, 11510 Puerto Real, Cádiz, Spain

[b] Instituto de Ciencias Marinas de Andalucía (CSIC), Campus Río San Pedro, s/n, 11510 Puerto Real, Cádiz, Spain

Received 26 May 2003; received in revised form 9 January 2004; accepted 9 February 2004

Abstract

The bioaccumulation of six metals (Fe, Mn, Zn, Cd, Pb and Cu) was studied as part of the monitoring of the Aznalcóllar mining spill (April 1998) on the Guadalquivir estuary and in other estuaries located in the Gulf of Cádiz. Fish, clams and oysters were collected during different seasonal periods along the years 2000 and 2001 in the Guadalquivir estuary to determine the bioaccumulation of the metals originated by the mining spill. Results were compared to the bioaccumulation of the same metals in fish and clams exposed in the laboratory to sediments collected in the same areas during autumn 2001. The bioaccumulation of these metals was compared to the concentration of metals measured in tissues of same taxas collected in the areas of the ría of Huelva and the Bay of Cádiz. Results show that the bioaccumulation of Zn and Cd in the organisms sampled in the Guadalquivir estuary was associated with the enrichment of these metals in the estuary from the mining spill and decreased along the time reaching the lowest values in autumn 2001. The metal Cu show different trends that are associated with other sources of contamination than the spill and related to the transport of this metal from Huelva to Guadalquivir estuary and/or to the use of this metal as plaguicide in the rice fields located in the area. The comparison between bioaccumulation results under field and laboratory conditions obtained in the different areas of study shows that these data can be used to discriminate between acute and chronic impacts associated with mining activities.

Keywords: Guadalquivir estuary; Sediment bioassay; Seasonality; Oyster; Clam; Fish

1. Introduction

Mine waters (a term encompassing all natural waters emanating from a mine site including waste rock piles and tailings dam leachates) are part of the water cycle but are rarely treated as such. This is despite the fact that short- and long-term pollution from active and abandoned mines is still one of the most serious threats to the water environment in many countries (Kroll et al., in press). Mainly the impact caused by these activities is related to the enrichment of heavy metals by the nearby ecosystems through different processes such as *Acid Mine Drainage* (natural process whereby sulphuric acid is produced when sulphides in rocks are exposed to air and water. The metal-laden acid will leach from the rock as long as its source rock is exposed to air and water and until the sulphides are leached out), *Heavy Metal*

* Corresponding author. Tel.: +34-956-016044/6159; fax: +34-956-016040.
E-mail address: angel.valls@uca.es (T.A. DelValls).

0045-6535/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.chemosphere.2004.02.015

Contamination and Leaching (Heavy metal contamination is caused when metals contained in excavated rock or exposed in an underground mine come into contact with water); *Erosion and Sedimentation* (Mineral development disturbs soil and rock in the course of constructing and maintaining roads, open pits and waste impoundments). All these three classes of processes are present in the mine activities carried out in the Iberian Pyrite Belt that affects the rivers located in the Southwest of the Iberian Peninsula. The chronic impacted area of Huelva is well-known around the world and different projects and studies have been carried out in the area. Special attention has been focused on the aquatic ecosystem after the Aznalcóllar mining spill (April, 1998) that implied a major concern in the area related to the impacts provoked by this kind of activities (Riba, 2003).

Once the heavy metals are introduced in the aquatic ecosystems it can affects the biological community. Since the aquatic organisms present a dynamic relation with the environment, these contaminants are incorporated and excreted by the organisms until a balance is accomplished. In spite of environmental factors, the physiologic states and the metabolism of the organisms affect the accumulation and excretion processes. Bioaccumulation of contaminants in organism tissues can be useful for assessing potential for trophic transfer of contaminants. These analyses can be conducted as appropriate with either field-collected, caged animals, or laboratory-exposed organisms (Chapman, 1997). However, integrated studies using both field-collected organisms and those exposed to samples under laboratory conditions can offer an added value to delineate origins of the bioaccumulation of heavy metals and to discriminate the natural factors potentially affecting the bioaccumulation of metals.

Estuaries are the most intensively used and the most vulnerable of coastal areas. Three different estuarine areas affected by mining activities in the Gulf of Cádiz were selected to characterize the bioaccumulation of heavy metals originated by mine activity (Fig. 1). The Guadalquivir estuary was impacted by an acute mining spill (April, 1998) caused by the breakage of a tailing pond in a pyrite mine located in 'Aznalcóllar' (Gómez-Parra et al., 2000). The ría of Huelva is a heavily industrialized area located at the mouth of two estuaries defined by the rivers Tinto and Odiel. The area receives acidic fluvial water discharges with high concentrations of heavy metals from the Roman Empire times, several

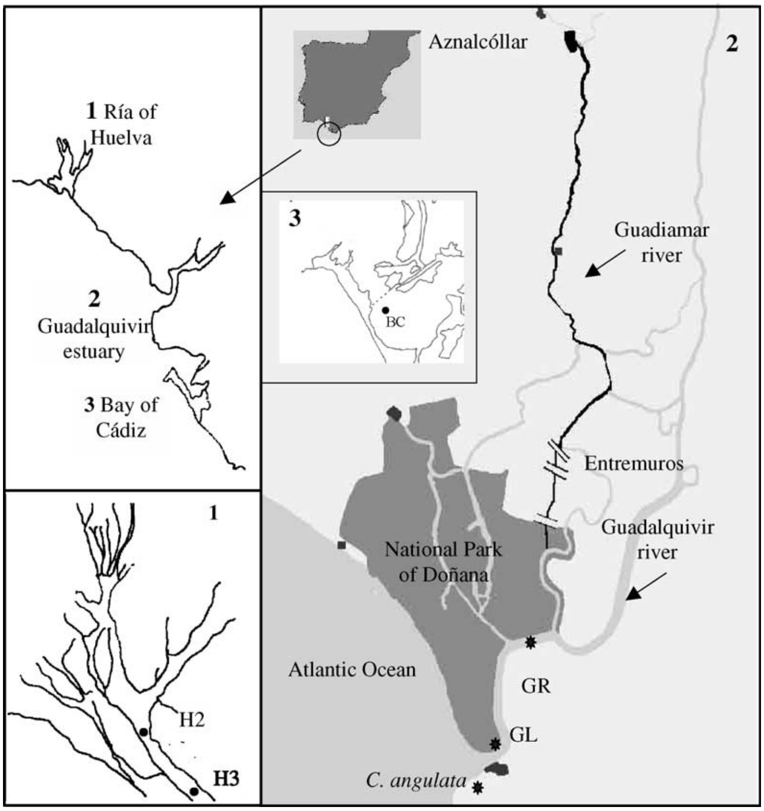

Fig. 1. Map showing the location of the sampling stations located in SW of Spain. In the Bay of Cádiz is located the station BC. In the Guadalquivir estuary are sampled the stations GL and GR and in the area of Huelva are selected H2 and H3.

centuries ago (Cabrera et al., 1992). Finally, the area of Cádiz is a low contaminated area without significant contamination and is selected in this study as not affected by mining activities (Riba, 2003). The main objective of this study was to compare the bioaccumulation of heavy metals both under field and laboratory conditions associated with metals originated by mining activities with a different source, acute from the mining spill and chronic from the continuous discharge of acidic waters. Besides, the comparison of results from field-collected animals to those obtained in laboratory-exposed organisms permit to identify sources of metal contamination.

2. Materials and methods

2.1. Approach

The present study was performed at five stations in three littoral and estuarine ecosystems located in the Gulf of Cádiz, SW, Spain (Fig. 1). In the Bay of Cádiz was selected one station (BC) as a negative control of pollution. Two stations were selected in the Guadalquivir estuary (GL and GR) as representative of different impacts originated by the mining spill (Riba et al., 2002a). Finally, two stations (H2, H3) were selected in the area of 'Huelva' as representative of a gradient of pollution (Riba et al., 2004a).

A summarized description of different surveys and propose of them are shown in Table 1. All stations were sampled for sediment (October 2001) and organisms (different surveys, see Table 1) to conduct heavy metal analysis and histopathological analysis (Riba et al., this issue) in their tissues. The concentration of metals (Zn, Cd, Pb and Cu) analyzed in the organisms tissues was selected to be representative of the mining contamination in the areas. These metals were demonstrated as tracers of the contamination associated with mining activities in the area of the Gulf of Cádiz in previous studies and also determined the transport of the metals from the spill to the estuary (Gómez-Parra et al., 2000; Riba et al., 2002b,c). Besides, metals Fe and Mn were measured in the surveys carried out in the Guadalquivir estuary during years 2000 and 2001 (Table 1) to trace the different geochemical origin of the metals analyzed.

Sediments were used for sediment toxicity tests under laboratory conditions in which the bioaccumulation of trace metals (Zn, Cd, Pb and Cu) was carried out on different organisms.

2.2. Sample collection

Sediment samples from the stations studied were collected with a 0.025 m^2 Van Veen grab. Only grabs that had achieved adequate penetration (2/3 of total volume) to collect the first 5 cm of the sediment and that showed no evidence of leakage or surface disturbance were retained and transferred to a cooler. When sufficient sediment had been collected from a particular station, the contents of the cooler were homogenized with a Teflon spoon until no color or textural differences could be detected. Then the coolers, chilled with ice, were transported to the laboratory. Samples were

Table 1
Summarized description of the different surveys and the approaches used to determine the relationship between bioaccumulation and histopathology in different littoral and estuarine areas in the Gulf of Cádiz described in this work and in that reported by Riba et al. (this issue)

Survey	Date	Histopathology	Bioaccumulation	Samples
Toxicity tests				
Fish	2001	Gills, digestive gland, external and gonads	Gills, gut, liver, muscle	All stations
Clams	2001	Gills, digestive gland, external and gonads	Soft body	All stations
Field survey				
Oysters	2000	All tissues	Soft body	GL
Oysters	2001	All tissues	Soft body	GL
Clams	2000	All tissues	Soft body	All stations
Clams	2001	All tissues	Soft body	All stations
Fish	2000	Gills and liver	Liver	GR
Fish	2001	Gills, liver and kidney	Liver	GR (BC, H2, H3)

The year 2000 is associated with three different surveys at summer (SM00), autumn (A00) and winter (W00) and the year 2001 is related to the surveys carried out in spring (SP01), summer (SM01) and autumn (A01) to collect organisms in the field. These values are results of the average values measured during different months for each seasonal period considered (e.g., June, July and August for SM00 or July and august for SM01). The toxicity tests were conducted in sediment samples collected in the same field survey to collect organism during autumn 2001 (A01).

received at the laboratory 6–7 h after collection and maintained in the cooler at 4 °C in the dark until they were used for toxicity testing. Sediment was filtered (0.5 mm) prior to performance of toxicity tests. Prior to sample collection and storage, all beakers were thoroughly cleaned with acid (10% HNO_3), and rinsed in double-deionized (Milli-Q) water.

Organisms were collected during different periods of time (Table 1) to determine the seasonal fluctuation of the bioaccumulation of the heavy metals. The organisms were selected taking into account different life styles and feeding habits, as well as their distribution in the studied sites and along the estuaries (Table 1).

Different surveys were carried out in the Guadalquivir estuary during two years (2000–2001) of monitoring to delineate the trends of the bioaccumulation of metals from the accidental spill and to discriminate the seasonal component by comparison to other areas and by means of sediment toxicity tests for the same period of time in the year 2001 (Table 1). All organisms were collected by shell-fisherman and/or by scuba divers.

2.3. Sediment toxicity tests

Two separate sediment bioaccumulation tests were used to measure tissue residues in laboratory-exposed organisms in the selected sampling stations. Tests were conducted using the estuarine clam *Ruditapes philippinarum* and the juveniles of the fish *Solea senegalensis*. The organisms were exposed during 10 and 30 days, respectively to whole sediment samples collected in the three estuarine areas during October 2001. Detailed conditions of the tests are reported by (Riba et al., 2004a). In each bioassay the temperature (20 ± 1 °C), pH (7.8–8.2), salinity (33.8 ± 0.2) and dissolved oxygen (>5 mg l^{-1}, 60% saturation) were measured and controlled every day.

Organisms from the tests were analyzed for chemical concentration in their tissues along the exposure time. Besides, organisms at the end of the bioassays were collected and analyzed to determine the histopathological lesions in different tissues (Riba et al., this issue).

2.4. Chemical analyses in organism tissues

Field-collected and laboratory-exposed organisms were depurated for 48 h before processing to avoid any sediment contamination. Then, organisms were dissected and damped out. The samples of field-collected *Crassostrea angulata* and *Scrobicularia plana* corresponded to soft body, those of the fish *Liza ramada* were from liver (Table 1). Samples from laboratory-exposed organism *R. philippinarum* correspond to soft body being those from *S. senegalensis* from muscle, gut, liver and gills (Table 1). All the samples from laboratory-exposed organisms were collected at the middle of the bioassay (15 and 5 days respectively for fish and clams) and at the end (30 and 10 days, respectively for fish and clams). Organisms were divided, in general, into three pools of 5–6 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, Fristch). Samples were digested according to the procedure by Amiard et al. (1987). Heavy metal concentrations (Fe, Mn, 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 $\mu g\ g^{-1}$ 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.

3. Results and discussion

3.1. Metal concentration in field collected organisms in the Guadalquivir estuary

In Figs. 2–4 are shown the concentration of six metals (Fe, Mn, Zn, Cd, Pb and Cu) in different tissues of three organisms, *C. angulata*, *L. ramada* and *S. plana*, respectively, collected in the Guadalquivir estuary at different periods during years 2000 and 2001. The oyster was collected in the mouth of the estuary to study the fluctuations in the bioaccumulation of metals with absence of influence from the mining spill. On the other hand, the use of the metal concentrations in the tissues of the fish collected in the area of the confluence of the rivers Guadiamar and Guadalquivir (GR2) was to assess the trend in the seasonality of bioaccumulation of metals in the area affected by the accidental spill. The clams were collected in the same area than the fish and in the other station located in the mouth of the estuary to determine the differences in the pattern of the bioaccumulation of metals along the estuary.

For *C. angulata* (Fig. 2) highest concentrations for all the heavy metals were measured in organisms collected in winter 2000, except for Cu that had two maximums for the period of winter 2000 (W00) and autumn 2001 (A01). In general, for all the metals measured in the oyster tissues a seasonal component can be detected with maximum values measured in winter, whereas minimum values were associated with organisms collected in summer. These results may suggest an increase of the metal availability in winter as results of the increase of rain flows from the contaminated river (Guadiamar) and from those fresh waters originated in the contaminated rivers 'Tinto and Odiel' from the 'ría of Huelva' in the same period of the year.

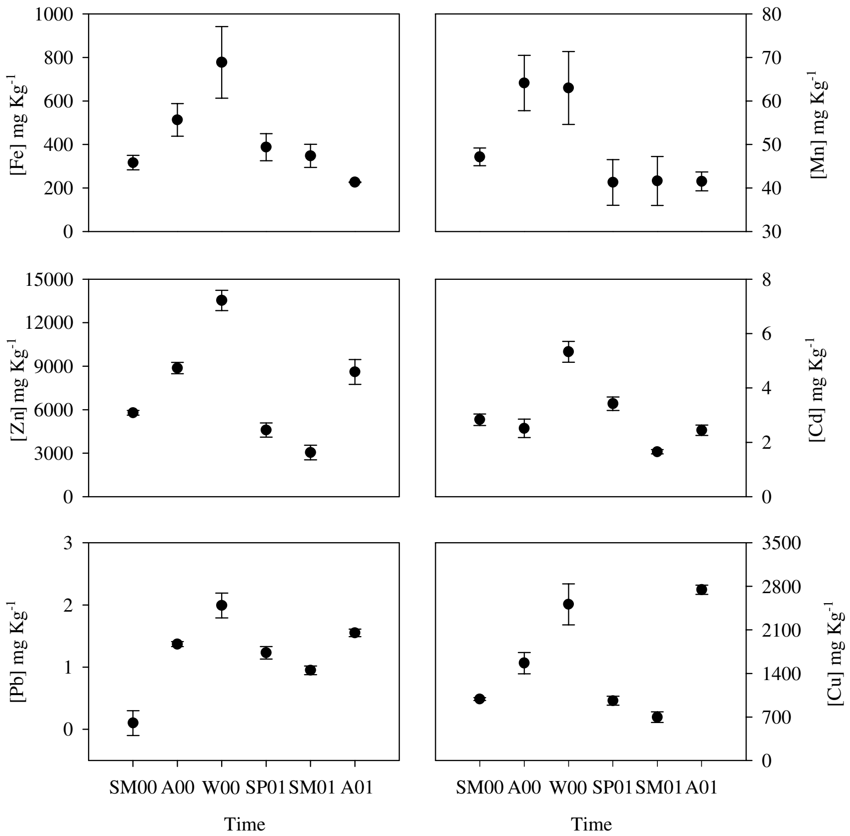

Fig. 2. Heavy metal concentration (-dry weight-) from summer 2000 (SM00) to autumn 2001 (A01), in the soft tissue of *C. angulata* at sampling station located in the coastal area nearby the mouth of the Guadalquivir estuary (GL).

The seasonal trend of the bioaccumulation of metals in the fish shows differences depending on the metal (Fig. 3). In this sense, Zn and Cd shows a similar pattern showing maximum concentrations in tissues of organisms collected during summer 2000 (SM00) and winter 2000 (W00), whereas Pb and Cu shows minimum concentrations in the animals collected in winter 2000 (W00), especially Cu that repeats the same pattern for the next year (again minimum in autumn 2001-A01-). Besides, the highest standard deviation for Zn and Cd were measured in the mentioned period of the year and may be related to a metal stress that produces a metal exchange between liver and muscle, and that varies at individual levels. In general, these differences among metals in liver of the fish can be related to a difference source in the entrance of these metals in the estuary as previously reported by Gómez-Parra et al. (2000). Therefore, the enrichment of metals Zn and Cd can be associated with the particle transport from upper the river Guadiamar that has some trace of toxic mud from the mining spill in some areas of the river (Riba, 2003), whereas the metals Pb and especially Cu should have other different sources pointed out by the mentioned authors such as from copper sulphates used as plaguicides in the rice fields and/or Cu and Pb from the adjacent chronic area of Huelva transported by the littoral current of the Gulf of Cádiz that flows from west to east along the coastal line (Braungardt, 2000).

The results of the concentration of metals in soft tissues of clams *S. plana* collected in two different areas of the estuary, GL in proximities of the mouth and GR in the area of the confluence of rivers Guadiamar and Guadalquivir shows a different pattern for these two sites (Fig. 4). There is an enrichment of metals in body of the clams associated with the particle transport from the Guadiamar river (Fe, Zn, and in particular Cd) in the organisms collected in the vicinity of station GR, whereas the other three metals shows, in general, higher concentrations in the body of the clams collected in station GL than in those organisms collected in GR. These results confirm the differences in the source and/or sources of contamination among the studied metals.

The concentration of the metals in the three species tissues were compared to those previously reported for the same organisms and in the same area by Blasco et al. (1999), which were obtained during the first months of

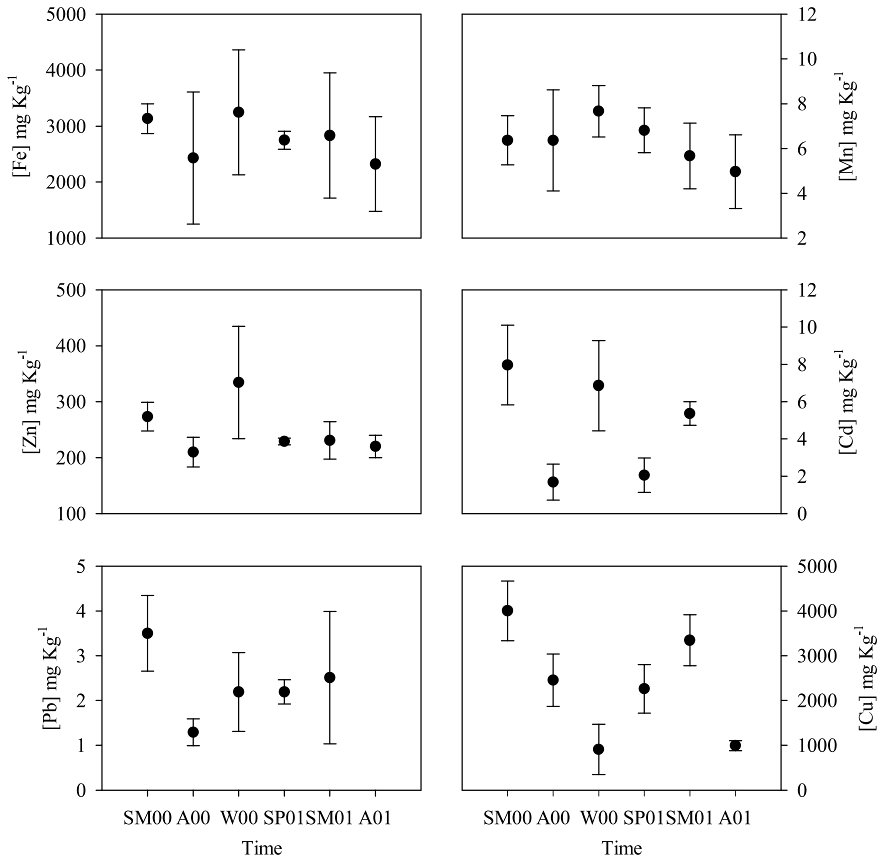

Fig. 3. Heavy metal concentration (-dry weight-) from summer 2000 (SM00) to autumn 2001 (A01), in the liver of *L. ramada* at sampling station located in the confluence of the Guadalquivir and Guadiamar rivers (station GR).

monitoring the impact of the mining spill on the estuary from May to September 1998 (Table 2). The range and the trends (summer and autumn) in the bioaccumulation of metals in *C. angulata* are similar to the results obtained in this study for all the metals, except for Cu that was higher than those previously analyzed in the year 1998 by Blasco et al. (1999). The comparison between metal concentration in fish during 1998 and in this study shows similar range of metals for Fe, Mn, Cu, Cd and Pb. However, the metal Zn showed higher range in its concentrations measured in the fish collected in 1998 (179–598 $mg\,kg^{-1}$) compared to that measured in this study (180–220 $mg\,kg^{-1}$) for the same period of the year (June–September). It could be related to the early impact of the mining spill that provoked and enrichment of metals in the whole estuary, water (Gómez-Parra et al., 2000), sediments (Riba et al., 2002b,c) and organisms (Blasco et al., 1999). Nowadays, the concentration of the metal Zn that was a tracer of the spill in the same fish collected in the same areas have clearly decreased.

The comparison of the data obtained using the clam *S. plana* to those previously reported in the area shows that some metals are in the same ranges for both studies such as Mn and Pb in the two stations (GL and GR). On the other hand, the concentrations of metals Fe and Cd analyzed in this study show a higher range measured in organisms collected in the station GR than those previously reported, being the same case for the metal Cu but in station GL. The comparison of the range of heavy metal Zn in both stations to those previously reported, shows that the concentrations obtained in this study are lower (GL, 500–620 $mg\,kg^{-1}$; GR, 575–1000 $mg\,kg^{-1}$) than those reported in 1998 (950–1600 $mg\,kg^{-1}$) as in the case of the fish *L. ramada*. They are new data that confirms those previously reported by Riba et al. (2004b) which inform that the estuary is recovering its initial *status quo* before suffer the impact of the spill. The slight increase of the concentration of Cd and Fe in organisms should be monitoring in the next year to confirm this recovery or the presence of a sequel in the estuary associated with the spill. These data confirm the potential extra sources to explain the enrichment of the metal Cu in the organisms collected in the estuary.

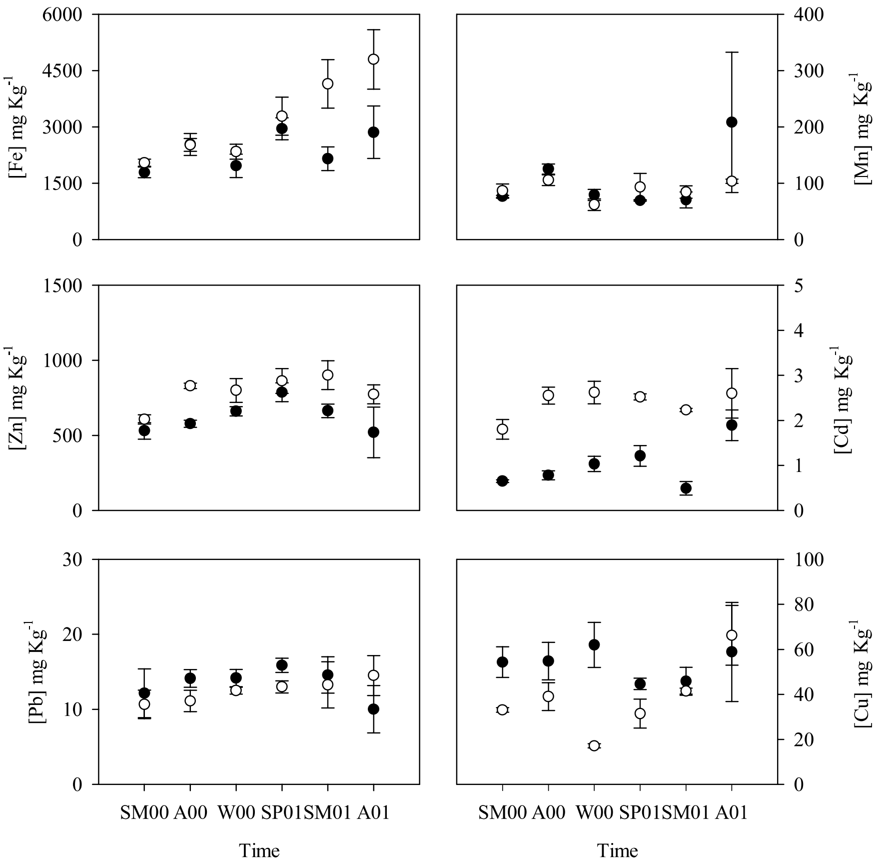

Fig. 4. Heavy metal concentration (-dry weight-) from summer 2000 (SM00) to autumn 2001 (A01), in the soft tissue of individuals of the clam *S. plana* collected at sampling stations GL (●) and GR (○) selected in the Guadalquivir estuary.

Table 2
Range of heavy metal concentrations (mg kg^{-1} dry weight) analyzed in specimens of *C. angulata* (soft body), *S. plana* (soft body) and *L. ramada* (liver) collected in the Guadalquivir estuary from 1998 (Blasco et al., 1999) to 2001 (this study)

Specie	Year	Cd	Cu	Fe	Mn	Pb	Zn
C. angulata	1998	1.6–4.8	536–1802	125–337	20–36	0.6–1.5	1523–9817
	2001	1.8–5.3	568–2806	180–380	38–49	0.9–1.6	1640–9870
L. ramada	1998	0.6–10	356–5240	997–3567	3–8	0.2–3.6	179–598
	2001	0.4–10	321–4944	1101–4189	3–8	0.7–4.1	180–220
S. plana	1998	0.2–2.9	19–24	889–2489	39–98	8.9–16	950–1600
	GR	1.6–3.9	19–33	1456–4987	41–102	9.1–17	575–1000
	GL	0.2–1.1	33–62	1487–3389	45–112	7.9–17	500–620

For the clam *S. plana* there were available two set of data for each station selected in the estuary: the confluence of the rivers Guadiamar and Guadalquivir (GR) and in the mouth of the estuary (GL), whereas for *C. angulata* data are reported for station GL and for *L. ramada* for station Gr. Data for this study are from the set of data shown in Figs. 2–4.

3.2. Bioaccumulation of metals in exposed-organisms to sediments in the laboratory

The results of the concentration of four metals (Zn, Cd, Pb and Cu) in four different tissues (gills, gut, liver and muscle) of the fish *S. senegalensis* are shown in Fig. 5 for the two stations selected in the Guadalquivir and for the stations selected in the Bay of Cádiz (BC) and the ría of Huelva (H#). The bioaccumulation of lead in all the tissues was lowest in the organisms exposed to sediments collected in the Bay of Cádiz. In general, the bioaccumulation of lead and zinc was

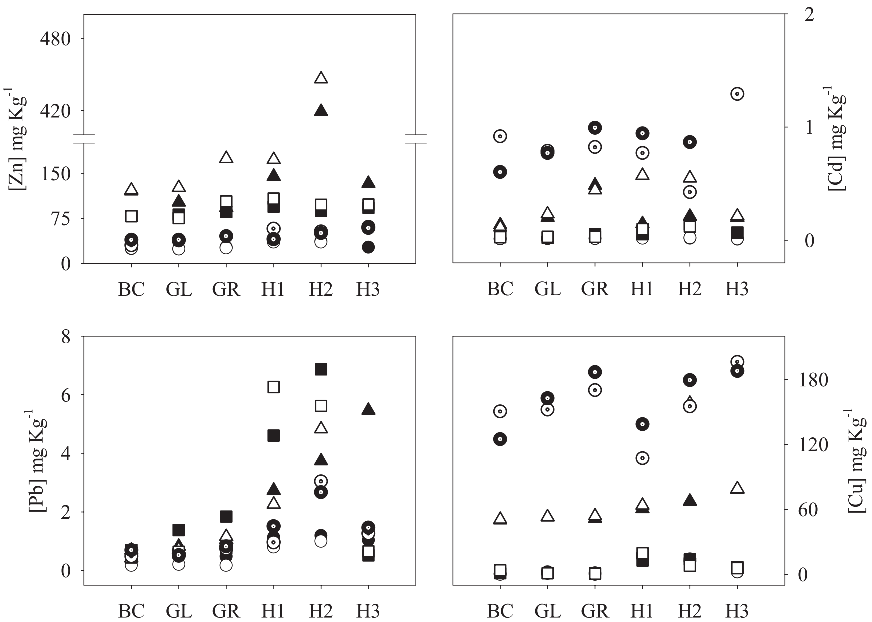

Fig. 5. Heavy metal concentration (-dry weight-) analyzed in different tissues of *S. senegalensis* during winter 2001 at day 15 of exposure and at the end of the bioassay (30). Results are shown for (●) muscle 15 day, (○) muscle 30 day, (▲) gut 15 day, (△) gut 30 day, (■) gill 15 day, (□) gill 30 day, (⊙) liver 15 day, (**O**) liver 30 day. Organisms were exposed to sediments collected in three different estuaries located in the Gulf of Cádiz: Bay of Cádiz (BC), Guadalquivir estuary (GL and GR), and ría of Huelva (H1, H2 and H3).

highest for all the tissues in organisms exposed to sediments collected in the ría of Huelva and mainly associated with the samples H1 and H2, being intermediate in organisms exposed to sediments collected in the Guadalquivir estuary and in general GR > GL. The other two metals, Cd and Cu showed similar values for all the stations except for station H1 and for the metal Cu that showed the lowest concentration of this metal in liver.

Some different patterns are measured for metals depending on the analyzed tissue. Thus, the concentration of Pb was highest in gills, that for Zn in gut and those for Cd and Cu in intestine tissues of the fish analyzed. The concentrations of Cu and Cd measured in liver can be related to a potential elimination of these metals through this tissue by changing them with muscle under an stress produced by metals, whereas a similar metal such as Zn is not concentrated in this tissue but in gut and then may not be eliminated through the same mechanism.

The concentration of Cd in the intestine tissues of fish exposed to sediments from the station GR located in the Guadalquivir estuary are similar to those obtained in fish exposed to sediments H1 and H2 from ría of Huelva. This same behaviour in the concentration of the metal Zn is measured in gills and in liver. In this tissue, the concentration of Cd and Cu measured at both stations located in the Guadalquivir was similar than that measured in organisms exposed to sediments from ría of Huelva (H1 and H2). These results may confirm the idea associated with different sources of enrichment of heavy metals in the Guadalquivir estuary depending on the metal studied that has been pointed out from the results obtained in the field surveys. The concentrations of Zn and Cd were higher in organisms exposed to sediments collected in the confluence of the rivers Guadiamar and Guadalquivir and thus associated with the mining spill. The results obtained for Cu confirms that this metal should have other different source of contamination than the spill and it may be associated with the contamination from the chronic area of the ría of Huelva and/or with the use of this metal as part of the list of plaguicides used in the area.

The results of metal concentrations measured in liver of the fish can be compared to those obtained in the Guadalquivir estuary using other specie of fish but in the same tissue. Concentrations of metals measured in liver of *L. ramada* during the field surveys were higher than those measured in the laboratory bioassays for any metal in any of the sediments from the three sites. Although, they are different species the comparison of the different set of data may informs that the source of metals is not only from sediments in the different areas but results of a complex mixture of different sources such as: dissolved concentration in water and the effect of natural variability in the bioaccumulation process. Besides, it confirms the widely accept mechanism for the accumulation of metals in the field as a result of a large period of exposure than that used in the bioassays,

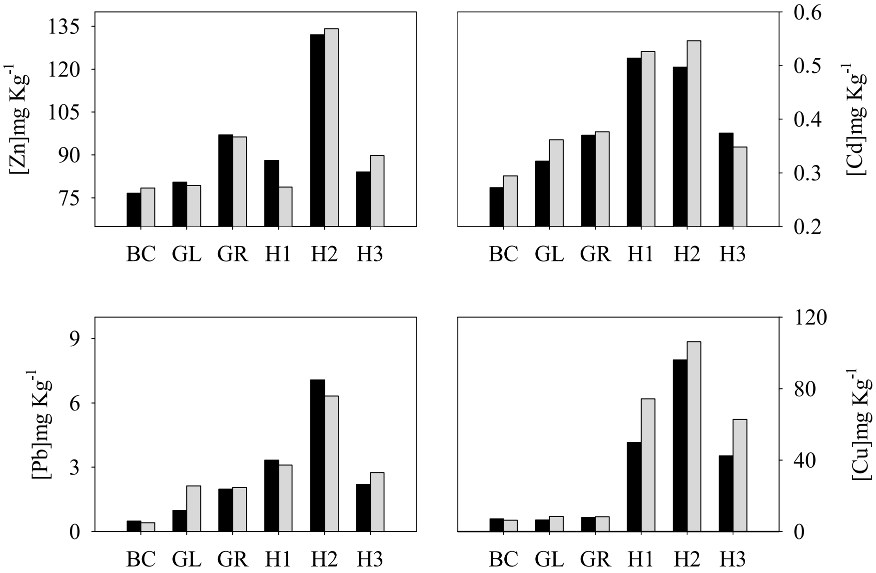

Fig. 6. Histogram of summarized results of heavy metal concentration analyzed in tissues of clams *R. philippinarum* exposed during winter 2001 to sediments collected in the three studied areas: Bay of Cádiz (BC), Guadalquivir estuary (GL and GR) and ría of Huelva (H1, H2 and H3) at day 5 (filled dark bars) and at the end of the sediment toxicity tests (10 day, filled grey bars).

although not difference was measured for the concentration of all the metals in all the tissues measured at 15 days than those measured at 30 days. It determines an steady state reached during the experiments about the middle of exposure period in the bioassay.

The results of the concentration of metals in the soft body of the clams *R. philippinarum* are shown in Fig. 6. The pattern was similar to that obtained in the fish bioassay for all the metals, being the highest concentration of all the metals measured in the soft tissues of organisms exposed to sediments from the ría of Huelva. Also, a parallelism in the pattern of the bioaccumulation of Zn in organisms exposed to sediment from station GR and compared to that measured in sediments from Huelva existed as in the case of fish, being the range for GR higher than for H1 and H3. A steady state was reached after 5 days of exposure time. These results confirm that the source of Zn in the Guadalquivir estuary is associated with the enrichment of Zn in sediments impacted by the acute mining spill.

3.3. Field and laboratory bioaccumulation

Results obtained in the concentration of metals Zn, Cd, Pb and Cu using two different clams under field (*S. plana*) and laboratory (*R. philippinarum*) conditions were compared for all the studied samples. To compare the results of concentration of metals measured in all the soft tissues of both organisms a previous normalization was performed. The process of normalization consists in two steps using the baseline concentrations in both organisms, *R. philippinarum* previous to the tests and *S. plana* in organisms collected from the same aquaculture farm than clams. The concentration of each metal measured in the baseline organisms was subtracted from the measured heavy metal concentrations for both organisms from field and those laboratory-exposed to sediments showed in Figs. 2–6. Then, these results were normalized using the concentration of metals measured in the clams collected in the Bay of Cádiz (*S. plana*), selected in this study as an area non-affected by mining activities, and exposed to their sediments (*R. philippinarum*). Thus, we approach to eliminate the component of difference among different species and between either field and laboratory conditions. The next expression was used to derive the new bioaccumulation percentages showed in Table 3.

$$\%[\mathrm{NM}]_i = \frac{[M]_i - [M]_{\mathrm{BC}}}{[M]_i} * 100$$

where $[M]_i$ is the concentration of metal in the soft tissue of the two clams analyzed in this study either for field or bioassay surveys, $[M]_{\mathrm{BC}}$ is the concentration of the metal in the soft tissue of the clams collected in the Bay of Cádiz. The normalized concentration of the four metals $[\mathrm{NM}]_i$ is expressed as percentage of concentration of metal respect to the clean area of the Bay of Cádiz and represents the increase of the heavy metal concentration compared to the background concentration. High values of the NMs correspond to enrichment of heavy metals in the organisms due to the concentration of metals either in the estuaries or in the sediments used in the bioassay.

Table 3
Summarized results of the percentage of metal concentration normalized against the baseline and reference concentrations in the field and laboratory-exposed organisms (%$[NM]_i$) used in this study (GL and GR, Guadalquivir estuary and H2 and H3 in the ría of Huelva)

Station	Zn	Cd	Pb	Cu
Field survey				
GL	3	76	49	16
GR	33	83	65	25
H2	56	87	89	92
H3	46	76	81	89
Bioassay				
GL	3	17	71	9
GR	20	24	78	16
H2	28	46	91	92
H3	11	22	83	87
GISBs				
GL	100	41	122	93
GR	85	41	113	91
H2	71	59	100	100
H3	63	45	99	98

The index of bioaccumulation associated with metals bound to sediments (GISBs) is shown for the four metals and in the studied areas.

The NMs are used to determine the influence of the metals bound to sediments from the areas studied (Guadalquivir estuary, GL, GR and ría of Huelva, H2 and H3) in the total concentration of metals bioaccumulated in the clams studied. Station H1 was not considered in this comparison because not clams were found in this location. The approach to derives and uses NMs takes into account some assumptions such as: (a) the concentration of the metals in the clams exposed under laboratory conditions are only associated with the concentration of metals bound to sediments (including those mobilized to the water during the bioassay), (b) the concentration of metals measured in the soft tissue of the clams collected in the field are result of different sources of metals, including those bound to sediments, (c) the natural variability affects the concentration of the metals measured in the soft tissues in the different areas in the same way, and (d) concentration of metals measured in different clams can be compared because they use the same mechanism to uptake the metals from the environment. In general, NMs calculated in the field collected organisms are higher than those derived for organisms exposed to sediments in the bioassays, except for Pb in the stations located in the Guadalquivir estuary that show higher values of NMs derived in the laboratory-exposed organisms. It is related to the low concentration of this metal measured in the soft tissue of clams exposed to sediments from the Bay of Cádiz (Fig. 6; 0.4 mg kg^{-1}) that derives NMs higher than 70% in the bioassay. The higher concentrations of NMs measured in field collected organisms was expected from the assumption of the model that associated the total concentration of a metal measured in a clam at field conditions as a sum of different processes.

To establish the influence of the metals bound to sediment in the total bioaccumulation (that under field conditions) a simple subtraction of the NMs derived in the bioassay from the NMs calculated in the field surveys and then for convenience (%) subtracted to a value of 100 determine a gross index of sediment bioaccumulation (GISB). The results shown in Table 3 calculate high values of GISB for Cu and Pb in all stations being the highest associated with the chronic area of Huelva, especially in the station H2 and about 100%. On the other hand, heavy metal Zn with an origin in the mining spill show higher values of GISB in the area of the Guadalquivir estuary than in the ría of Huelva. The GISBs derived for the metal Cd show the highest value in the station H2 located in the ría of Huelva although the GISBs derived for the other stations in both areas show similar values. These results may confirm that the heavy metals originated in the ría of Huelva (Cu and Pb) could reach the Guadalquivir estuary influencing their bioaccumulation in the organism collected in the mouth of the estuary. On the other hand, the metals Cd and Zn have a different source of contamination and mainly associated with the spill. The contribution of the concentration of metals bound to sediments to the total bioaccumulation measured in collected organisms is a complex process and involves homeostasis, natural variability (including pH and salinity changes) and seasonality among other considerations. The simple approach here described is useful to discriminate between trends among metals and areas chronically and acutely impacted by mining activities.

4. Final remarks

This study presents the results of a combined chemical and biological assessment of bioaccumulation both under field and laboratory conditions at different periods during years 2000 and 2001 and at different sites located in the Gulf of Cádiz. The conclusions obtained are summarized below, based on the utilization of samples located in the Bay of Cádiz as negative control or baseline condition:

(a) The bioaccumulation of heavy metals Zn and Cd in the organisms studied in the Guadalquivir estuary was identified with an origin in the Aznalcóllar mining spill. These two metals still in high concentrations in the organisms collected in the Guadalquivir estuary although their concentrations have decreased in the last years. The other metals considered in this study have other different sources of contamination than from the spill.
(b) The use of clams either collected under field conditions (*S. plana*) and laboratory-exposed to sediments (*R. philippinarum*) from the same areas located in the Gulf of Cádiz (Bay of Cádiz, ría of Huelva and the Guadalquivir estuary) permits to discriminate between different origins of contamination by mining activities, chronic against acute impacts.
(c) The bioaccumulation of metals bound to sediments can be used to discriminate the contribution of sediment as a source of metals from the total factors that affect the bioaccumulation of them under laboratory condition with the propose to discriminate between different origins of contamination.

Acknowledgements

Thanks are due to John Rotiz for critically reading a previous version of this manuscript. Authors want to thank Pablo Vidal and Dr A. Arias for their help during sampling collection and treatments. The described work was supported as a part of projects PICOVER (Junta de Andalucía) and REN2002/01699 (Spanish Science and Technology National Program).

References

Amiard, J.C., Pineau, A., Boiteau, H.L., Metayer, C., Amiard-Triquet, C., 1987. Application of atomic absorption spectrophotometry using Zeeman effect, to the determination of eight trace elements (Ag, Cd, Cr, Cu, Mn, Ni, Pb and Se) in biological materials. Water Res. 21, 693–697.

Blasco, J., Arias, A.M., Sáenz, V., 1999. Heavy metals in organisms of the river Guadalquivir estuary: possible incidence of the Aznalcóllar disaster. Sci. Tot. Environ. 242, 249–259.

Braungardt, C.B., 2000. Metal biogeochemistry of a mine contaminated estuarine-coastal system in SW Spain. Ph.D. thesis, University of Plimouth, 353 pp.

Cabrera, F., Conde, B., Flores, V., 1992. Heavy metals in the surface sediments of the tidal river Tinto (SW Spain). Freseni Environ. Bull. 1, 400–405.

Chapman, P.M., 1997. Is bioaccumulation useful for predicting impacts. Mar. Pollut. Bull. 34, 282–283.

Gómez-Parra, A., Forja, J.M., DelValls, T.A., Senz, I., Riba, I., 2000. Early contamination by heavy metals of the Guadalquivir estuary after the Aznalcóllar mining spill (SW Spain). Mar. Pollut. Bull. 40, 1115–1123.

Kroll, A.M., Betlem, G., Amezaga, J.M. Mine water pollution and European policies. In: DelValls, Blasco (Eds.), Integrated Assessment and Management of the Ecosystems Affected by the Aznalcóllar Mining Spill (SW, Spain). UNESCO/IOC/Technical report, in press.

Riba, I., 2003. Evaluación de la calidad ambiental de sedimentos de estuarios afectados por actividades mineras mediante métodos integrados. Ph.D. thesis, Universidad de Cádiz, Spain, 384 pp.

Riba, I., Blasco, J., Jiménez-Tenorio, N., González de Canales, M.L., DelValls, T.A. Heavy metals and effects: II. Histopathology–bioaccumulations relationships caused by mining activities in the Gulf of Cádiz (SW, Spain), this issue.

Riba, I., Casado-Martínez, M.C., DelValls, T.A., Forja, J.M., Gómez-Parra, A., 2004a. Sediment quality in the Atlantic coast of Spain. Environ. Toxicol. Chem. 23, 271–282.

Riba, I., Conradi, M., Forja, J.M., DelValls, T.A., 2004b. Sediment quality in the Guadalquivir estuary: lethal effects associated with the Aznalcóllar mining spill. Mar. Pollut. Bull. 48, 144–152.

Riba, I., DelValls, T.A., Forja, J.M., Gómez-Parra, A., 2002a. Tiered testing of sediments from the Gulf of Cádiz after the Aznalcóllar mining spill using sediment quality Triad approach. In: Augusto, P., Robert, E., Hinchee, Marco, P. (Eds.), Management Contaminated Sediments. Venice, Italy, pp. 163–172.

Riba, I., DelValls, T.A., Forja, J.M., Gómez-Parra, A., 2002b. Monitoring the impact of the Aznalcóllar Mining Spill on recent sediments from the Guadalquivir estuary, Southwest Spain. Bull. Environ. Contam. Toxicol. 69, 129–138.

Riba, I., DelValls, A., Forja, J.M., Gómez-Parra, A., 2002c. Influence of the Aznalcóllar mining spill on the vertical distribution of heavy metals in sediments from the Guadalquivir estuary (SW Spain). Mar. Pollut. Bull. 44, 39–47.