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RESEARCH ARTICLE

Bioavailability and toxicity of metals from a contaminated sediment by acid mine drainage: linking exposure–response relationships of the freshwater bivalve *Corbicula fluminea* to contaminated sediment

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Abstract Streams and rivers strongly affected by acid mine drainage (AMD) have legal vacuum in terms of assessing the water toxicity, since the use of conventional environmental quality biomarkers is not possible due to the absence of macroinvertebrate organisms. The Asian clam *Corbicula fluminea* has been widely used as a biomonitor of metal contamination by AMD in freshwater systems. However, these clams are considered an invasive species in Spain and the transplantation in the field study is not allowed by the Environmental Protection Agency. To evaluate the use of the freshwater bivalve *C. fluminea* as a potential biomonitor for sediments contaminated by AMD, the metal bioavailability and toxicity were investigated in laboratory by exposure of clams to polluted sediments for 14 days. The studied sediments were classified as slightly contaminated with As, Cr, and Ni; moderately contaminated with Co; considerably contaminated with Pb; and heavily contaminated with Cd, Zn, and specially Cu, being reported as very toxic to Microtox. On the fourth day of the exposure, the clams exhibited an increase in concentration of Ga, Ba, Sb, and Bi (more than 100 %), followed by Co, Ni, and Pb (more than 60 %). After the fourth day, a decrease in concentration was observed for almost all metals studied except Ni. An allometric function was used to determine the relationship between the increases in metal concentration in soft tissue and the increasing bioavailable metal concentrations in sediments.

Keywords *Corbicula fluminea* · Metal bioaccumulation · Acid mine drainage

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Introduction

Acidic drainage is one of the biggest environmental problems caused by mining of sulfide-rich mineral deposits, and it results from the oxidation of pyrite and other sulfide minerals. Mine drainage is frequently acidic and contains high concentrations of sulfates, metals, and metalloids. If adjacent to hydrographic systems, acid mine drainage (AMD) can constitute a serious threat to the aquatic organisms and can contaminate not only surface and ground waters but also sediments and soils.

In the southwest of the Iberian Peninsula, thousands of years of mining in the Iberian Pyrite Belt (IPB) (Nocete et al. 2005) have resulted in enormous metal wastes that seriously degrade the environment. The generated AMD affects numerous watercourses, reservoirs, and even entire watersheds. Some examples can be observed in the Huelva province (SW Spain) such as the Odiel and Tinto Rivers (Nieto et al. 2013; Sarmiento et al. 2009a, b). The Odiel River Basin is generally recognized as a fluvial system with a catastrophic ecological situation due to chronic and severe pollution from

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AMD, so much so that it affects 37 % of the length of the drainage network (Sarmiento et al. 2009a).

The main aim of The Water Framework Directive (2000/60/EC) is to achieve a good ecological and chemical status in European waters before 2015. Because of the special characteristics of the Odiel and Tinto Rivers, the European objectives of the Directive have been deferred until 2027 for these rivers. In this sense, the implementation of a passive treatment of AMD process has been designed and financed by a LIFE+ project. However, in rivers highly contaminated by AMD, the use of conventional environmental quality biomarkers is not possible due to the absence of macroinvertebrate organisms. In addition, rivers in decontamination process, such as the Odiel River, have a huge gap in terms of water toxicity assessment, making impossible the estimation of the environmental recovery.

Among the common approaches used to study environmental contamination, the use of bivalves as bioindicator species has proved to be a valuable and informative technique (Hedouin et al. 2011). The Asian clam *Corbicula fluminea* has been widely used as a biomonitor of metal contamination in freshwater systems (e.g., Fletcher et al. 2014; Reis et al. 2014; Zuykov et al. 2013), including the AMD-polluted environments (Andrés et al. 1999; Audry et al. 2005; Porter et al. 2010). Asian clams are an effective biomonitoring tool (Doherty and Cherry 1988), and because of their ease of execution and sensitivity, in situ testing is possible using these species (Soucek et al. 2000). The Asian clam is capable to survive in polymetallic polluted environments and low pH (Arini et al. 2014).

The aim of our work is to determine the relevance of using the clam *C. fluminea* as bioindicator species of metal contamination in the Iberian Pyrite Belt. The ability of this species to bioaccumulate under natural conditions has already been assessed, as well as their ability to inform about the contamination status of their surrounding environment. Bonnail et al. (2016) assessed the metal contamination using *C. fluminea*, in three sediments from watercourses corresponding to different metallic environments from Iberian Pyrite Belt. Results showed that the bivalve is a good biomonitor of Cu and Pb in highly contaminated environment, while the bioaccumulation response was negative for As, Cd, and Zn. However, numerous works show the high capacity of *C. fluminea* to bioaccumulate As, Cd, and Zn (e.g., Chen and Liao 2012; Arini et al. 2015).

In the light of these results, a new laboratory bioassay has been carried out by exposing the clam to sediment from the bottom of an AMD-contaminated reservoir under laboratory conditions. In this case, the studied sediments are considered under anaerobic conditions (Sarmiento et al. 2009c), so that metal mobility, bioavailability, and bioaccumulation by the clams could be different to studies previously described.

These experiments were carried out under controlled conditions simulating as closely as possible those in the natural environment. Laboratory experiments cannot reproduce exactly the conditions in the field. However, the *C. fluminea* transplantation in our study system is not allowed due to this bivalve is considered an invasive species.

Materials and methods

Sediment sampling and clam collection

The Sancho Reservoir is an artificial and polluted reservoir located in the Odiel River basin (southwestern of the Iberian Peninsula), whom water and sediments are affected by AMD from lixiviates of Tharsis mine, one of the biggest massive sulfide deposits at the IPB, which leachates are highly polluted (Caraballo et al. 2011). Values of pH between 4 and 7 can be observed along the water column, as well as concentrations up to 1.4 mg l^{-1} of Fe, 2 mg l^{-1} of Zn, 3.5 mg l^{-1} of Al, 124 mg l^{-1} of SO$_4$$^{2-}$, 70 μg l^{-1} of Co, 12 mg l^{-1} of Pb, etc. (Sarmiento et al. 2009c). The effect caused by the AMD loads into the reservoir is especially important in the bottom sediments. The most bioavailable fraction from these sediments shows element concentrations up to 2.3 μg kg^{-1} of As, 23.5 mg kg^{-1} of Fe, 1.3 mg kg^{-1} of Mn, 3.6 μg kg^{-1} of Pb, 0.1 mg kg^{-1} of Zn, etc. (Sarmiento et al. 2009c).

Bottom sediments were collected near the reservoir walls using a Ponar Dredge sampler. The samples were deposited into hermetic plastic recipient and stored in dark and refrigerate conditions until analysis.

More than 500 adults of freshwater clams *C. fluminea* (20–33-mm shell length and 1.05–2.23-g wet weight tissue) were collected from an irrigation reservoir close to Huelva City (southwestern Spain). Water remaining in the artificial reservoir is drinkable and suitable for strawberry irrigation. Healthy clams were selected based on the following criteria: significant weight, not easily opened by hand and green bound in the valve. The bivalves were then transported to the laboratory in plastic recipients filled with water from the reservoir and avoiding anoxic conditions and overheating. In the laboratory, the clams were stored overnight in the transport containers until room temperature acclimation avoiding heat shock. Then, the clams were transferred to aquariums filled with Natura® commercial mineral water, which was previously acclimatized to the laboratory temperature (20 ± 2 °C) and was oxygenated for 5 days. A portion of 0.03 g L^{-1} of commercial *Artemia* sp. was added for feeding, and oxygen was input though a diffuser.

Bioassay tests

Sediment samples were subjected to a Microtox® toxicity test following the protocol for the basic solid-phase

test (BSPT) according to the standard operating procedure (AZUR Environmental 1998). Briefly, 7 g of sediment were tested as suspensions prepared with 35 ml of commercial Microtox® solid-phase test diluent and diluted to a series of nine concentrations in the cuvettes. The reconstituted bacterium was added to the dilutions which were incubated for a period of 15 min at 15 °C. The modification of the BSPT reported by Campisi et al. (2005) was carried out, and an average from three values of the EC_{50} was obtained for each sample. A noncontaminated sediment sample was used as a control.

In the laboratory, the sediment was mixed (1:4 *v/v* sediment/water) with commercial freshwater with the following composition (mg l^{-1}): 197 HCO_3, 50.6 SO_4, 13.9 Cl, 65.1 Ca, 13.7 Mg, 8.2 Na, and 4.7 SiO_2. The rest of the analyzed elements were below the detection limits. Three aquariums were filled with a mixture of the Sancho sediment and mineral water commercial, and a fourth chamber was maintained just with water to preserve blank clams. A whole-sediment contact bioassay was carried out for 14 days of exposure with the freshwater clam *C. fluminea*. Each selected clam was weighed and settled homogeneously over them (30–35 individuals). Oxygen saturation was permanently maintained by oxygen diffuser. The photoperiod was maintained with artificial light: 15 h of light/9 h of darkness. Clams were sampled in different times (days 4, 6, 10, 12, and 14) along the experimental exposure (5–6 individuals of each aquarium). Clams taken from each aquarium were a sample and replica when it was possible. After 72 h in clean water for depuration, clams were weighed again and frozen until processing. There were no significant changes in the weights of clams after the experiment, and the mortality test (percentage of survival) was null along the bioassay.

Analysis procedure of sediments and soft tissues

Metal fractionation in sediments was determined following the BCR three-step sequential extraction procedures outlined by Rauret et al. (1999), and adding aqua regia extraction as a fourth step. Water samples and the extracts from sediment digestion were analyzed (As, Cd, Co, Cr, Cu, Fe, Ni, Pb, Sn, V, and Zn) by inductively coupled plasma atomic emission spectrometry (ICP-AES, Yobin-Ybon Ultima2). Internal check was performed on the sequential extraction by comparing the total amount of metals extracted by different reagents during the sequential extraction procedure with the results of the pseudototal digestion. The recoveries were greater than 80 %. Multielemental reference standards and blanks were employed at the beginning and at the

end of each sequence. Detection limits were calculated using average and standard deviations from ten blanks and were as follows: 500 µg l^{-1} for Zn, Cu, and Fe; 1 µg l^{-1} for Cr and As; 2 µg l^{-1} for Pb and Co; and 5 µg l^{-1} for the rest of the elements. The relative standard deviations were less than 10 % for all the analyzed elements, indicating good repeatability of the procedures.

Soft tissues were removed from the shells and were rinsed with Milli-Q water. Between 20 mg of freeze-dried homogeneous fraction of soft tissue placed in Savillex® was completely acid digested with 2 ml HNO_3 (65 %) suprapur and 1 ml H_2O_2 by using a thermoregulatory plate (110 °C). This procedure was repeated twice. In the second time, peroxide was not required and the temperature decreased till 80 °C. The extracts were diluted with 10 ml Milli-Q water to ~1 % acid. Trace element concentrations (V, Cr, Fe, Co Ni, Cu, Zn, As, Cd, Sn, Pb, Ga, Se, Mo, Sb, Ba, Tl, and Bi) were quantified using an Agilent Technologies 7700 inductively coupled plasma-mass spectrometer (ICP-MS). Oyster tissue and Certified Reference Material TORT-2, Lobster Hepatopancreas Reference Material for trace metals of the National Research Canada (NRC), were used in trace element recovery and analytical method validation. The agreement of the analysis results and certified values was higher than 90 %.

Multielemental reference standards and blanks were employed at the beginning and at the end of each sequence. Detection limits were calculated using average and standard deviations from ten blanks and were 0.15 µg l^{-1} for Fe, Cu, Zn, and Co, and less than 0.03 µg l^{-1} for the rest of the analyzed elements. The relative standard deviations were less than 10 % for all the analyzed elements, indicating good repeatability of the procedures.

Some freeze-dried soft tissue samples, taken in several days of the bioassay, were cut in 5 mm thin and graphite covered by using an evaporator EMITECH K250X model. An environmental scanning electron microscope (ESEM) (20 keV of acceleration voltage and the beam current to 80 nA) equipped with a backscattering electron detector solid-state detector (SSD) and an energy-dispersive X-ray (EDAX Genesis 2000) spectrometer was used for qualitative and semiquantitative analyses of the internal soft tissue.

Data analysis

Kinetics of uptake and decrease in the concentrations of the soft tissues were expressed in terms of change of metal concentrations over time. The data obtained for metal uptakes per unit of time was modeled using a simple linear regression model (Eq. 1). Kinetics of decrease in the concentration of elements was described by either a simple

linear regression model (Eq. 2) or single-component exponential equation (Eq. 3) (Hedouin et al. 2011).

$$C_t = C_i + K_u t \qquad (1)$$

$$C_t = C_i - K_e t \qquad (2)$$

$$C_t = C_i e^{-K_e t} + A \qquad (3)$$

where A is a constant (mg kg^{-1}), C_t and C_i are the concentrations in soft tissue at time t (days) and initial concentration, respectively (mg kg^{-1}), and K_u and K_e are the uptake and decrease in the concentration rate constants (mg kg^{-1} day^{-1}), respectively. Model constant and their statistics (ANOVA test and Pearson's coefficient, $\alpha = 0.05$ level) were estimated by iterative adjustment of the model using the nonlinear curve-fitting routines in the OriginPro software 2015.

For statistical analysis, mean values for each experimental day were calculated for measured parameters: one-way ANOVA followed by the Dunnett's posttest (GraphPad Prism version 5.00 software) to find significant differences on the different days regarding the control concentration, and the multiple comparison test of Tukey (OriginPro software 2015). The level of significance for statistical analyses was always set at $\alpha = 0.05$.

To evaluate the efficiency of metal bioaccumulation in the studied bivalves, the biosediment accumulation factor (BSAF) was calculated. The BSAF is defined as the ratio of the metal concentration in the organism to that in the sediment (Zhao et al. 2012).

$$BSAF = (C_m - C_i)/C_s \qquad (4)$$

where C_m and C_i are the maximum and initial concentrations in the organism, respectively, and C_s is the average concentrations in the associated sediment of a given metal.

Results and discussion

Geochemical and toxicological characterization of the sediment

Numerous studies support the idea that the greatest reactive fraction from the geochemical matrix of sediments correspond to the most bioavailable form of the metals (Nieto et al. 2007; Riba et al. 2002; Sarmiento et al. 2009c). In BCR sequential extraction, fraction 1 (F_1) corresponds to the most mobile fraction of the metals, easily soluble. Fractions 2 (F_2) and 3 (F_3) may be a threat depending on the environmental conditions. Fraction 2 represents metals bound to oxides that can be released if conditions change from oxic to anoxic. Fraction 3 is made up of metals bound to organic compounds and sulfides, which may be released under oxidizing conditions. Tessier et al. (1979) and Luoma (1989) reported that metal bioavailability was strongly influenced by the concentrations of iron oxides in sediments. The concentration of the toxic elements in the mobile phases are comprised into the three first fractions ($F_1 + F_2 + F_3$), i.e., the most bioavailable form of the mineral content.

Table 1 shows the results obtained from the BCR sequential extraction as well as metal proportion composition in the most bioavailable fractions ($F_1 + F_2 + F_3$) in percentage over the total concentration. Iron is the most abundant element in the sediment, by far (43 mg g^{-1}), followed by Zn, Cu, and Pb (483, 372, and 126 µg g^{-1}, respectively).

The extracted percentages of the total concentrations of some elements in each sequential extraction step are presented in Fig. 1. Iron, zinc, and copper displayed high concentrations in the most bioavailable fraction (F_1), (3981, 240, and 80 µg g^{-1}, respectively), whereas Sn, Cd, and Cr did not overcome the 3 % (less than 0.5 µg g^{-1}). A considerable proportion of As (19 %) and Co (35 %) was also extracted in step 1. Almost half of the Pb (60 %) was extracted in the second step. Copper was mainly associated with reducible and oxidizable fractions (33 and 38 % for steps 2 and 3, respectively), and Cd was totally extracted in the first three fractions (19, 33, and 48 %, respectively). Trace elements such as Sn, Cr, and V were mainly extracted in the residual fraction (more than 74 % of total concentration) due to lithogenic composition associated to the silicate minerals. Fe and As are also extracted in step 4 (more than 61 % of total); however, it could be due to the primary sulfides are not totally extracted in the step associated to the sulfides (step 3) and some could be removed with the residual fraction (Förstner 1985).

Table 1 Amount of some elements released in the different steps of the BCR sequential extraction procedure (data in mg kg^{-1} and percentage of the bioavailable fraction of the elements (%F_{1+2+3})) and contamination factors for some studied metals

	F_1	F_2	F_3	F_4	% F_{1+2+3}	C_f^i
As	3.60	1.70	1.40	12.3	35.3	0.66
Cd	0.40	0.70	1.00	nd	100	7.00
Co	5.10	2.20	4.60	2.70	81.5	1.04
Cr	0.50	0.80	2.80	15.0	21.5	0.18
Cu	80	122	140	29.2	92.2	17.7
Fe	3981	11,811	820	26,125	38.9	–
Ni	3.10	1.10	3.80	9.1	46.8	0.95
Pb	4.30	75.2	5.60	40.5	67.8	4.19
Sn	nd	nd	0.20	17.2	1.15	–
V	1.30	2.50	1.50	15.6	25.4	–
Zn	240	164	49	30	93.8	9.66

nd nondetected, C_f^i contamination factor
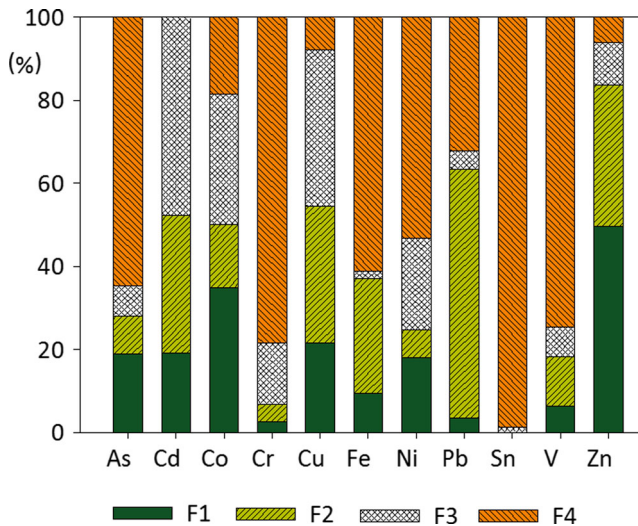
Fig. 1 Percentage of the elements extracted in each step of the sequential extraction procedure for the studied sediments (F_1, easily soluble fraction; F_2, reducible fraction; F_3, oxidizable fraction; F4, residual fraction)

In the sediment, the concentrations of metals and metalloids in the first fraction (F_1), in relative abundance, are the following: Zn (50 %), Co (35 %), Cu (21 %), Cd and As (19 %), Ni (18 %), Fe (9 %), V (6 %), Pb (3 %), Cr (2 %), and Sn (0 %). This sediment is therefore toxic and harmful since this extracted fraction elements concerns the most bioavailable fraction, therefore the most hazardous for the environment (Vives et al. 2007) consisting of exchangeable metals and those soluble in water or in slightly acidic conditions.

Figure 1 shows higher concentration of elements (e.g., As, Cd, Co, Cu, and Ni) than the oxidant sediments studied by Bonnail et al. (2016) in the third fraction (F_3). These elements are associated to newly formed Fe sulfides, attenuating the mobility of them (Sarmiento et al. 2009c). However, oxidation of these sediments during the bioassay occurs, which releases toxic elements back into the water experiment.

As a result, the concentrations of toxic elements in the most bioavailability phase (based on the sums of the first three fractions ($F_1 + F_2 + F_3$) are the following: Cd (100 %), Zn (94 %), Cu (92 %), Co (81 %), Pb (67 %), Ni (47 %), Fe (39 %), As (35 %), V (25 %), Cr (21 %), and Sn (1 %).

Håkanson (1980) proposed the contamination factor (C_f^i) in order to assess soil contamination by the use of reference concentrations in the surface layer of bottom sediments corresponding to preindustrial activity. In this work, we used the concentration of elements obtained from unpolluted sediments taken in a fresh natural stream belonging to the South Portuguese Zone from Sarmiento et al. (2011). The C_f^i is a single-element index, and four categories are defined from low contamination factor indicating low contamination ($C_f^i < 1$) to very high contamination ($C_f^i \geq 6$). The sum of

contamination factors for all the elements examined represents the contamination degree (C_d) of the environment, and four classes are recognized from low degree of contamination ($C_d < 8$) to very high degree of contamination ($C_d \geq 32$). The assessment of the overall contamination of sediment based on the degree of contamination (C_d) for the metal contents was 41 (Table 1), indicating very high degree of contamination (Hakanson 1980). According to the contamination factor (C_f^i) values, the sediment was classified as slightly contaminated with As, Cr, and Ni; moderately contaminated with Co; considerably contaminated with Pb; and heavily contaminated with Cd, Zn. and specially Cu.

Geochemical results obtained are in agreement with those obtained by Sarmiento et al. (2009c). The percentage of bioavailable fraction of metals was mostly the same, except for Cd, which was not detected by Sarmiento et al. (2009c).

Table 2 shows the EC_{50} results obtained through to the Microtox® test in four aliquots of the sediment collected in the reservoir. The sample showed EC_{50} values ranging between 434 and 502 mg l^{-1} (0.40 and 0.46 %) and can be reported as very toxic to Microtox, using the classification rated by Brouwer et al. (1990).

Metal concentration in *C. fluminea*

Prior to bioassay, a total of 12 individuals were analyzed in order to assess the baseline metal concentration in the collected clams (control, $n = 12$). Table 3 summarizes metal concentrations analyzed in soft tissue prior and along to bioassay. Metal concentrations in the control bivalves were elevated (in mg kg^{-1}, e.g., Fe 119 ± 42.9, Cu 41.5 ± 8.91, Zn 113 ± 41.8, Pb 1.05 ± 0.51, As 6.82 ± 1.38, $n = 12$). After bioassay, the most abundant trace metals analyzed were Fe (up to 321 mg kg^{-1}), Zn (219 mg kg^{-1}), Cu (84 mg kg^{-1}), and As (10 mg kg^{-1}), followed in much lower

Table 2 Microtox EC_{50} results for sediment sample the Sancho Reservoir

Sample	EC_{50}			
	mg g^{-1}	95 % C.L.	%	Classification[a]
Aliquot-1	485	342–691	0.42	Very toxic
Aliquot-2	454	230–787	0.41	Very toxic
Aliquot-3	434	259–684	0.40	Very toxic
Aliquot-4	502	289–1086	0.46	Very toxic
Blanck	10,320	8684–12,270	9.50	Non toxic

[a] Brouwer et al. (1990)

Table 3 Metal concentrations in soft tissue of *Corbicula fluminea* before and during the bioassay

	Control		Bioassay		
	Mean ($n = 12$)	Mean ($n = 25$)	SD	Min	Max
As (mg kg^{-1})	6.82 ± 1.38	7.27	1.68	4.57	10.1
Ba (mg kg^{-1})	2.62 ± 1.51	3.29	2.58	0.55	11.2
Bi (µg kg^{-1})	4.49 ± 1.88	6.16	3.27	2	14.4
Cd (µg kg^{-1})	314 ± 130	355	132	190	643
Co (µg kg^{-1})	356 ± 112	495	156	328	919
Cr (mg kg^{-1})	0.64 ± 0.19	0.83	0.32	0.3	1.54
Cu (mg kg^{-1})	41.5 ± 8.91	50.6	16.4	30	83.9
Fe (mg kg^{-1})	119 ± 42.9	153	69.8	50	321
Ga (mg kg^{-1})	0.67 ± 0.33	0.86	0.63	0.21	2.73
Mo (µg kg^{-1})	323 ± 139	273	86.6	190	462
Ni (mg kg^{-1})	0.31 ± 10.14	0.74	0.5	0.26	2.00
Pb (mg kg^{-1})	1.05 ± 0.51	1.02	0.6	0.26	2.58
Sb (µg kg^{-1})	9.21 ± 3.87	16.3	6.61	8.44	34.5
Se (mg kg^{-1})	0.86 ± 0.28	0.80	0.27	0.33	1.18
Sn (µg kg^{-1})	162 ± 58.9	154	53.3	70.8	268
Tl (µg kg^{-1})	4.82 ± 2.79	3.23	1.9	0.94	8.58
V (µg kg^{-1})	101 ± 34.6	96.2	45.6	30	226
Zn (mg kg^{-1})	113 ± 41.8	108	44.8	62.3	219

n samples number, *SD* standard deviation

concentrations by Pb (1.0 ± 0.6 mg kg^{-1}), Cr and Se (0.8 ± 0.3 mg kg^{-1}), and Ni (0.7 ± 0.5 mg kg^{-1}; mean ± SD, on a dry weight basis).

On the fourth day of the exposure, the clams exhibited metal increase in relation to control (concentration at day 0 in Fig. 2) except for As, Se, Mo, and Tl. However, the metal increase is only significant in V, Co, Ni, Pb, Ga, Sb, Ba, and Bi following the Dunnett's test. The increase in concentration of Ga, Ba, Sb, and Bi is more than 100 %, followed by Co, Ni, and Pb (more than 60 %).

During the exposure, only the concentration of Ni showed a significant lineal increase with time ($K_u = 0.083$ mg kg^{-1} day^{-1}; $p < 0.002$; $r = 0.99$). Although on day 14 a decrease in the concentration of Ni was observed, it was not significantly lower than at day 12 (Fig. 2).

After the fourth day, a decrease of the concentrations was observed for almost all metals studied except Ni. Several authors suggest that *C. fluminea* has a lower metal bioaccumulation power when they are exposed to highly metal polluted environments (Bartfiel et al. 2001). The assessment sediments have been referred as very high degree of contamination, so the bivalve could be in a stress situation and stop accumulating or even decrease the initial concentration in the tissue (Chen et al. 2012). However, the clam seems to stop the accumulation of all metals except Ni. Several authors consider bivalves as good indicators of contamination by Ni (i.e., Chalkiadakiab et al. 2014; Hedouin et al. 2007; Zaroogian and Johnson 1984). Hedouin et al. (2007) observed that Ni bioconcentration was directly proportional to the Ni concentration in seawater for several species of bivalves, consistent with our study. Peltier et al. (2009) found maximum concentration of Ni, Cu, Cd, and Zn during the first 28 days, suggesting that the rapid accumulation occur after introduction into the contaminated environment. However, there are no data between days 0 and 28.

The largest concentration decreases were found in Ga, Ba, Pb, Bi, V, Tl, Sn, and Fe by more than 50 %. Concentrations of Pb, V, and Bi showed a significant linear decrease over time ($K_e = 0.12$, 0.007 mg kg^{-1} day^{-1} and 0.49 µg kg^{-1} day^{-1}, respectively; $p < 0.05$; $r > 0.8$). The kinetics of decrease in the concentration of Fe, Ga, Cr, and Ba were best fitted by an exponential model ($K_e = 0.25$, 0.70, 0.32, and 0.65 mg kg^{-1} day^{-1}; $p < 0.02$; $r > 0.96$).

After the decrease of the concentration, elements such as Zn, Cu, Co, Cd, and Sb showed again a significant increase of the concentration from the tenth day of exposure (Fig. 2), whereas random patterns of metal bioaccumulation and decrease in the concentration were observed for the rest of the elements (Fig. 2).

Fig. 2 Relationship between Fe and metal concentrations in the soft tissue of Corbicula fluminea before and during the bioassay. Figure shows the average, maximum and minimum concentrations analysed for each sampling day
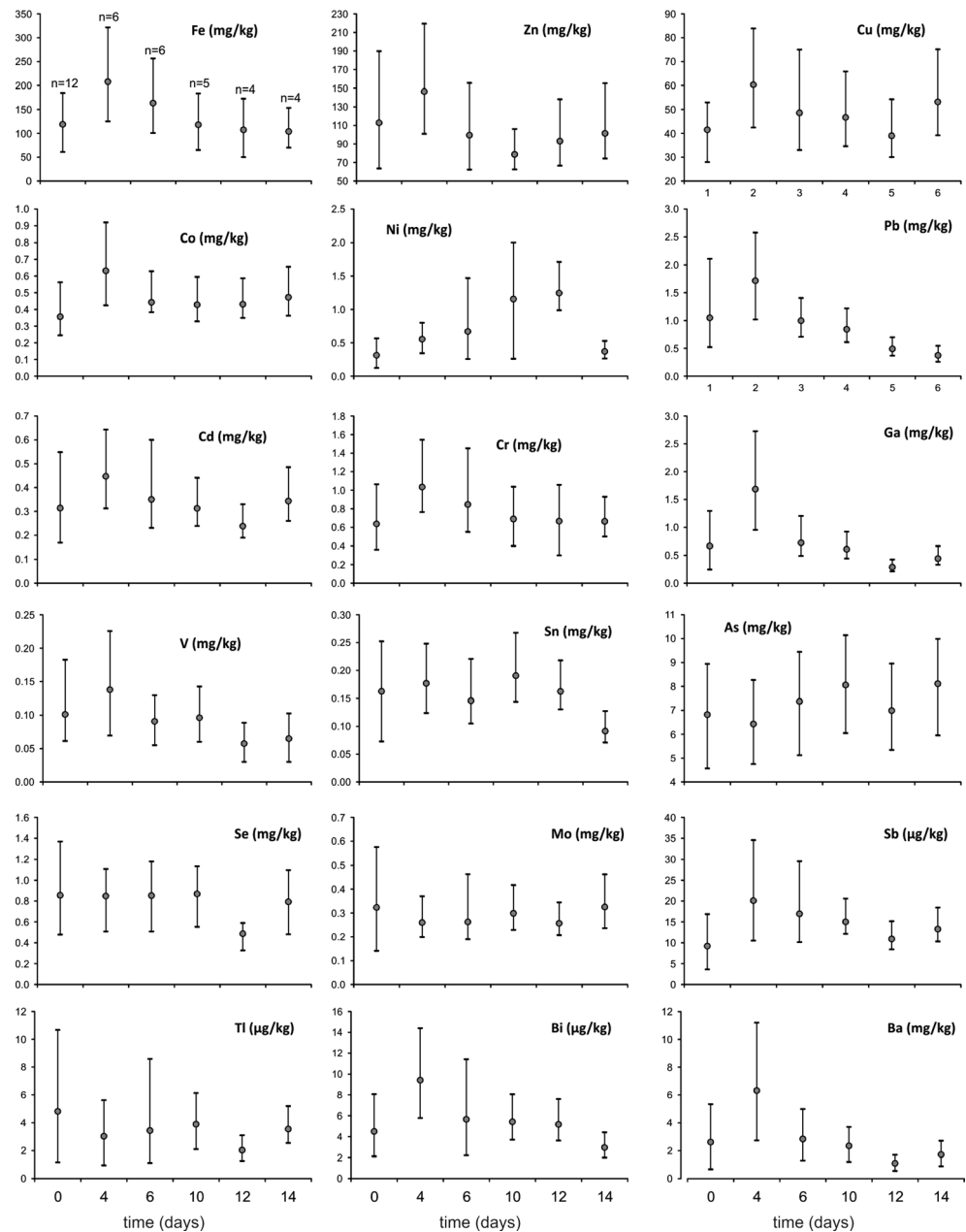

Several authors accept the metabolic regulation in bivalves as the metal is ejection into pellets, granules, and debris forms (Arini et al. 2014; Bilos et al. 2009). Accumulation of metal particles was observed in ESEM photography coupled to EDS analysis of a cross section in soft tissue of several exposed individuals after and along the bioassay (Fig. 3). The characterization of some of these particles includes elements such as Fe, Cu, Zn, and S, characteristic features of AMD systems. It is possible that C. fluminea could be able to remove and accumulate metals when it is exposed to an extremely contaminated environment. The patterns that it follows are according to the assimilation/removal rate for each element, thus regulating the stress to high pollution. The nickel is the only studied element that does not maintain a clear pattern, thus being the most useful element for the use of the C. fluminea as a bioindicator of contamination by AMD.

Table 4 shows the correlation matrix between analyzed metals in soft tissue using Pearson coefficients (bold values indicate $p < 0.05$). The highest correlations are found between elements associated with AMD-polluted environments as well as Cu, Zn, As, Cd, and Pb. Almost all the metals show a high correlation with

Fig. 3 ESEM images (under high vacuum mode, with acceleration voltage of 20 keV and the beam current of 3.5 nm) from a histological transversal cut showing the internal distribution of heavy particles (*white dots*) within the soft tissue of *Corbicula fluminea* after sediment contact exposure bioassay. Metal composition of bioaccumulated particles were characterized by SSD analyses and quantify by a RX detector, providing graphs similar to figure
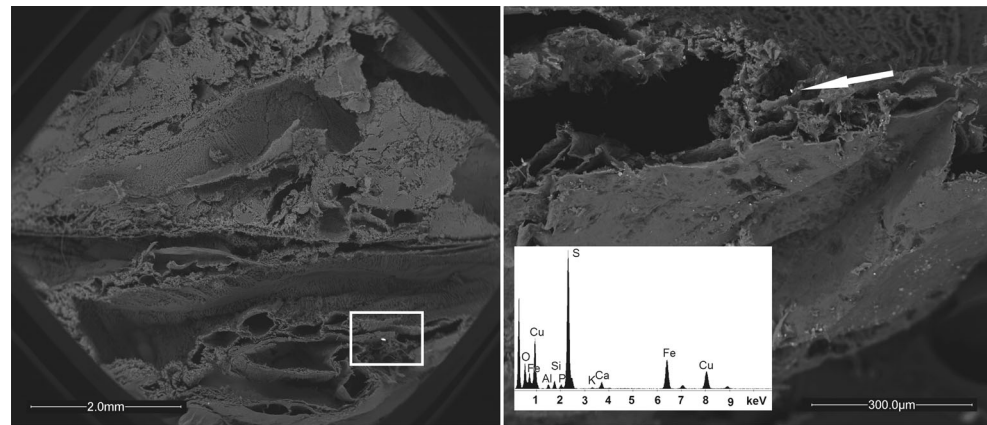
Fe ($r > 0.8$), except Ni, As, Se, Mo, and Tl. Figure 4 displays the relationship between Fe and elements in which a significant linear correlation was observed at the fourth day (black filled squares), especially for Co and Ni. Given that the highest concentrations for most of the analyzed elements have been observed for the fourth day of exposure, it is possible to set up an exposure period of only 4 days as enough for a proper monitoring.

Sediment-tissue relationship

To evaluate the efficiency of metal bioaccumulation, taking into account only the net accumulated metal concentration after exposure, the BSAF values were calculated. According to the obtained values, *C. fluminea* displayed a greater accumulation capacity for the following items: As > Sn > Ni > Cr > Zn > Cd > Cu > Co > Pb > V > Fe.

Previous ranking suggests that clam has a greater capacity to accumulate the metals for which the sediments are assessed to be slightly contaminated (according to the contamination factor, the sediments were classified as slightly contaminated with As, Cr, and Ni).

Heavy metal concentration in *C. fluminea* was ranked in decreasing order as Fe > Zn > Cu > As > Pb > Ni > Cr > Co > Cd > Sn > V, while concentration in surface sediments were ranked as Fe > Zn > Cu > Pb > Co > Ni > As > V > Cr > Cd > Sn. Figure 5 reports the maximum concentration accumulated

Table 4 Correlation matrix calculated using Pearson coefficients for metals analyzed in soft tissue of *Corbicula fluminea* along the bioassay

	V	Cr	Fe	Co	Ni	Cu	Zn	As	Cd	Sn	Pb	Ga	Se	Mo	Sb	Ba	Tl	Bi
V	*1*																	
Cr	*0.66*	*1*																
Fe	*0.41*	*0.76*	*1*															
Co	0.38	*0.82*	*0.85*	*1*														
Ni	*0.45*	*0.52*	0.28	0.30	*1*													
Cu	0.28	*0.70*	*0.86*	*0.85*	0.25	*1*												
Zn	0.26	*0.71*	*0.89*	*0.84*	0.15	*0.90*	*1*											
As	−0.10	0.27	*0.45*	*0.46*	0.25	*0.66*	*0.57*	*1*										
Cd	0.30	*0.65*	*0.94*	*0.81*	0.13	*0.94*	*0.91*	*0.60*	*1*									
Sn	0.27	*0.63*	*0.75*	*0.69*	*0.61*	*0.74*	*0.70*	*0.59*	*0.69*	*1*								
Pb	*0.47*	*0.72*	*0.90*	*0.78*	0.20	*0.76*	*0.81*	0.22	*0.83*	*0.72*	*1*							
Ga	*0.51*	*0.74*	*0.85*	*0.80*	0.11	*0.72*	*0.82*	0.22	*0.79*	*0.60*	*0.95*	*1*						
Se	0.05	0.12	0.29	0.30	−0.18	0.38	*0.42*	*0.58*	0.42	0.29	0.34	*0.43*	*1*					
Mo	0.07	*0.44*	*0.44*	0.43	0.28	*0.71*	*0.63*	*0.74*	*0.60*	*0.57*	0.29	0.27	0.24	*1*				
Sb	0.40	*0.69*	*0.91*	*0.72*	0.25	*0.74*	*0.79*	0.52	*0.87*	*0.64*	*0.77*	*0.78*	0.34	*0.46*	*1*			
Ba	0.38	*0.70*	*0.85*	*0.81*	0.07	*0.75*	*0.87*	0.35	*0.81*	*0.64*	*0.92*	*0.97*	0.55	0.34	*0.78*	*1*		
Tl	0.19	*0.49*	*0.48*	0.34	0.34	*0.62*	*0.54*	*0.53*	*0.57*	*0.55*	0.40	0.34	0.12	*0.87*	*0.56*	0.36	*1*	
Bi	0.32	*0.63*	*0.94*	*0.77*	0.22	*0.77*	*0.85*	0.35	*0.87*	*0.78*	*0.92*	*0.82*	0.27	0.35	*0.79*	*0.82*	0.38	*1*

Italic values indicate *p*<0.05

Fig. 4 Relationship between Fe and metal concentrations in soft tissue of *Corbicula fluminea* before and during the bioassay. Figure shows the average, maximum, and minimum concentrations analyzed for each sampling day
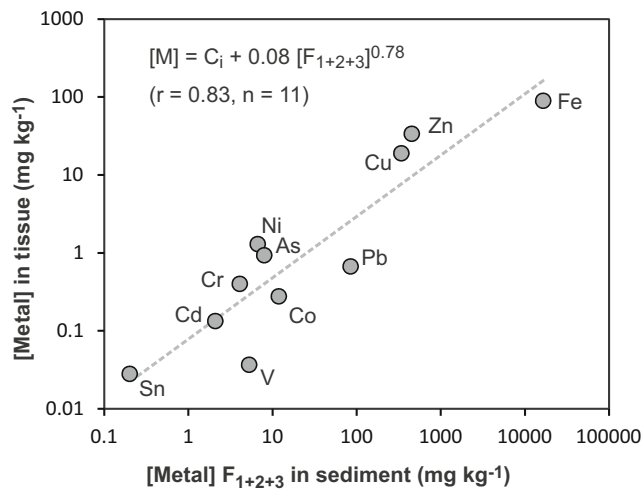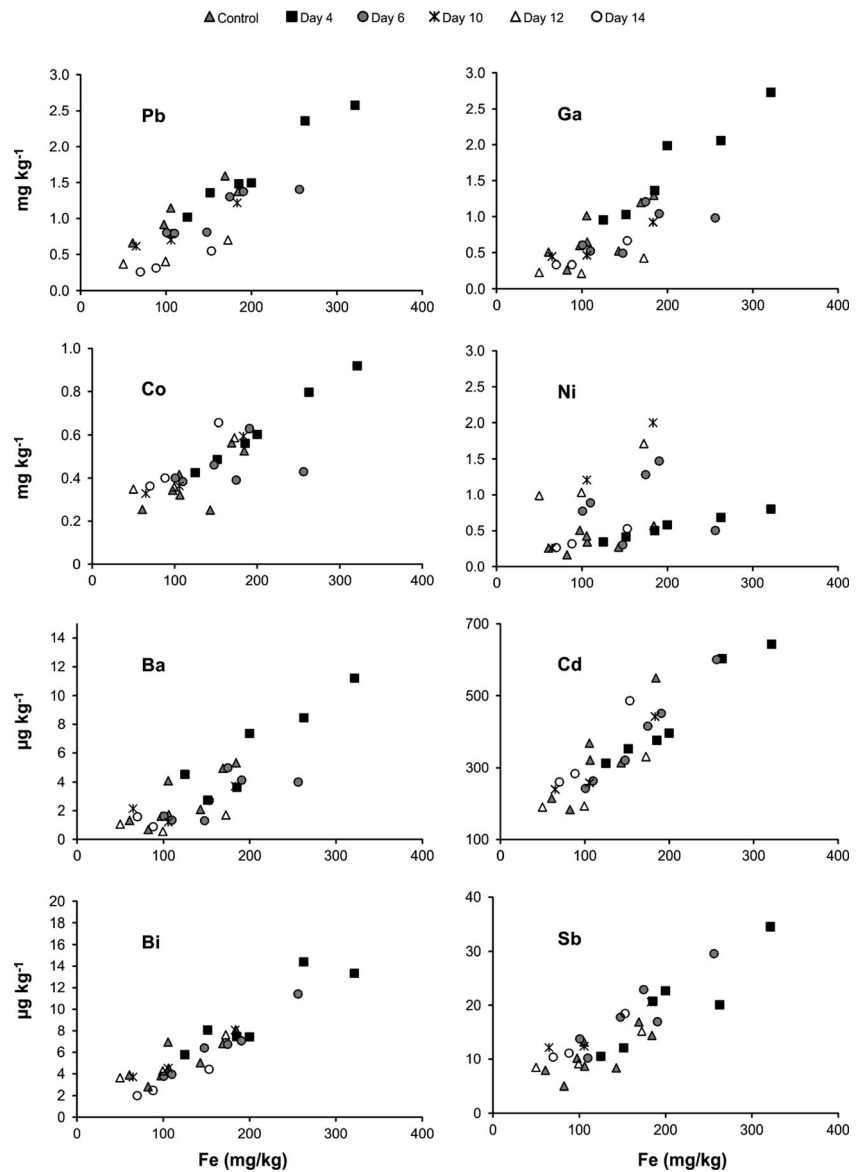
Fig. 5 Metal accumulated in tissue versus bioavailable metal concentration in the studied sediments

by the clam for each metal (C_m–C_i) against metal concentration in bioavailable form contained in the sediment. An allometric function based on power regression (Freundlich model) was used to determine the relationship between the observed increases in metal concentration in soft tissue with increasing bioavailable metal. It reflects differences in efficiency of uptake, being greater when the metal concentration in sediment is low (e.g., for Sn, Cd, and Cr), and strongly decreasing when the metal concentration in the environment is high (e.g., for Fe, Zn, and Cu).

Conclusions

This study indicates that the clam *C. fluminea* can be recommended for a monitoring of toxic metals in strongly

contaminated environmental systems, where the use of another environmental quality biomarkers are not possible. Biomonitoring studies using bioassays would be an efficient solution to survey environmental levels of metal contaminants in areas lacking resident bivalves. The present results show that the clams exposed to contaminated environment are able to accumulate most metals studied in a few hours, although the used clams have a high initial metal concentration.

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