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ORIGINAL PAPER

Integrative assessment of sediment quality in lower basin affected by former mining in Brazil

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Abstract The Ribeira de Iguape River (Southeast Brazil) is metal contaminated by mining activities. Despite it has been cataloged as "in via of restoration" by the literature, this basin is still a sink of pollution in some segments of the fluvial system. This study aimed to assess the sediment quality in the lower part of the RIR basin. The employed approach was based on biological responses of the freshwater clam *Corbicula fluminea* after 7-day exposure bioassays using as the reference site the Perequê Ecological Park. Toxic responses (burial activity and lethality) and biochemical biomarkers (GST, GR, GPx, LPO, MTs, AChE and DNA damage) were evaluated and then integrated with metal bioavailability and chemical concentrations to address the sediment quality in the area through the weight-of-evidence approach. A multivariate analysis identified linkages between biological responses and contamination. Results pointed that, despite being below the benchmarks of the US Environmental Protection Agency, there is slight metal contamination in the lower part of the basin which induces oxidative stress in *C. fluminea;* other toxic responses were sometimes attributed to As and Cr bioaccumulation. The sediment quality values (TEL–PEL values in mg/kg) were calculated for the current study for As (0.63–1.31), Cr (3.5–11.05), Cs (1.0–1.17), Cu (6.32–7.32), Ni (6.78–7.46), Ti (42.0–215), V (1.77–8.00). By comparison with other international guidelines, the sediment quality of the lower basin of the Vale de Ribeira does not identify a significant environmental risk.

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Keywords *Corbicula fluminea* · Ribeira de Iguape River · Biomarkers · Mining contamination · Weight of evidence

Introduction

The Ribeira de Iguape River (RIR) basin is an important region which encompasses the major remnant of continuous Atlantic Rainforest of Brazil that covers an extension of about 25,000 Km^2 (De Freitas Melo et al. 2012) between the states of São Paulo (61%) and Paraná (39%) (Theodorovicz and Theodorovicz 2007) in the Southeastern Brazil (Fig. 1). According to Morais et al. (2013), 21% of the remaining fragments of Atlantic Rainforest (7% from the original) are located on RIR valley and about half is under any kind of legal protection. The main course has got 470 km and springs in Paranapiacaba Ridge (Abessa et al. 2014). This fluvial network flows through three geological dominium before meeting the Atlantic Ocean: metamorphic rocks, magmatic and igneous, and sedimentary covertures (CETESB 1999); containing mineral reserves that promoted extraction activities. The first mining vestiges in the region remind to gold extraction in 1675 (Sánchez 2002), but the intensive lead and silver mining extraction are dated in the later eighteenth century (Morães 1997). Those extractions dated from sulfated minerals of galene ore ($PbSO_4$) from the Panelas Mine (Adrianópolis, in Paraná) and Furnas and Lageado mines (Iporanga, São Paulo). Despite mining activities were ceased in 1995, about 89,000 m^3 of metal-rich residues is still deposited close to the river (Franchi 2004; Morais et al. 2013). Mining residues are uncontrollably discharging into the RIR and increasing the concentrations of metal in waters and sediments (Guimarães and Sígolo 2008a). Mining discharges characterization from metal-rich residues spreads from the upper part of the basin registry values (mean ± SD in mg/kg): 3656 ± 20 Ba, 2730 ± 70 Cu, 214 ± 5 Cr, 34,018 ± 967 Pb, 118,004 ± 280 Zn (Guimarães and Sígolo 2008a). Phosphate rocks are also exploited and processed for manufacturing NPK fertilizers in the region of Cajati (São Paulo) (Bitar 1990), and this a secondary contamination input in the basin. Nevertheless, the RIR counts with six legally protected areas in the region, because the "Vale do Ribeira" involves the larger portion of preserved rainforest in Brazil. The most recent study (Abessa et al. 2014) has concluded that, in spite of an existing natural restoration process, there are still sediments enriched in Pb and other elements, especially downstream of the former mining activities. Natural events, such as the rainstorms, still have a role in carrying metals to the subtropical catchment; being the mean lead concentration in Sete Barras city (lower RIR) close to the effects range low (ERL) values as the US sediment quality guidelines (SQGs) (35.8 mg/kg).

Some other ecotoxicological studies have previously characterized the basin. Between 2000 and 2010, the State Environmental Agency CETESB monitored the water toxicity, detecting no toxic effects to *Ceriodaphnia dubia*; in contrast, whole sediment toxicity tests were positive for *Hyalella azteca*. In 2010, the interface between sediment–water showed a general absence of acute toxicity in bioassays using *Daphnia similis* (Abessa et al. 2012). These findings indicate a potential toxic risk of the sediments and emphasize the use of more accurate methods to detect the adverse effects related to metals-rich mining residues.

Thereby, the use of weight-of-evidence (WoE) approach allows a proper characterization of the contaminated areas and the bioavailability of elements susceptible to cause toxic responses assessed by a biological model, providing thus the identification of the cause of pollution and its quantitative measurement (Riba et al. 2004). In contrast, the biomarkers are considered indicators of "early warning" effects associated with sediment contamination (Martín-Díaz et al. 2004). Up to date the ecotoxicological risk for nontarget organisms is sparsely documented, with the focus predominantly on the acute toxicity of magnified expectable environmental concentrations. The monitoring approaches using biomarkers provide information on short-time adverse alterations that occur in organisms after contaminants have been taken up either via respiratory surfaces, via ingestion, or exposed at non-lethal concentrations, which is relevant in realistic scenarios. Such alterations can be measured as biochemical, physiological or histological responses induced by pollutants at a sub-organism level (Cajaraville et al. 2000). The use of biomarkers does not replace the most common chemical or ecotoxicological strategies, but integrates them providing qualitative and/or semiquantitative information

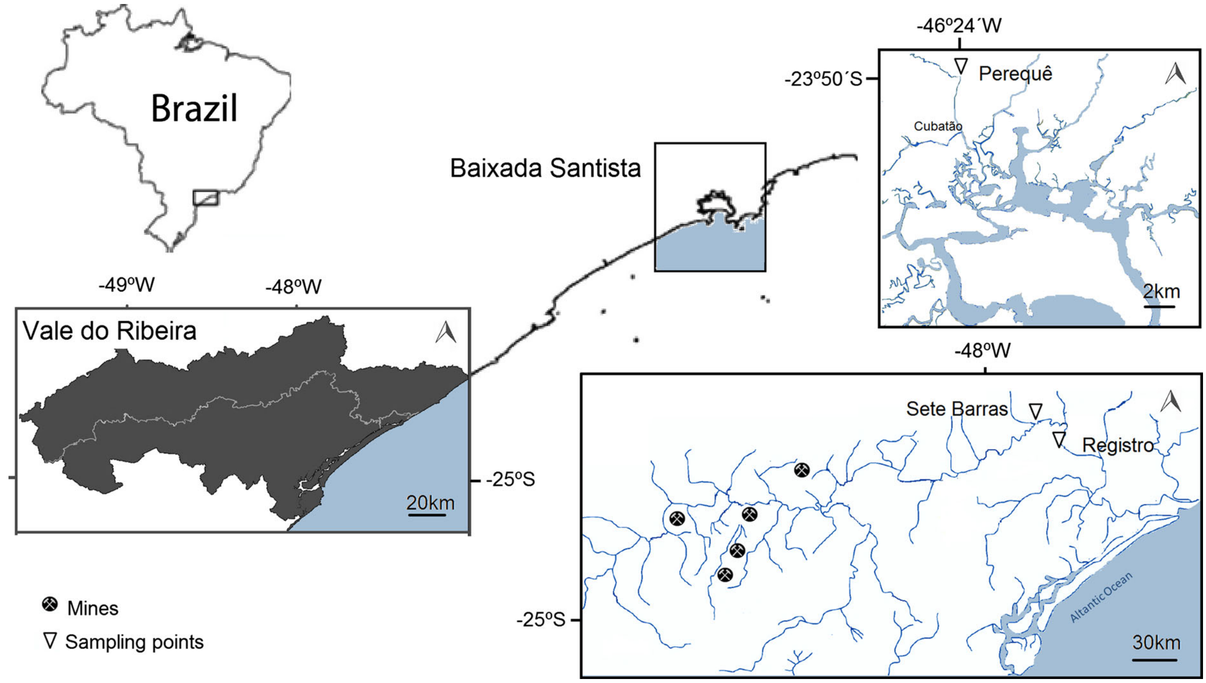

Fig. 1 Map of the study area, showing the location of the sediment sampling sites in the lower Ribeira de Iguape River Basin and in the Perequê Ecological Park and the locations of some mines along the Vale do Ribeira

about the insult and possibly its nature; they also determine the synergic effect of a mixture of pollutants, even when the single contaminants occur in low amount (Donnini et al. 2007). They represent the defense mechanisms through activation of cysteine-rich proteins for detoxification of metal contamination such as metallothionein (MTs), phase I and II metabolisms of detoxification including the enzyme glutathione S-transferase (GST); and changes in the enzymatic activity of glutathione peroxidase (GPx) and glutathione reductase (GR), both enzymes that play role against oxidative stress (Martín-Díaz et al. 2008). Structural and functional effects can be identified as damage through rupture of DNA strands and membrane lipid peroxidation (LPO).

The present study aimed to integrate sediment quality assessment based on biological effects registered by the freshwater clam *Corbicula fluminea* as a line of evidence in the WoE approach to determine the sediment quality in the RIR. To achieve the main aim, the following approaches were employed: (1) sediment characterization to determine metal contamination; (2) the toxicity using sublethal responses (burial) and a battery of biomarker responses measured in *C. fluminea*; (3) bioaccumulation of metal(loid)s in soft tissues to evaluate the bioavailability status and link them with environmental contamination in the lower part of the RIR basin.

Materials and methods

Sampling

Two sites located in the lower part of the RIR basin (Paraná) were selected (Fig. 1): Sete Barras (SB; 24°23′16″S, 47°55′33″W), in the middle of the basin, is characterized with strong flooding episodes. In contrast, Registro (R; 24°29′15″S, 47°50′37″W), in the lower basin, is affected by the salt plumb influenced by high tides and flooding episodes as a result of the confluence of many tributaries. A third site in the Perequê Ecological Park (P; 23°50′29″S, 46°24′57″W) was selected as reference sediment sampling site from the clean tributary of the Cubatão River known as Rio do Ouro (São Paulo). Superficial sediment samples were handy collected from the river banks and transported in dark conditions into plastic recipients with a layer of water from the adjacent river.

Individuals of *Corbicula fluminea* were handy collected in SB and transported to the laboratory in water from the adjacent river and kept overnight at room temperature (25 ± 2 °C) acclimatization. Then, they were introduced into aired commercial mineral water for 2 days for gut depuration before the bioassay tests.

Sediment toxicity tests

Sediment toxicity assays were conducted in 1L test-chambers, each replicate containing an aliquot of sediment samples (SB, R, P) and commercial water (1:4 v/v). Individuals ($N = 15$) of the freshwater clam *C. fluminea* were exposed for 7 days under continuous conditions of oxygen saturation, room temperature and photoperiod (10 h light: 14 h dark). No feeding took place during the exposure bioassay. Survival and reburial activity were monitored along the test. The sublethal endpoint of burial activity was recorded as the median effective time of half of the population buried (ET_{50}) calculated as described by DelValls et al. (2002). After exposure, clams remained 24 h in clean water for gut depuration before being frozen.

Sample analyses

Sediment characterization

Sediment samples were dried at 60 °C, homogenized in mills and analyzed for the total organic carbon (TOC), nitrogen (N) and sulfur (S) by using an elemental analyzer model Vario MACRO Cube. For the analysis of elements in the sediments, the pseudo-total digestion described by the International Organization for Standardization was carried out based on the standard procedure ISO 11466 (1995). Detailed sediment proceed is described in Bonnail et al. (2017).

Metals soft tissue

Clams were dissected, and the extracted soft bodies were destined for the different purposes (metal bioaccumulation and biomarker response analyses). For the metal bioaccumulation measurements, individual or pull of two clams (till reach 20 mg dw homogenized freeze-dried soft tissues) were acid-digested using a temperature-control microwave (Milestone ETHOS 1600 Microwave Labstation) with 5 mL HNO_3 (hiperpur Panreac®) and 2 mL H_2O_2 (33%) in a closed vessel as described in Bonnail et al. (2016a).

Analytical method

The trace metal analysis of sediment and clam digestions took place by means of an Agilent Technologies 7700 Series inductively coupled plasma mass spectrometer (ICP-MS) and an Iris Intrepid Model atomic emission spectroscopy (ICP-AES). Two certified reference materials were used in the trace metal recovery and the analytical method for the biological tissue: the certified Oyster Tissue Standard Reference Material® 1566b from the National Institute of Standards and Technology (NIST) and the TORT-2 Lobster Hepatopancreas Reference Material® for trace metals from the National Research Council of Canada (NRC). The agreement of the analysis results and certified values was higher than 90%.

Biomarkers responses

For enzymatic activity response, clams were individually homogenized in a buffer solution of 50 mM TRIS, 1 mM dithiothreitol (DDT), 1 mM EDTA, and 50 mM sucrose and 150 Mm KCl (pH 7.6). A part of the homogenate fraction was preserved for lipid peroxidation (LPO) and DNA strand damage measurements; other part was centrifuged (15,000 rpm for 30 min at 4 °C) providing a supernatant fraction (S_{15}) destined for glutathione reductase (GR), glutathione peroxidase (GPx), glutathiones S-transferase (GST) and acetylcholinesterase (AChE) analyses. Total protein content in homogenized and S_{15} fraction was analyzed according the dye-binding principle (Bradford 1976).

Biomarker assays were carried out attending different protocols: LPO (Wills 1987); glutathione metabolism determination of GPx (McFarland et al. 1999); GST and GR (McFarland et al. 1999 adapted by Martín-Díaz et al. 2009); AChE (Ellman et al. 1961); DNA strand breaks (Olive 1988); and metallothionein protein content (Viarengo et al. 1997). Further detailed protocols are provided in Bonnail et al. (2016b).

Data analyses

Statistical analysis

A one-way ANOVA with Dunnett's posttest was performed using GraphPad Prism version 5.00 software (San Diego California, USA) to test the significant ($\alpha < 0.05$) inter-site differences among the collection of variables: (1) percentage of clam survival after exposure; (2) metal concentration in tissue of the freshwater clam (As, Ba, Cd, Cr, Cu, Co, Cs, Fe, Ga, Ge, Ni, Sb, Sc, Se, Sn, Sr, Pb and Zn) after seven-day sediment toxicity tests; (3) each biomarker response (LPO, AChE, GST, GR, GPx, DNA damage, MTs) between controls (C_{24h}) and the treatments (SB, R, P).

Multivariate Analysis Approach (MAA)

Relationships among variables at each study site (SB, R, P) were explored using the multivariate technique factor analysis (FA), in which principal component analysis (PCA) was used as the extraction procedure following methods described by other authors (DelValls and Chapman 1998; DelValls et al. 2002; Riba et al. 2004; Morales-Caselles et al. 2009; Martín-Díaz et al. 2009). The geochemical characteristics of the sediments (TOC percentage, fines percentage, metal concentrations—As, Ba, Co, Cr, Cs, Cu, Ni, Pb, Sn, Sr, Ti, U and V), toxic responses based on reburial activity (ET_{50}) and *C. fluminea* biomarker responses of toxicity bioassays (activities of GST, GR, GPx, AChE, MTs, LPO and DNA damage; $n = 48$) and metal(loid) bioaccumulation in soft tissue (As, Ba, Co, Cr, Cs, Cu, Ni, Pb, Sn, Sr, Zn, $n = 15$) were integrated using the FA/PCA. The analysis was conducted on the matrix (varimax normalized rotation) and included any principal component axis. The resulting sorted rotated factor loadings are correlated through new coefficients, in the present study, a component loading cutoff of 0.45. A Pearson correlation coefficient was used to determine general relationships ($\alpha < 0.05$) among biomarkers and sediment chemical contents, as well as between contaminant concentrations in sediments and organisms. PCA and correlation analysis were carried out by means of the statistical packages of XLSTAT-pro (v. 5.1) software tool.

Sediment Quality Triad (SQT)

The set of variables used for the MAA (except Fe and Mn) were applied to develop an adaptation of the classical sediments quality triad (Chapman 1996; DelValls and Chapman 1998), where any of the axes of an isometric system corresponds to the indexes of contamination (I_{cont}), toxicity (I_{tox}) and bioaccumulation (I_{bio}). The SQT approach was performed with a double normalization of the original set of data of the chemical composition with the percentage of TOC and the sediment characterization (%fines) and then to the minimum (ratio to reference, RTR) in order to calculate the I_{cont}. While a single normalization of toxicity and bioaccumulation to the values obtained for the reference station (RTR) provided the I_{tox} and the I_{bio}. In this case, the reference station was SB, due to comparative purposes for being the place closest to the contamination source (mines) from Iguape River. The area comprised between the formed triangles in the isometric system determines the pollution index (P_{triad}), which is represented by the unitary triangle for SB with a P_{triad} value of 1.32.

Results and discussion

Sediment characterization

Results from sediment characterization and metal concentrations are summarized in Table 1. Sediments were classified as fine in SB, muddy in R and sandy in P, with an important percentage of fines close to the mouth of the RIR (14%). The fines percentage varied according to the OM ($r = 0.720$) and N percentage ($r = 0.988$); however, it showed negative correlation with sulfate content ($r = -0.858$). The metal concentrations in sediment showed a increasing gradient in RIR toward the estuary for most of the studied elements (Al, As, Ba, Ca, Co, Cr, Cu, Fe, Ga, K, Li, Mg, Na, Ni, P, Pb, Sc, Si, Sr, Th, Ti and U); just Mn, B, Be and V were found in greater concentrations in the nearest emission point. Some elements such as Bi, Cd, Sb, Se and Sn were below the detection limits. In contrast, many element concentrations in sediments from P were above the average concentrations of RIR (As, Cr, Cs, Cu, Fe, Ga, K, Li, Mg, Na, Ni, P, Rb, Si, Sn, Ti and V).

A preliminary evaluation of sediment potential toxicity risk was made by contrasting obtained metal(loid) concentrations with the international freshwater sediment guidelines proposed by the USEPA (2007). Initially, the studies sites appear to not present any risk to biota due to the low concentrations of the different metals, except for Co (Bonnail et al., 2017). This element overpasses the thresholds

Table 1 Results of the sediment characterization: organic matter (OM); total organic content (TOC); nitrogen (N); Sulfur (S) and total metal concentration in mg/kg in sediments from the stations: Sete Barras (SB) and Registro (R) in the RIR; and the Natural Park Perequê (P) in São Paulo state

	Sediment			*Corbicula fluminea soft tissue*									
	SB	R	P	C24h		C10d		SB		R		P	
Fines (%)	1.83	14.2	0.47										
OM (%)	1.40	1.60	0.60										
TOC (%)	0.68	0.44	0.30										
N (%)	0.06	0.09	0.05										
S (%)	2061	0.90	1163										
Metals (mg/kg)				*av*	*SD*	*av*	*SD*	*av*	*SD*	*av*	*SD*	*av*	*SD*
Al	2903	3732	10576										
As	0.56	0.63	1.31	1.92	0.14	1.88	0.12	2.77	0.39*	2.35	0.06	2.57	0.08*
B	4.84	0.37	0.10										
Ba	41.6	63.1	42.1	7.30	2.86	6.24	2.01	8.88	3.44	10.6	4.68	5.07	2.16
Be	0.38	0.38	0.33	0.01	0.001	0.034		0.011		0.048	0.041	bdl	
Bi	Bdl	bdl	bdl	0.02	0.012	bdl		bdl		0.137		bdl	
Ca	643	941	130										
Cd	Bdl	bdl	bdl	0.61	0.14	0.53	0.07	0.59	0.19	0.35	0.05	0.43	0.13
Co	**6.67**	**6.75**	**4.62**	0.89	0.08	0.71	0.10	0.76	0.11	0.69	0.02	0.64	0.16*
Cr	3.26	3.55	11.05	5.66	0.54	7.28	1.42	5.50	1.33	5.32	0.77	5.47	0.71
Cs	0.43	1.00	1.17	0.050	0.013	0.038	0.003	0.032	0.006	0.103	0.118	0.047	0.013
Cu	4.67	6.21	7.32	55.1	8.67	67.6	12.3	46.2	1.26	48.6	11.8	42.8	6.11
Fe	2045	2480	10071	485	15	396	30	489	85	420	42	430	48
Ga	0.54	0.70	2.25	0.97	0.71	0.51	0.29	1.47	0.70	1.64	1.09	0.63	0.48
K	535	1264	3165										
Li	1.32	2.35	6.01	0.18	0.08	0.26	0.04	0.09	0.02	0.24	0.14	0.13	0.04
Mg	1089	2155	3993	n									
Mn	394	362	129	5.76	1.06	5.50	1.01	7.46	1.50	9.05	1.24*	7.54	1.40
Na	57.8	61.6	67.1										
Ni	5.37	6.78	7.46	1.46	0.08	1.71	0.27	1.71	0.98	2.21	0.77	1.08	0.22
P	20.5	30.3	39.5					nm					
Pb	10.1	18.9	3.87	1.19	0.10	1.40	0.09	1.16	0.19	1.56	0.45	0.88	0.10
Sb	Bdl	bdl	bdl	0.09	0.03	0.13	0.07	0.04	0.03	0.09	0.07	0.03	0.01
Sc	0.75	1.31	2.33	0.14	0.02	0.08	0.02	0.09	0.02	0.17	0.11	0.10	0.02
Se	Bdl	bdl	bdl	0.36	0.04	0.34	0.04	0.29	0.06	0.34	0.06	0.35	0.15
Si	1.43	10.5	24.7										
Sn	Bdl	bdl	0.30	156	46	231	58	94	5	155	93	123	20
Sr	4.49	7.48	1.18	8.26	1.69	8.21	1.75	7.99	2.05	10.50	2.16	5.13	1.83
Th	0.89	1.60	1.68	0.06	0.035	0.003	0.002	0.014	0.008	0.072	0.100	0.791	bdl
Ti	26.0	42.0	215										
U	0.28	0.48	0.26	0.31	0.04	0.22	0.01	0.19	0.03	0.23	0.08	0.19	0.07
V	1.82	1.77	8.00	0.27	0.01	0.19	0.04	0.27	0.09	0.38	0.10	0.28	0.09
Zn	29.7	47.4	34.4	84.4	11.3	86.6	9.29	76.5	6.60	70.6	13.7	67.7	14.9
Zr	0.18	0.22	0.42	0.52	0.17	0.33	0.05	0.10	0.03	0.23	0.18	0.18	0.09

Results of metal concentration in soft tissue of *Corbicula fluminea* in the controls (C_{24h} and C_{10d}) and after the sediment bioassay in organisms exposed to sediments from stations SB, R and P are also shown as mg/Kg wet weight (or dry weight)

bdl below detection limit, *nm* not measured

In bold: values over-passing the freshwater USEPA thresholds (2007) for metals in sediment

* Significant difference of metal bioaccumulation with respect to the C_{24h} samples (ANOVA—Dunnett's test, $p < 0.05$)

established by the USEPA in the three stations (Table 1).

Biological responses

Four different biological responses of *C. fluminea* were analyzed in this study: the mortality events, the reburial activity (ET_{50}), the biomarker response and the metal bioaccumulation.

Clam mortality. The percentage of clam survival for each triplicate assay in every treatment was negative; i.e., no significant differences were found in the lethal toxic response compared with those measured in the control ($p < 0.05$). Results were consistent between replicates, and clams appeared to have a great tolerance for all the sediment samples.

Clam reburial. The reburial toxicity results in the first 48 h of exposure for each sediment tests are summarized in Fig. 2. The ET_{50} average values were 250 min for SB, 133 min for R and 337 min for P. Clams exposed in sediments from station R displayed a faster burial response than from the other two stations. Station P showed slowest burial rate, and in some of the replicates low than 50% were buried.

Biochemical biomarker responses. Results of biomarker analyses in 7-day sediment toxicity test with *C. fluminea* are shown in Fig. 3. Significant ($p < 0.05$) GPx and GST activity enhancement regarding the control clams are observed in organisms undergoing exposure to the three sediments (SB, R and P). GR activity was significantly induced ($p < 0.05$) in the Asian clam from SB and P. DNA strand damage and AChE activity barely responded to sediment exposure in a significant manner compared with controls.

The correlations between biomarkers are collected in Table 2. The antioxidant systems (linked to the GPx and GR) are activated to avoid the stress produced by xenobiotics to eradicate the ROS (Di Giulio et al. 1989). GPx and GR activities increased for the three-location exposure, showing strong positive correlations ($p < 0.05$) between them and negative with the GST. The GST is the enzyme indicative of the phase II of xenobiotic metabolism (conjugation for excretion). On the other hand, the LPO is shown as the effect of oxidative stress: So, the activation of GR and especially GPx ($r = 0.770$, $p < 0.01$) indicates the activation of protection mechanisms against membrane damage by LPO. The high TBARS concentrations, as determination of LPO after exposure to contaminated sediment in SB and P, showed a significant relationship to DNA strand damage ($r = 0.774$, $p < 0.01$), despite this damage did not seem significant against the controls ($p > 0.05$) for any of the samples. So that, the failure of antioxidant defenses to detoxify ROS production can lead to significant oxidative damage including enzyme inactivation, protein degradation, DNA damage and finally cell death (Halliwell and Gutteridge 1999; Aguirre-Martínez et al. 2015).

AChE assays did not display any significant activity after exposure. Previous studies have demonstrated the inhibitory effect of metals in AChE activity (Frasco et al. 2005; Bonnail et al. 2016b). However, concentrations of xenobiotics in the studied sediments did not cause any modification in its activity.

The MT expression level is dose-dependent and sensitive to the concentration of heavy metals with four key functions: bioaccumulation and detoxification of toxic metals; homeostatic regulation of metals;

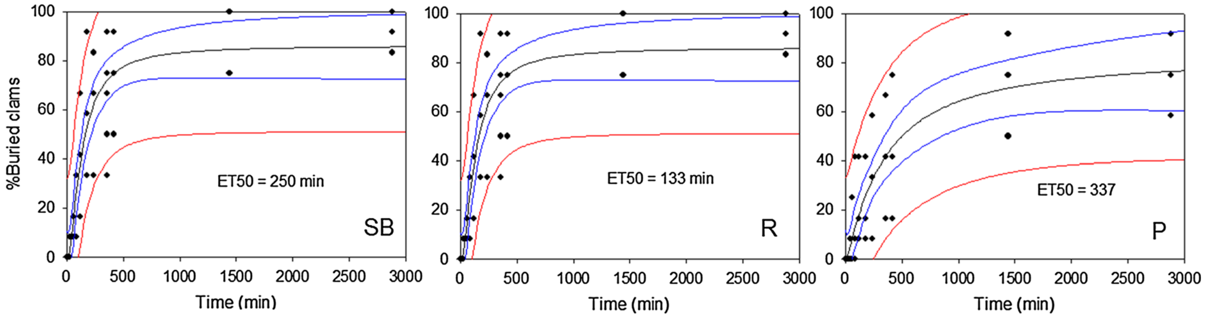

Fig. 2 Clam reburial results of the toxicity assays of exposure to sediments from SB, R, P. The percentage of clams buried that is represented for each triplicate (*dots*) versus the time of exposure (up to 48 h)

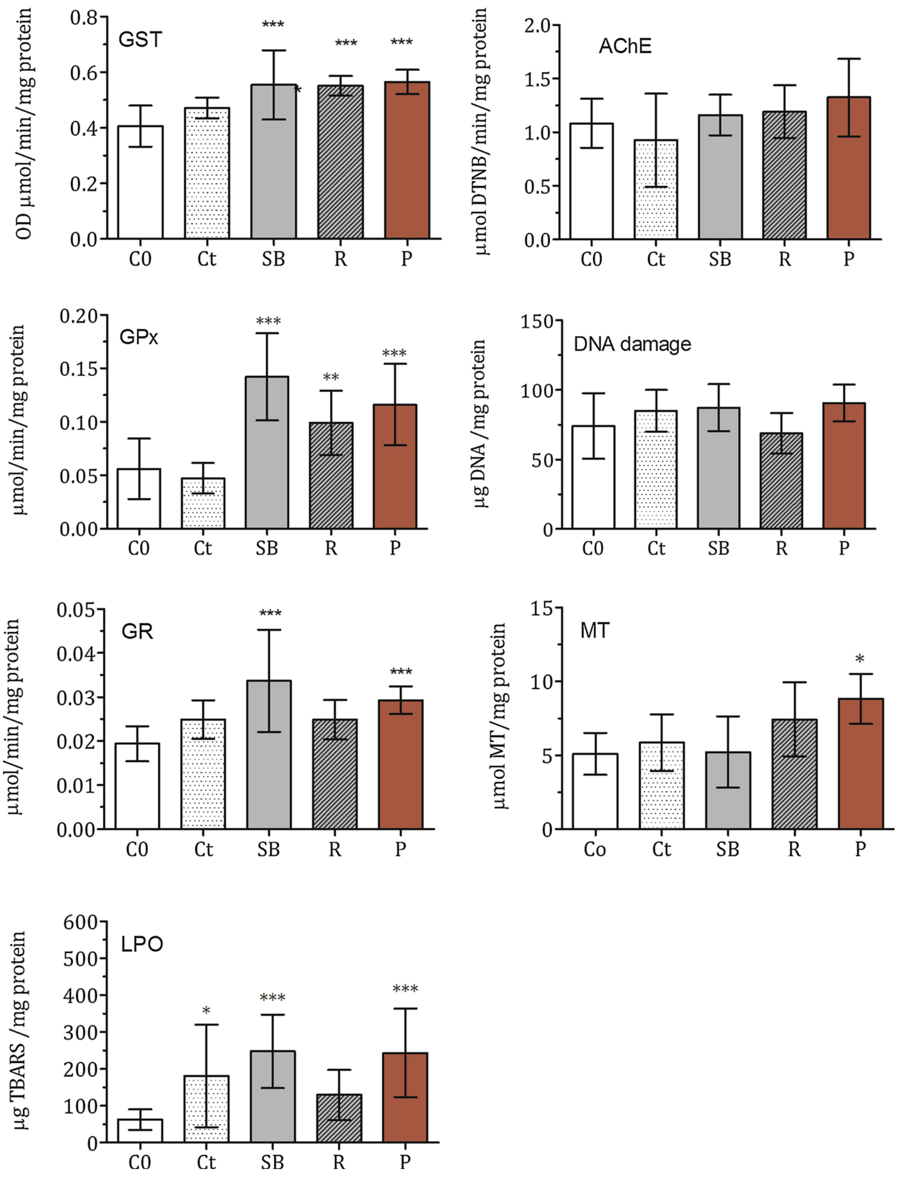

Fig. 3 Representation of glutathione S-transferase (GST), glutathione peroxidase (GPX), glutathione reductase (GR), acetylcholinesterase (AChE), DNA strand damage and lipid peroxidation (LPO) activities determined in the whole tissue of *Corbicula fluminea* after a 7-day exposure to sediments from the RIR (SB and R) and the Perequê Ecological Park (P). Asterisks represent significance level (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) compared with the control treatment at the beginning (C_0) and at the end (Ct) of the tests

Table 2 Pearson coefficients correlating (r) biomarker responses in whole tissue of *C. fluminea* after exposure

	AChE	*MTs*	*GST*	*GPx*	*GR*	*DNA S. Damage*
MTs	−0.184					
GST	0.847***	0.465*				
GPx	−0.558*	−0.127	−0.620**			
GR	−0.648**	0.532*	−0.591**	0.553**		
DNA S. Damage	−0.558*	−0.890**	−0.671**	0.857***	0.558**	
LPO	−0.193	−0.295	−0.142	0.770**	0.216	0.776**

Asterisks show significance level (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

protection against oxidative stress; and neuroprotective mechanisms (Cheung et al. 2005; Mao et al. 2012). The notable MT levels in P (Fig. 3) are coinciding with significant ($p < 0.05$) increase in the oxidative stress (GR, GST, GPx) and the induction of LPO. Therefore, the MT may provide an important role as protection against oxidative stress in order to adapt to the increasing metal concentration in the environment. This activity displayed significant negative correlations ($r = -0.890$, $p < 0.01$) with DNA damage. DNA alterations may also be induced by an interaction with oxygen radicals or as a consequence of apoptosis or necrosis processes (Viarengo et al. 2007). Results of the experiment did not reveal any increase in DNA strand breaks in clams in spite of strong correlations with the antioxidant system activation and neurological inhibition (Table 2).

Metal bioaccumulation Results of metal bioaccumulation in 7-day sediment-exposed *C. fluminea* are shown in Table 1. Arsenic bioaccumulation was significant ($p < 0.05$) in organisms exposed to SB and P sediments. But, Co and Rb were significantly bioaccumulated after 7-day exposure to P sediments. Clams in contact with sediments from R registered a significant Mn concentration in soft tissue of the clam. Co displayed significant negative concentration ($p < 0.05$) after P sediment exposure with respect to the control clams. Metal bioaccumulation for the rest of the studied metals (Ba, Cd, Cu, Cu, Cs, Fe, Ga, Ge, Ni, Sb, Sc, Se, Sn, Sr, Pb and Zn) showed to be not significant ($p < 0.05$) when compared with both controls after exposure.

The concentration of metals accumulated in *Corbicula fluminea* was compared with metal concentrations of the Asian clam collected in the area in previous studies from Guimarães and Sígolo (2008b) and Abessa et al. (2015). In general, values of Cd, Cr, Pb and Zn in tissues of *C. fluminea* were lower in the current study. Guimarães and Sígolo (2008b) found almost the double concentrations of Cr Pb and Zn in tissue of clams collected in SB; however, Cd and Zn concentrations were doubled in R in Guimarães and Sígolo study (Table 3). While Cu concentration in tissue shows an increasing tendency toward the estuary for both studies (this study: 46 < 48, and Guimarães and Sígolo (2008b): 17 < 35), values from the current study were significantly greater. Abessa et al. (2015) reported greater values than this study; especially for lead, clams registered five times the concentration of this element in SB in the sampling survey from 2014.

Triad representation

The indexes of contamination (metal concentrations—As, Ba, Co, Cr, Cu, Ni, Pb, Sr, Ti, U, V, Zn—normalization of the original set of data to organic concentration—I_{cont}), toxicity (reburial and biomarker response—I_{tox}) and metal bioaccumulation in *Corbicula fluminea* (I_{bio}) derived from normalized values and represented in an isometric system configure the classical representation in triangles known as "Triad" (Fig. 4). For this study, even though SB is the station supposedly more contaminated due to the proximity to mining discharges in the RIR, it was chosen as reference site because its I_{cont} demonstrated that this site was the least contaminated. The station R, also

Table 3 Metal concentration values (in mg/kg dw ± SD) found in soft tissue of *Corbicula fluminea* after sediment exposure (in this study) and collected in Sete Barras (SB) and Registro (R)

	SB	R
Cd		
a	0.58 ± 0.18	0.35 ± 0.05
b	0.82 ± 0.02	0.71 ± 0.02
c	0.71 ± 0.15	
Cu		
a	46.2 ± 1.2	48.5 ± 11.7
b	17.6 ± 0.3	35.3 ± 0.7
c	75.94	
Cr		
a	5.5 ± 1.33	5.32 ± 0.76
b	9.64 ± 0.07	3.59 ± 0.12
c	8.80 ± 1.05	
Pb		
a	1.15 ± 0.19	1.55 ± 0.45
b	3.77 ± 0.077	0.51 ± 0.08
c	5.57 ± 0.77	
Zn		
a	76.5 ± 6.6	70.5 ± 13.7
b	181 ± 1.7	116.8 ± 3.3
c	138 ± 15.2	

a: This study

b: Guimarães and Sígolo (2008b)

c: Abessa et al. (2015)

located in RIR, displayed a high value of the contamination index (2.22) as a consequence of Cs (11% of the index), Pb (9.3%), Sr, Ti, Zn and U (approximately 8% of the index value, respectively). Nevertheless, the I_{cont} of P (9.39) showed an elevated value for the same elements, with Sn as the main contributor of the sediment contamination (51% of the index) followed by Ti (14%).

The toxicity index was obtained from the physiological response of the reburial activity (ET_{50}) and the biochemical responses after exposure (AChE, GPx, GST, GR, LPO, MTs and DNA strand damage). The station R displayed the minimum toxicity index value (0.85), being even lower than the value of the reference station SB. Toxic responses were significantly shown by clams in SB, since the toxicity index value calculated for R was 15% lower than SB.

The bioaccumulation results are usually associated with the bioavailability of contaminants in the environment (Martín-Díaz et al. 2008). Thus, the greatest bioavailability of contaminant was determined for R (1.30), whose greater contributor for the index was Cs bioaccumulation (24% or the index), coinciding with the pointed as maximum contaminant, followed by Pb, Sr and Ba (10% of the contribution approximately), which are the other contaminants characterizing the basin. The I_{bio} calculated for P was below the reference station, but Cs and Sn contributed to its calculation (14 and 13%, respectively). In contrast, a lack of data did not allow calculating the case of titanium in P.

In summary, the new indexes normalize the contribution of each parameter (contamination, toxicity and bioaccumulation) by ranking them attending the pollution-environmental degradation index (P_{triad}). The station P in Cubatão River would be 8.99 times more degraded than the station SB from the RIR, while the station R could be considered 2.23 more degraded than the upstream station SB in the Ribeira de Iguape. It has been noted in the relative low toxicological and bioaccumulation indexes calculated that do not correspond in a 100% to the metal contamination. This is because the chemical contamination has two potential different origins (mining and industry); therefore, there is no balanced between metal contamination in the Ecological Park of Perequê (P) and its comparison with the Vale do Ribeira de Iguape (RIR). The chemical levels in this station pointed the greatest pollution-degrading systems associated with other type of contamination; on the other hand, clams displayed the greatest stress associated with bioaccumulation levels below the reference station. This method of data integration is not considered strong enough used alone due to the influence of particular variables in any of the three axes, and additional integration methods such as multivariate analysis must be used to confirm the integration (Riba et al. 2004).

Data integration

The classical triad representation provides a comparative approach against the considered less contaminated station in the study. So that, to integrate all the data and obtain better interpretations, the multivariate analysis approach was conducted to explore variable distributions and analyze the SQT component relationships.

Data integration was conducted using the PCA to the original data set of chemical concentrations and sediment characterization, biological effects and metal bioaccumulation representing the original variables (Table 1; Figs. 2, 3). This clustered the initial

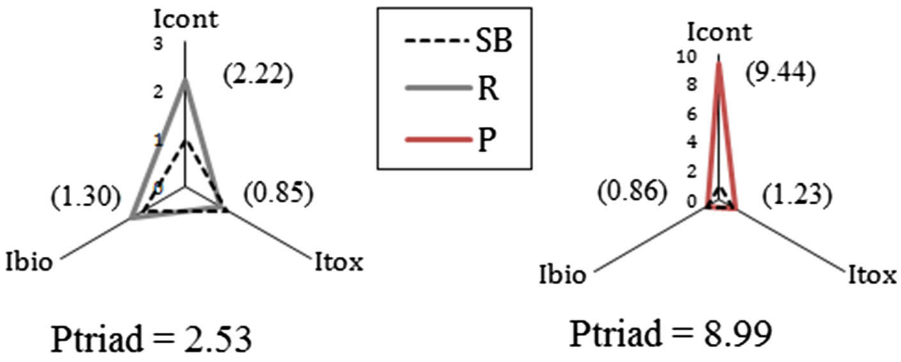

Fig. 4 Classical sediment quality triad ratio-to-reference (RTR) presentation based on the studied stations of the RIR (R and B) and the Perequê sediment (P)

variables into two factors (Table 4), which explained 100% of the original variance. For this work, only variables whose coefficient was above 0.45 were considered components into each factor.

Factor 1 (#1) explained 54.77% of the original variance, and it grouped with positive loading OM, TOC and Co, Mn, Pb and Sr concentrations in sediment and Ba, Co, Cu, Fe, Ni, Pb, Sr, Zn concentration in soft tissue of *C. fluminea* and negative loading of some metal concentrations in sediment (As, Cr, Cs, Cu, Fe, Ni, Sn, Ti and V) and reburial activity and biomarker responses of AChE, MTs and GST. This factor is defined as toxicity of those metals with negative loading when related to biomarker of exposure to metals. Negative values of this factor #1 point certain toxicity in the stations, whereas positive values of the score of factor #1 are associated with bioaccumulation of Pb, Co and Sr related to their total concentration in sediment. Also it is associated with a bioaccumulation of other metal(loid)s not linked with the total concentration in sediment.

Factor 2 (#2) accounted for 45.22% of the original variance. This factor stood for percentage of fines and metal concentration in sediments of Ba, Cs, Pb, Sr, U and Zn and bioaccumulation of Ba, Cs, Cu, Mn, Ni, Pb, Sn and Sr, while it exhibited a negative loading for biomarker responses (glutathione activities, DNA damage and LPO) and As and Cr bioaccumulated in soft tissue. This factor is not identifying with toxicity associated with concentration of metals in sediments. Notwithstanding, positive values of this factor #2 describe metal contamination that provokes bioaccumulation of Pb, Ba, Cs and Sr derived from total concentration in sediment; except for Pb, whose origin is apparently different and more reactive. The loading of other metals permit to define that there is bioaccumulation of other metals not associated with the total concentration in sediment. The negative score of this factor #2 points that toxicity is not associated with the contamination of the metals in sediment, but it is with the bioaccumulation of two metalloids, As and Cr. This might be due to a greater bioavailability of these elements in sediment.

Table 4 Sorted rotated loading (coefficient) matrix of 40 variables on the two principal factors from the multivariate analysis of the single results obtained from the chemical composition, the results from the toxicity bioassays and the bioaccumulation of the sediments from the RIR and P

	#1	#2
% Variance	54.77	45.22
Fines		0.872
OM	0.902	
TOC	0.914	
AsS	−0.984	
BaS		0.969
CoS	0.958	
CrS	−0.975	
CsS	−0.843	0.538
CuS	−0.935	
FeS	−0.979	
MnS	0.990	
NiS	−0.894	
PbS	0.641	0.767
SnS	−0.968	
SrS	0.731	0.682
TiS	−0.984	
US		0.934
VS	−0.966	
ZnS		1.000
ET$_{50}$	−0.902	
AChE	−0.997	
MTs	−0.921	
GST	−0.867	−0.498
GPx		−0.931
GR		−0.969
DNA$_{\text{Damage}}$		−0.916
LPO		−0.972
AsB		−0.972
BaB	0.842	0.539
CoB	0.951	
CrB		−0.996
CsB		0.997
CuB	0.779	0.627
FeB	0.603	
MnB		0.975
NiB	0.758	0.652
PbB	0.640	0.768
SnB		0.975
SrB	0.738	0.675

A graphical representation of the estimated factor values corresponding to each case (studied site) is presented in order to confirm the descriptions of these new factors for *Corbicula fluminea* after 7-day exposure period (Fig. 5). The environmental degradation of each station is defined by each factor description. The

Table 4 continued

	#1	#2
ZnB	0.891	

The new factor, defined factors #1 and #2 are numbered in order of decreasing variance. The new variables represent a loading >0.45

S sediment, *B* bioaccumulated

station of SB is characterized by a positive score of #1 and negative of #2 that describes that there is a bioaccumulation of metals grouped by #1 (Co, Pb, Sr) without toxic effect. The negative value obtained for F2 in SB explains the bioaccumulation of As and Cr that might be responsible for the toxicity on the clam related to the biomarker analysis. The prevalence of this factor also evolves the stress responses provoked in the Asian clam due to exposure of the sediments. Downstream in the RIR, in R, the positive values for both factors indicate bioaccumulation of all metal without effect. This is supporting the idea that contaminants "rest" in the lower part of the basin under mobile forms (Bonnail et al. 2017) to be dumped into the ocean with flooding; this effect agrees with Abessa et al. (2015) study. Finally, the station located in the Natural Park (P) displayed negative scores for both factors; toxic responses are attributed to other contamination sources. Therefore, in spite of being a "non-contaminated" station, it is comparatively more environmentally degraded than the RIR stations. Furthermore, the metal bioaccumulation registered in soft tissue of the clam might have a different contamination source.

Freshwater sediment quality values

Sublethal toxicological responses and metal bioaccumulation in the clams are associated with metal pollution. The presence of metal contamination in the basin is affecting the environmental risk. Therefore, some international organizations, such the NOAA or the Canadian Council, recommend determining interim site-specific sediment quality guidelines based on interactions between chemical mixtures and observed effects as supportive toxicological information related to each region. A monitoring program using multiple biological responses and chemical measurements is able for the identification of impacted environments. The guidelines developed in the current study were obtained as outlined in DelValls et al. (2002). This method allows calculating two concentrations, the maximum (PEL—probable effects level) and the minimum (TEL—threshold effect level), linked to the appearance of biological effect based on the contribution of the new variables (factor score). The sediment quality values calculated link the effect provoked by the metal(loid) concentrations (As, Cr, Cs, Cu, Fe, Ni and Sn and Ti) found in factor #1.

Sediment quality assessments using sublethal responses of benthic organisms, such as changes in the metabolism of contaminants, neurotoxicity, oxidative stress, genetic changes and effects on growth and reproduction, have been used to evaluate contaminated areas (Choueri et al. 2009; Ramos-Gómez et al. 2011a, b). These integrative values have previously implemented by other authors in marine areas (DelValls et al. 2002; Álvarez-Guerra et al. 2007; Martín-Díaz et al. 2008). In contrast, for freshwater environments the values calculated by other authors are adapted in many regions and implemented by many organisms and agencies such as Australia and New Zealand (adapted by Long et al. 1995); extensively used are the values from USEPA (adapted from Smith et al. 1996, and Macdonald et al. 2000); in Europe, The Netherlands counts with contaminated land policies for contaminated freshwater sediment (Visser 1993, 1995), while new values are proposed in England and Wales (Hudson-Edwards et al. 2008) for mining areas; in Spain these areas might be assessed by *C. fluminea* bioaccumulation (Bonnail et al.

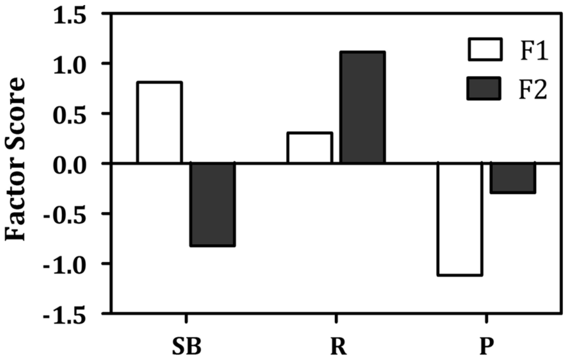

Fig. 5 Representation of factor scores estimation for each of the studied cases evaluated using the clam *Corbicula fluminea* after a multivariate analysis approach (MAA) was applied to the chemical concentration and characteristics of the sediments to link with the biological adverse effect determined via biomarker responses and metal bioaccumulation in the Asian clam

Table 5 Site-specific freshwater sediment quality values (FSQVs in mg/kg) for toxic metal(oid)s (As, Cd, Cs, Cr, Cu, Ni, Pb, Sb, Zn) proposed for the Ribeira de Iguape River lower catchment based on biological responses of the Asiatic clam

	As		Cr		Cu		Cs		Ni		Ti		V	
	TEL	PEL	TEL	PEL	TEL	PEL	TEL	PEL	TEL	PEL	TEL	PEL	TEL	PEL
This study	0.63	1.31	3.5	11.0	6.21	7.32	1.00	1.17	6.78	7.46	42.0	215	1.77	8.00
Visser (1993,1995)	29	55	100	380	36	190			35	210				
Long et al. (1995)	2.0	25	80	370	65	270			21	52				
Smith et al. (1996)	2.0	25	80	370	65	270			18	36				
MacDonald et al. (2000)	9.79	33	43.4	111	31.6	149			23	49				
Hudson-Edwards et al. (2008)	5.9	17	37.3	90	36.7	197			18	36				
Huh et al. (2014)		93		270		390								
Bonnail et al. (2016c)	6.0	171	15.6	38.3	22.5	451			8.94	18.4				

2016c); Korea proposed sediment management standards (Huh et al. 2014).

Values obtained in the current study are based on toxic stress of the Asian clam after 7 days of exposure. The freshwater sediment quality values (FSQV) derived from the this study are overall below FSQV previously calculated (Table 5). The TEL values calculated that provoked effect in the clam corresponded with the metal concentration from R, while the probable effect level (PEL) values displayed affinity with metal concentrations from P. By comparison, low risk is associated with the metal concentration of the RIR; metal concentrations in the basin are much lower than the thresholds established by other authors (values overpass hundred folders for Cr, Cu).

The freshwater sediment quality values calculated in this study for the Brazilian sediments are below the SQGs determined by other environmental agencies (Table 5). Therefore, the toxic element thresholds demonstrate no metal pollution. Just the TEL values of As are similar to Long et al. (1995) and Smith et al. (1996). The lack of FSQV in the regulatories for some elements (Cs, Ti and V) does not allow comparison. The SQG calculated in this study has some restrictions. Values are just comparable in freshwater environments in spite of the proximity with estuarine areas. It is necessary to highlight that thresholds obtained in this study are based on three low contaminated areas carried with one species at different response levels (biochemical, physiological, individual and population). Therefore, these guidelines might be applied to low-polluted areas containing this species. So, further studies should be conducted to improve values; furthermore, other species responses might complete this type of studies. In the current work, these values might be indicative of an environmental stress because the effects measured in the clams correspond to exposure (oxidative stress). To summarize, the sediments of the RIR lower basin constitute a stressed ecosystem but not significant demonstrated pollution or environmental degradation.

Conclusions

The weight-of-evidence approach employed to determine sediment quality assessment of the lower basin of the Ribeira de Iguape River has been applied to three lines of evidence (contamination, toxicity and metal bioaccumulation) using the toxic responses of *C. fluminea*. This was achieved by applying the classical sediment quality triad and a multivariate analysis to a set of stations in the RIR lower basin and the Pequerê Ecological Park. A source of metals has been identified in the Rio do Douro (Pequerê) which seems producing biological effects over the clam after 7-day exposure to sediments. Meanwhile clams exposed to sediment from Sete Barras bioaccumulated As and Cr provoking enzyme stress, in spite of these concentrations do not become from the sediment contamination associated with the total concentration. Although, downstream in Vale do Ribeira (Registro) there is some degradation present due to contamination of Ba, Cs, Pb, Sr, V, and Zn despite that the clam registered bioaccumulation of some elements (Co, Pb and Sr); but Pb bioaccumulation in tissue might have a different origin not associated with the metal concentration in sediment.

This study allowed calculating the sediment quality values of some metals linked to the minimum (TEL) and severe (PEL) effects caused in the clam as: 0.63–1.31 mg/kg As, 3.5–11 mg/kg Cr, 6.21–7.32 mg/kg Cu, 1–1.17 mg/kg Cs, 6.78–7.46 mg/kg Ni, 42–215 mg/kg Ti and 1.77–8 mg/kg V. These values are responsible for the measured effects (exposure effect biomarkers) and were below the thresholds of international regulatories.

Long-term restoration of the RIR basin is determined by low concentrations of metals in sediment. Nevertheless, the MAA revealed metal contamination of As, Cr, Cs, Cu, Fe, Ni, Pb, Ti and V in the lower part of the basin, promoting biomarker stress in clams exposed to Sete Barras sediments and bioaccumulation of Pb, Co and Sr in clams exposed to Registro sediment, although lead displayed a different origin than total metal concentration in sediment. The As and Cr found in tissue from Perequê sediment-exposed clams were associated with toxicity caused by a different contamination source.

In summary, it has been identified a stress in the area of study associated with the link of some metals and biomarkers of exposure that identify a moderate environmental risk in the studied sediments.

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