



Water and sediment ecotoxicological assessment in a river affected by former mining activities

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Abstract. During most of the 20th century, the Ribeira de Iguape River Basin experienced intense activity related to mining. As consequence, it is estimated that the Ribeira de Iguape River received the discharges of approximately 5.5 tons-month⁻¹ of residues rich in As, Cd, Pb, Cu, Cr and Zn. In this study samples of sediment and water were collected at five sampling sites distributed along the river, with the purpose of evaluating environmental quality. To achieve that, chemistry analysis was performed for As, Cd, Pb, Zn, Ni, Mn, Fe, Cr, Al and Cu, together with acute toxicity tests with *Daphnia similis*. The results showed that for all the samples, metals concentrations were below the quality criteria adopted by the State Environmental Agency for both waters and sediments; additionally, no toxicity was observed. The absence of acute toxicity and the low levels of metals are indicative of satisfactory environmental conditions, corroborating to the literature reporting to decreasing contamination by lead and other metals. However, for sediments, higher concentrations of metals were found along the river, in comparison to the reference site, indicating recovery was not totally achieved.

Key words: toxicity, *Daphnia similis*, Ribeira de Iguape river, contamination, metals

Resumo. Avaliação ecotoxicológica de águas e sedimentos em um rio afetado por atividades pretéritas de mineração. Durante a maior parte do século XX, a bacia hidrográfica do Rio Ribeira de Iguape foi palco de intensa atividade relacionada à mineração. Como consequência, estima-se que o Ribeira de Iguape recebeu descargas de aproximadamente 5,5 toneladas/mês de resíduos ricos em As, Cd, Pb, Cu, Cr e Zn. Neste estudo, amostras de sedimento e água foram coletadas em cinco pontos de amostragem distribuídos ao longo do rio, com o objetivo de avaliar a qualidade ambiental. Para tal, análises químicas foram realizadas para As, Cd, Pb, Zn, Ni, Mn, Fe, Cr, Al e Cu, assim como testes de toxicidade aguda com o microcrustáceo *Daphnia similis*. Para todas as amostras, as concentrações de metais estiveram abaixo dos limites máximos pela Agência Estadual de Meio Ambiente, tanto para águas quanto para sedimentos, além disso, não foi observada toxicidade aguda. A ausência de toxicidade aguda e os baixos níveis de metais fornecem indícios de condições ambientais satisfatórias, corroborando a literatura que relata uma diminuição histórica da contaminação por chumbo e outros metais. No entanto, para os sedimentos maiores concentrações de metais foram encontradas ao longo do rio, em comparação com o sítio de referência, indicando que a recuperação não foi totalmente alcançada.

Palavras chave: toxicidade, *Daphnia similis*, rio Ribeira de Iguape, contaminação, metais

Introduction

The Ribeira de Iguape River (RIR) Basin is situated between the Southeast of São Paulo State and the Northeast of Paraná State, comprising 31 municipal districts on approximately 28,000 km² (Lopes Jr. 2007). The river flows towards the Cananéia-Iguape Estuarine Complex, an important estuarine protected area, which was recognized as Atlantic Rainforest Reserve of Biosphere, in 1993, and as a World Natural Heritage site, by UNESCO, in 1999. This region is ecologically very important, as it comprises the major remnant of continuous Atlantic Rainforest of Brazil; from the 7% resting fragments of this biome in the country 21% are located on Ribeira de Iguape River valley; about of 50% under any kind of legal protection.

Between 1918 and 1995, the RIR valley represented an important region for mineral production, comprising nine major mines which actively worked on lead extraction. From these 9 mines, six were located at Paraná State (Panelas, Rocha, Barrinha, Perau, Canoas and Paqueiro) and three were located in São Paulo (Furnas, Lajeado and Espírito Santo) (Corsi & Landim 2003).

Since the beginning of mining activities in the region, metals rich residues, consisting in the tailing and metallurgical slags of blast furnace (Guimarães & Sigolo 2008), rich in As, Ba, Cd, Pb, Cu, Cr and Zn, were directly dumped into the Ribeira de Iguape River. Mean concentrations of Pb, Zn, Cu, Cr and Ba in such residues were 34,018.00; 119,004.33; 2,730.33; 214.17 and 3,656.00 mg kg⁻¹, respectively (Guimarães & Sigolo 2008). Cassiano (2001) estimated that about 5.5 tons per month of residues were discharged into the river along the mining period. This situation persisted until early 1990's decade, and afterwards, the material started to be disposed directly on the ground, on the river banks, exposed to the weathering (Guimarães & Sigolo 2008). The mining activities have ceased in 1995, however, about 89,000m³ of metal rich residues were kept deposited on the ground, close to the river (Franchi 2004).

During the 1980's and 1990's decades, literature reported the occurrence of high levels of metals, especially Pb, in waters and sediments from RIR and from the Cananéia-Iguape Estuarine Complex (Eysink *et al.* 1988, Corsi & Landim 2003, Moraes *et al.* 2004). Since then, there are indications that the Pb contents in waters and sediments from RIR have been decreasing along time (Cunha *et al.* 2005, Lopes Jr. 2007). However, in such investigations, the observed concentrations exceeded the background values proposed to the region (Morgental *et al.* 1975, 1978). More recently, the

São Paulo State Environmental Agency – CETESB – stated that the river is under a process of natural restoration (CETESB 2007), after control actions induced by such agency.

Restoration of an ecosystem can be defined as the process involved in the return of such ecosystem to a state that is as similar as possible to its natural condition (Bradshaw 1997, Garten & Ashwood 2004), and for Ribeira de Iguape River, such process may be closely related to the natural removal of contaminated materials and the dilution promoted by hydrological processes. Despite official reports suggesting environmental restoration, other recent studies have detected persistence of metals in RIR sediments and biota (Guimarães & Sígolo 2008, Rodrigues *et al.* 2012, Melo *et al.* 2012), evidencing that contamination by metals is a not totally resolved problem for RIR, and showing that further studies should be conducted. In addition, the soils in RIR basin favor Pb desorption (Bushle *et al.* 2010), mainly during floods and rainstorms. Thus, the region downstream to mining may still be considered environmentally vulnerable to contamination.

In this context, chemistry analyses of waters and sediments provide information for the management of aquatic environments and the water quality (Förstner 2004). Chemical approach alone quantifies the substances concentrations in the environment, but does not provide information on the bioavailability or potential effects to biota. In its turn, ecotoxicological approach is capable to detect harmful effects to aquatic organisms, but do not indicate which contaminants are responsible for such effects. Thus, the combined use of chemistry and ecotoxicological approaches may be considered more appropriate to evaluate the environmental quality (Chapman *et al.* 1999).

This investigation aimed to evaluate the quality of waters and sediments from Ribeira de Iguape River, an environment affected by mining residues, through the integrated employment of chemistry analyses and toxicity tests.

Materials and Methods

Study Area

The study area is part of RIR basin, between 24°00'S-24°45'S and 47°30'W- 49°30'W, regarding the upper and mid RIR portions. The sampling sites comprised: (P1) or reference site, before the confluence with Criminosas River, this site was situated upper to mining activities and ore processing; (P2) located just after Rocha mine; (P3) close to Ribeira City downtown; (P4) after Plumbum processing plant; and (P5), after the confluence with Pardo River.

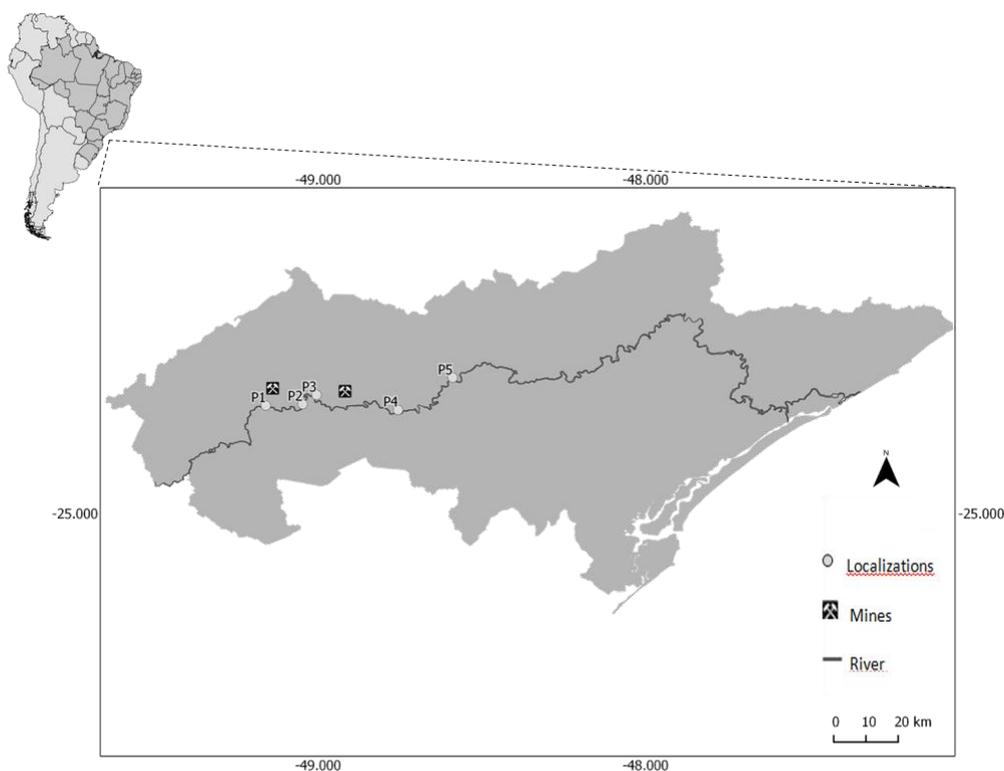


Figure 1. Map showing the localization of sampling sites in Ribeira de Iguape River.

Sampling

The sampling survey was conducted in October 2010. The techniques used to collect waters and sediments, as well as to transport and to store them followed the procedures established by National Guidelines (CETESB 2011). Water samples were collected, in duplicate, directly with polycarbonate bottles (250 ml each), from the river banks. In field, pH, dissolved oxygen and Eh were measured using appropriate devices. Water samples for chemistry were acidified with nitric acid addition ($\text{pH} < 2$), whereas those for toxicity tests were stored frozen. Surficial sediment samples (2-cm surface layer) were collected with small plastic shovels from the river banks, at the water level, and conditioned in sealed plastic bags. Immediately after collection, samples were transferred to thermic containers with ice, and transported to the laboratory. In laboratory, sediment samples to chemistry analyses were frozen at -20°C , whereas those for ecotoxicological tests were stored refrigerated at 4°C .

Sedimentological Analyses

The grain size distribution was obtained by dry sieving method (McCave & Syvitski 1991).

Initially, 150g aliquotes from each sediment sample and the slag were previously dried at 60°C for at least 72h. Then, the material was sieved for 10 minutes in a set of different mesh sizes (2mm, 1,7mm, 1 18mm, 600um, 150um, 75um e $< 75\text{um}$) installed on a shaker. The fractions retained in each mesh were weighted in analytical balance and recorded. The analysis of organic matter was made by the method of loss by ignition in a muffle and gravimetry (Luczak *et al.* 1997).

Chemical Analyses

Water samples were analyzed by Inductively Coupled Plasma Mass Spectroscopy with source (ICP-MS) according to USEPA 6020 (USEPA 1994) and SMEWW 3125B (APHA 1998). Whole sediment samples were analyzed by inductively coupled plasma optical emission spectrometry (ICP-OES) as described by USEPA 6010 (1986) and SMEWW 3120B (APHA 1998), after acid extraction by aqua regia. Concentrations of aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), nickel (Ni) and zinc (Zn) were measured. For water samples, QA/QC procedures involved the use of fortified samples for Lithium, Vn, Cu, Zn, Mb,

Antimony, Pb and evaluation of recoveries, and the use of blanks; quantification limits (QL) were $0.1 \mu\text{g}\cdot\text{L}^{-1}$. For waters, QA/QC consisted of analyzing a standard sample fortified with metals (Standard Water - LCS), and use of blanks; quantification limits were $0.1\mu\text{g}\cdot\text{L}^{-1}$. Recoveries ranged from 83 to 118% and were within the acceptable ranges. For sediments, QA/QC consisted of analyzing a standard sample fortified with metals (Standard Soil - RTC - CRM023) and blanks; QL were 0.5 mg kg^{-1} for most of elements and 0.05 mg kg^{-1} for Cd. Recoveries were within the acceptable ranges, and ranged between 90 and 110% for most elements and 82% for Cd. The laboratory is accredited by ISO17025 standard.

The metals concentrations in sediments were compared to Canadian Sediment Quality Guidelines (SQG) for freshwater (CCME 2002), considering TEL (*threshold effect level*) and PEL (*probable effect level*). The metals contents in water samples were compared to National Standards for freshwaters (Brasil 2011), considering RIR as a class 2 water body; e.g., a watershed which waters may be used for drinking after standard treatment (Brasil 2011). Moreover, to evaluate trends of increase in metals concentrations in waters and sediments collected down the river, the levels of metals in waters and sediments were compared to those obtained in the reference site.

Acute Toxicity Tests

Acute toxicity tests were conducted for waters and sediments (the later by using sediment-water interface approach) with the cladoceran *Daphnia similis*, following the protocol NBR- 12713 from Brazilian Association of Technical Standards (ABNT 2009). Initially, the test chambers were prepared, using 20 ml glass test tubes filled with 10 ml water samples or, in case of SWI test, 2 ml sediment plus 8 ml dilution water (Cesar *et al* 2004); in the SWI test a nylon mesh was positioned on the sediment surface, avoiding the animals to be in direct contact with sediments. In each chamber, 5 neonates of *Daphnia similis* were introduced. Four replicates were prepared for each sample. For both water and sediment toxicity tests, additional replicates were prepared to monitor physical-chemical parameters. In these replicates, measurements of pH, dissolved oxygen and Eh were made in the beginning and at the end of experiments. Control group consisted in organisms exposed to dilution water (reconstituted mineral water, the same water used for culturing test organisms). For SWI tests, as a QA/QC procedure, an additional control was prepared (control net) containing dilution water plus the mesh used in the SWI tests. Water and SWI

controls consisted in 4 replicates each as well. The experiments were kept inside an incubator chamber, at photoperiod (8:16 dark-light) and temperature ($21 \pm 2^\circ\text{C}$). The animals were not fed during the experiments. After 48h, mortality and immobility rates were observed. The results obtained for each sample were compared to the controls, by paired Student *t*' test.

Data Integration

Geochemical and ecotoxicological data were integrated by multiple Pearson correlations and principal component analyses (PCA), with the aid of PAST free software (Hammer *et al.* 2001). PCA was conducted considering sediment toxicity, 9 metals (Cd was not detected), TOC and 7 textural classes of sediment. Data for PCA was log-transformed prior to the analysis. For data interpretation the axes explaining $> 90\%$ variances were considered. The adopted cut-off value was 0.5. No data standardization was employed since this treatment produces similar results than log transformation (Baxter 1995).

Results and Discussion

Water Chemistry

Physical-chemical characteristics of water samples are presented in Table I. The water pH values were generally high, reaching 9.25 (P1), excepting for P2 water, which pH was neutral (7.05). The dissolved oxygen contents were high in all samples, ranging from 11.37 to 11.82 mg L^{-1} ; such values were above the minimum levels established for class 2 rivers (D.O. $> 6 \text{ mg L}^{-1}$) (Brasil 2005). The pH and D.O. values in water samples were within the ranges previously observed for this area (Lopes Jr. 2007), and are consistent to alkaline character of RIR basin. According to Rodrigues (2008), regarding to transport and releasing of chemical elements, pH values are extremely important to the characterization of metals mobility throughout electron exchanges between environmental elements; thus the values found in the present study suggest that conditions do not favor the metals mobility. As the study area is situated in a region rich in carbonate rocks, high pH values would be expected for waters and sediments. Still, such high pH values tend to decrease metals solubility, fostering the removal of dissolved forms and their precipitation or adsorption onto suspended particles (Salomons & Stigliani 1995).

The concentrations of metals in RIR water samples are presented in Table II. In general, concentrations were low (below QL). Due to that, water chemistry data were not used for further integrative analyses. Only for Fe and Al the levels

were above the National Standards recommended for class 2 rivers (Brasil 2011). These elements occurred in high concentrations in all the samples, reaching up to 3X the national standards for Al and 8X those recommended for Fe. Manganese also

showed values exceeding National Standards (Brasil 2011) at P2 and P3. However, according to CETESB (2008), concentrations of Fe, Al and Mn are naturally high in RIR Basin.

Table I. Physical-chemical parameters of waters from Ribeira de Iguape River.

Sample	pH	OD (mg/L)
P1	9.25	11.82
P2	7.05	11.37
P3	8.43	11.55
P4	8.45	11.81
P5	8.89	11.43

Moraes *et al.* (2004) and Cunha *et al.* (2005) suggested that low concentrations of metals in waters were due to the typical alkaline characteristics and low Eh values found in this water body. In 2001, Pb concentrations exceeded the standards, and between 2002 and 2005, conditions

were satisfactory (CETESB 2002, 2003, 2004, 2005). From 2006 to 2009, Pb values exceeding legal limits were observed in at least two monitored sites (CETESB 2006, 2007, 2008, 2009), whereas in 2010, the official report indicated one episode of Pb above the limits (CETESB 2010).

Table II. Concentrations of metals in water samples from Ribeira de Iguape River. Values in bold font indicate exceedences to Brazilian Standards for water quality (Brasil 2011).

Metals	National standards*	Concentration (mg L ⁻¹)				
	mg L ⁻¹	P1	P2	P3	P4	P5
Pb	0.01	< 0.0005	0.0040	0.0028	0.0028	0.0024
Ni	0.025	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cr	0.05	0.0004	0.0051	0.0058	< 0.0001	< 0.0001
Cd	0.001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Cu	0.009	< 0.0001	0.0039	0.0062	< 0.0001	< 0.0001
Zn	0.18	< 0.0001	0.0089	0.0198	0.0035	< 0.0001
Al	0.1	0.2808	1.58	2.31	0.3137	0.3177
Fe	0.3	0.8942	3.42	7.07	0.7623	0.7664
As	0.01	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Mn	0.1	0.0508	0.2490	0.3059	0.0525	0.0492

* according to Brazilian National Standards (Brasil, 2011) for class 2 freshwaters

Sedimentological and Chemistry Analyses

Grain size distribution analyses showed that sediments presented a preponderantly sandy composition, with variable percentages of gravel and fines, and predominance of fine and very fine sands (Table III). The organic matter contents were relatively low (Table III), ranging between 0.85% (P5) and 4.96% (P3).

The concentrations of metals in sediments

were relatively low (Table IV). The lowest Pb concentration was observed in the reference sample (3.5 mg kg⁻¹), which was even lower than the proposed background for RIR basin (Morgental *et al.* 1975, 1978). On the other hand, the sediment sample from P4 presented the highest concentrations of all analyzed elements. The Cd levels were below the quantification limits for all samples. Arsenic occurred in low concentrations and was not detected

in two samples (P1 and P3). The concentrations of all analyzed metals were below TEL, when compared to the Canadian SQGs (CCME 2002). Regarding to the concentrations of Al, Fe and Mn,

the RIR basin presents naturally high levels of these metals (Lopes Jr. 2007), and the observed values are compatible to those founds by CETESB (2010) and Alba *et al.* (2008).

Table III. Grain size distribution and organic matter in sediments from Ribeira de Iguape River and in mining slags deposited by the river.

	Grain Size (%)							Organic Matter (%)
	G	VCS	CS	MS	FS	VFS	S+C	
P1	28.21	3.19	2.82	2.35	27.03	27.10	9.30	1.74
P2	3.86	2.67	9.86	17.12	32.10	23.67	10.72	1.21
P3	1.46	0.00	5.03	10.79	31.16	31.62	19.94	4.96
P4	1.08	4.48	8.72	7.02	41.70	15.44	5.56	1.41
P5	15.44	1.43	2.02	5.97	24.73	30.39	20.01	0.85

G: gravel; VCS: very coarse sand; CS: coarse sand; MS: medium sand; FS: fine sand; VFS: very fine sand; S+C: silt and clay (mud)

The comparison of metal concentrations in sediment samples with the values obtained for the reference site analyzed metals provided additional information on the contamination. In P4 sediments, all analyzed metals were enriched; Pb concentrations were 7 times greater whereas Cu and Zn

concentrations were twice the values in P1. The values decreased towards down the river (P5). Regarding to Pb, in all samples the levels were higher than those observed in the reference sediments (P1).

Table IV. Concentrations of metals in sediment samples from Ribeira de Iguape River (dry weight).

Metals	TEL	PEL	Concentration (mg kg ⁻¹)				
	mg kg ⁻¹		P1	P2	P3	P4	P5
Pb	35	91.3	3.5	6.2	4.6	25	15
Ni	18	35.9	4.6	4	2.7	8.5	5.2
Cr	37.3	90	11	9.4	5.7	14	8.7
Cd	0.6	3.5	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05
Cu	35.7	197	7.3	7.8	5.2	15	9.1
Zn	123	315	20	22	17	49	35
Al			2783	2626	2323	4960	2947
Fe			10082	8533	5917	12615	7540
As	5.9	17	< 0.5	0.8	< 0.5	2.4	0.9
Mn			182	265	161	287	253

Morgental *et al.* (1975, 1978), based on 1296 sediment samples from potential control areas in RIR basin, proposed background levels for Pb in sediments for the RIR basin, which were between 12 mg kg⁻¹ and 16 mg kg⁻¹. Such values were similar to that reported to the reference site (P1) by Guimarães (2007). If background values are considered, only

sediment from P4 presented Pb contents above such values (25 mg kg⁻¹), whereas for the other samples, Pb concentrations were below background values (Morgental *et al.* 1975, 1978). Besides, the monitoring program made by State Environmental Agency has systematically registered values lower than the background; however, at same time, Pb

concentrations exceeding TEL have been routinely observed by such monitoring (CETESB 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010) in RIR sediments. On the other hand, low concentrations of Pb in sediments from RIR were recently found by Melo *et al.* (2012).

Sediment and Water Toxicities

During both toxicity tests, water conditions in test chambers were considered appropriate, within the ranges recommended by the test protocol (ABNT 2009). No water or sediment samples exhibited significant acute toxicity (Table VI). The absence of

acute toxicity in RIR waters are in consonance to the chemistry data, as since 1997 the concentrations of most metals have been considered adequate, when compared to legal standards (CETESB 2000). Such results corroborate the lack of acute toxicity in the monitoring conducted by CETESB. However, such monitoring has systematically detected chronic toxicity in RIR sediments (CETESB 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010), suggesting recovery of sediment quality is not totally achieved.

Table V. Results of acute toxicity tests of waters and sediments from Ribeira de Iguape River to *Daphnia similis*.

Sample	Water		Sediment-Water Interface	
	Mean immobility plus standard deviation	Conclusion	Mean immobility plus standard deviation	Conclusion
P1	0 ±(0)	NT	0.25 ± (0.5)	NT
P2	0 ± (0)	NT	0.50 ± (0.6)	NT
P3	0.50 ± (0.6)	NT	0 ± (0)	NT
P4	0.50 ± (1.0)	NT	0.25 ± (0.5)	NT
P5	0.25 ± (0.5)	NT	0 ± (0)	NT
Control	0 ± (0)	NT	0.50 ± (0.6)	NT

NT – not toxic / T – toxic

Data Integration

Previous studies (Salomons 1994, Fialkowski *et al.* 2003, Angelo *et al.* 2007, Karbassi *et al.* 2008 and Besser *et al.* 2009a) have shown that areas under influence of mining activities present environmental fragilities or contamination (Yau & Gray 2005). Besser *et al.* (2009) verified that Pb levels in river sediments could produce chronic toxicity even when below reference values. In this study, no acute toxicity occurred for water and sediments. However, positive correlation between sandy fractions and metals may be related to the nature of the mining residues, which are thick (mainly gravel and coarse sands) and are comminuted when residues are transported downstream (Guimarães 2007).

Despite acute toxicity was not observed, mortality rates among organisms exposed to sediment correlated positively to all detected metals ($p < 0.05$) (Table VII), which suggests a slight influence of metals, although not significant enough to produce acute effects. The PCA results indicated that the first 3 axes explained more than 83% variances (63.81%; 19.57% and 10.05%, respectively). Axis 1 accounted for 63.81% variances and showed association of metals,

Daphnia immobility, and sand contents (excepting very fine and medium sands); Axis 2 accounted for 19.57% variances and basically showed associations between grain size aspects themselves and the Axis 3 (10.05% variances) evidenced some association between Cu, Zn and muds (Table VIII). A cluster analysis was used to evaluate similarity between samples, and the result grouped sites P2 and P5, with intermediary levels of metals (including Pb), closer to P1 (clean site). P3 was separated from the others, as it presented lower levels of contaminants (and intermediary concentration of Pb), high levels of OM and fines. P4 separated alone as the most contaminated sample (Figure 2).

Still regarding to sediments, samples were predominantly composed by fine and very fine sands, indicating the sampling sites did not constitute depositional areas. The low concentrations of metals in sediments, and the lack of acute toxicity possibly could be explained by the sandy nature of sediments. However, some association among metals, fine sands and *D. similis* mortalities were evidenced (Tables VII-VIII). In addition, RTR values indicated some enrichment in Pb levels, in comparison to the reference site, which could be detected in all samples, suggesting the influence of

former mining activities, at P2 and especially at P4. Sample from P4 exhibited enrichment trends for other elements present in mining residues, as Cu and Zn. The worse conditions observed at P4 probably are related to the concentrate residues of Plumbum

plant, and the further decreasing of metals levels at P5 can be associated to the diluting effect caused by Pardo River, as this site was located down to the confluence of both rivers.

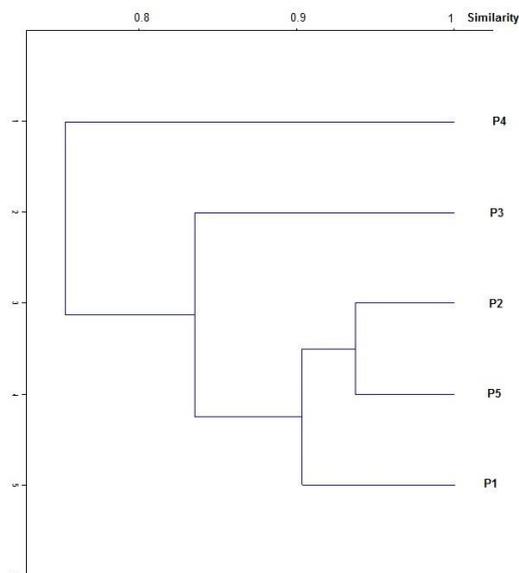


Figure 2. Cluster analysis (Bray Curtis, UPGMA) with Ribeira e Iguape River sampling sites, considering sediment properties, metals concentrations and toxicity.

Table VI. Pearson's correlations among sediment geochemical parameters and toxicity (weaker correlations are marked in italic; stronger correlations are marked in bold – for $p=0.05$).

	G	VCS	CS	MS	FS	VFS	S+C	% OM
% OM	-0.50	-0.67	-0.09	0.14	0.03	0.45	0.45	-
Pb	0.18	0.53	0.26	-0.20	0.62	-0.69	-0.34	-0.43
Ni	0.47	0.81	0.28	-0.35	0.64	-0.84	-0.65	-0.57
Cr	0.63	0.98	0.34	-0.35	0.58	-0.88	-0.89	-0.66
Cu	0.34	0.78	0.39	-0.21	0.71	-0.87	-0.63	-0.55
Zn	0.28	0.61	0.23	-0.26	0.58	-0.71	-0.40	-0.52
Al	0.34	0.77	0.39	-0.27	0.78	-0.88	-0.66	-0.40
Fe	0.61	0.98	0.37	-0.34	0.64	-0.90	-0.92	-0.56
As	0.09	0.67	0.53	0.02	0.76	-0.86	-0.54	-0.49
Mn	0.01	0.62	0.54	0.24	0.48	-0.72	-0.45	-0.76
Mortality	0.37	0.94	0.63	-0.06	0.79	-0.96	-0.97	-0.42

G: gravel; **VCS:** very coarse sand; **CS:** coarse sand; **MS:** medium sand; **FS:** fine sand; **VFS:** very fine sand; **S+C:** silt and clay (mud)

The results found in this investigation present coherence when compared to published data, which suggest that metals levels in RIR sediments and waters are apparently decreasing (CETESB 2007, 2008, 2009, Cunha *et al.* 2005, Lopes Jr. 2007). Thus, the results give support, at least partially, to official reports from the State Environmental Agency on natural attenuation in RIR

catchment (CETESB 2007). Natural restoration has been observed worldwide in rivers contaminated by metals from mining activities (Bradshaw 1997, Moore & Langner 2012); however this process may be slow and depends on several factors (Cooke and Johnson, 2002). Despite that, in RIR, literature indicates that suspended sediments seem to be still enriched by Pb (Guimarães & Sígolo 2008) as well

as bioaccumulation of metals in bivalves still occurs (Guimarães & Sígolo 2008a, Rodrigues *et al.* 2012), showing that the restoration process is not complete. This is corroborated by the remaining enriched levels of metals in sediments, especially Pb, which

suggests a residual influence of mining contamination on RIR. Besides, lacking of acute toxicity does not necessarily indicates absence of degradation, since the monitoring made by CETESB has detected chronic sediment toxicity.

Table VII. Vectors obtained by PCA using geochemical and ecotoxicological data for sediments from Ribeira de Iguape River (cut-off value >0.40).

Variables	PC1	PC2	PC3
G	0.40	0.88	0.26
VCS	0.90	0.13	0.38
CS	0.48	-0.83	0.28
MS	-0.18	-0.94	0.06
FS	0.74	-0.51	0.09
VFS	-0.94	0.25	-0.21
S+C	-0.79	0.02	-0.61
%OM	-0.59	-0.25	0.03
Pb	0.84	-0.02	-0.53
Ni	0.97	0.16	-0.19
Cr	0.95	0.22	0.23
Cu	0.97	0.01	-0.23
Zn	0.88	0.06	-0.48
Al	0.96	0.02	-0.17
Fe	0.94	0.18	0.30
As	0.92	-0.22	-0.33
Mn	0.77	-0.29	-0.29
Mortality	0.89	-0.11	0.43

G: gravel; VCS: very coarse sand; CS: coarse sand; MS: medium sand; FS: fine sand; VFS: very fine sand; S+C: silt and clay (mud)

In addition, Mahiques *et al.* (2009) identified correlation between the input of metals and changes in infaunal benthic community in the Estuarine Complex of Iguape and Cananéia, at the mouth of RIR, detecting a continuous enrichment of Pb and Cr in estuarine sediments, which was, in its turn, directly related to the former mining activities, situated upper RIR; according to such authors, after 10 years of the last mine closure, the region still receives large amounts of metals. Moraes *et al.* (2004) estimated that about 840,000 tons of Pb are transported downstream river each year, mostly associated to fines. Such transport of large amounts of metals, especially Pb, may explain the concentrations exceeding TEL observed in the

downstream estuarine areas (Eysink *et al.* 1988, 1990, Mahiques *et al.* 2009). According to Costa *et al.* (2009) rainstorms have major role in removing surface soils and contaminants along the basin. Thus, there are enough evidences indicating that despite the natural restoration process which is in progress along RIR, Pb may still represent any threat to aquatic biota, especially those from the estuarine areas situated downstream.

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