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Ecotoxicological monitoring of urban reservoir sediment located in the coastal region of northeastern Brazil

Monitoramento ecotoxicológico do sedimento de reservatório urbano localizado em região costeira do nordeste do Brasil

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RESUMO

A lagoa de Extremoz, no nordeste do Brasil, é um reservatório de água urbano, utilizado para atividades domésticas e industriais. O objetivo deste trabalho foi analisar a qualidade do sedimento de fundo de três estações de amostragem (T1, T2 e T3) com níveis de ocupação humana heterogênea. As amostras de sedimentos dessas estações foram coletadas mensalmente (fevereiro à novembro de 2014). Foram analisados os teores de metais (Cd, Cu, Cr, Pb, Ni, Fe e Zn), conteúdo de matéria orgânica (MO), carbonato ($CaCO_3$), granulometria e toxicidade aguda do sedimento em bioensaios de dez dias. com o anfípode *Hyalella azteca*, bem como dados mensais de precipitação e ocupação do entorno do corpo d'água. O número de eventos tóxicos para *H. azteca* foi maior nos meses chuvosos (março, maio, junho, julho e setembro) e na estação mais urbanizada (T2). Algumas variáveis apresentam correlação positiva, como os teores de metais pesados, M.O. e silte-argila. Isso é esperado porque a M.O. e as frações finas do sedimento têm uma área superficial específica mais alta e, portanto, mais locais de ligação para os metais pesados.
Palavras-chave: Hyalella Azteca; Bioensaio; Metais pesados; Contaminação ambiental

ABSTRACT

The Extremoz lake, Northeastern Brazil, is an urban water reservoir, used for domestic and industrial activities. The objective of this work was to analyze the quality of the bottom sediment of three sampling stations (T1, T2 and T3) with heterogenous human occupation level. The samples of sediment from these stations was collected monthly (February to November 2014). This analysis involved the concentration of metals (Cd, Cu, Cr, Pb, Ni, Fe and Zn), content of organic matter (OM), carbonate ($CaCO_3$), granulometry and acute toxicity of sediment in ten days bioassays with the amphipod *Hyalella azteca*, as well as monthly rainfall data and the occupation of the surroundings of the water body. The number of toxic events to H. azteca is higher in the rainy months (March, May, June, July and September) and in the more urbanized station (T2). Some variable exhibit positive correlation, as the heavy metals, OM and the silt-clay grain sizes. This is expected because the OM and the fine fractions of the sediment have a higher specific surface area and hence more binding sites for the heavy metals.
Keywords: Hyalella Azteca; Heavy metal; Bioassay; Environmental contamination

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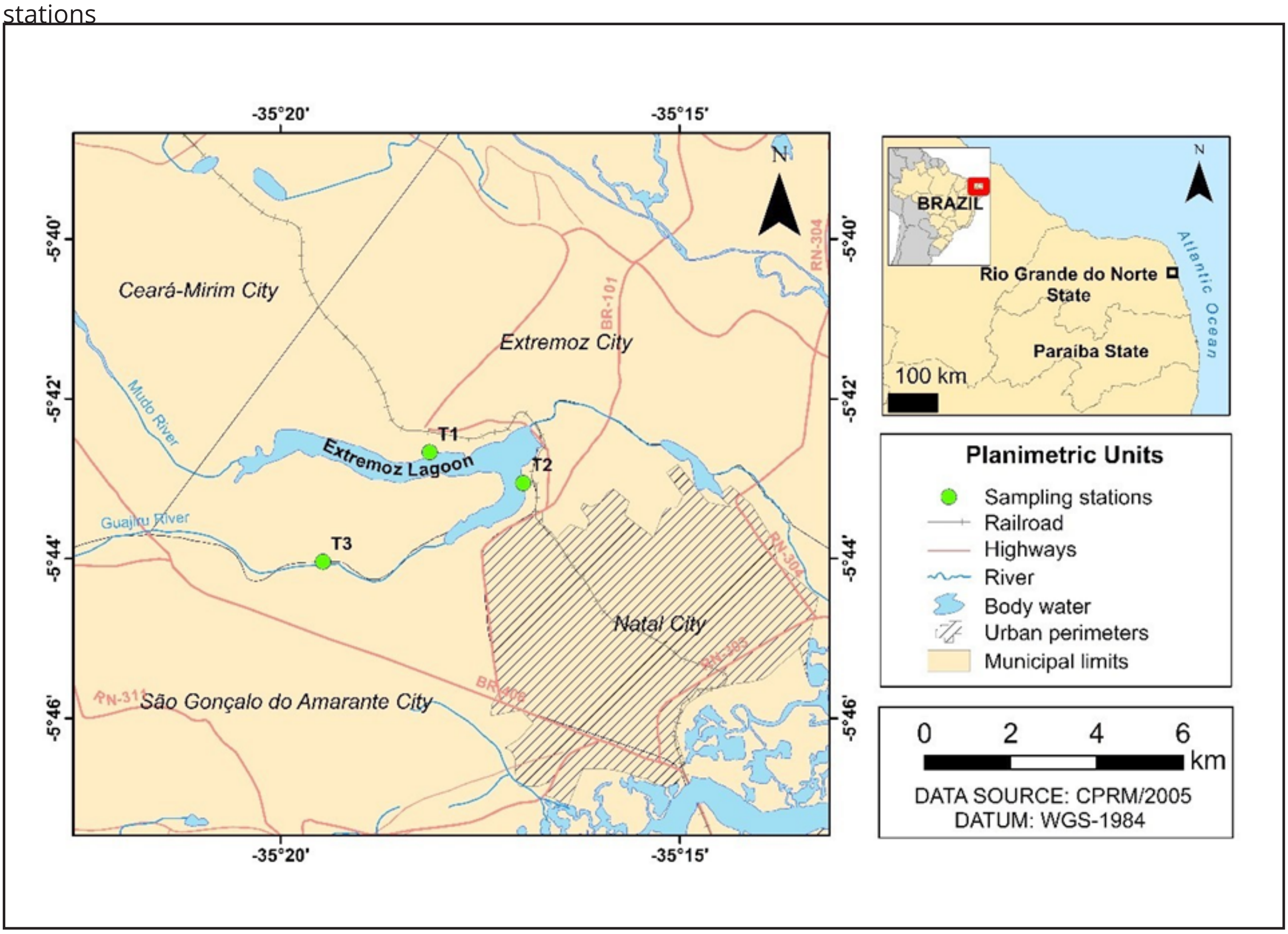
1 INTRODUCTION

Regions of intense urban growth depend on the availability and quality of water, although the urbanization process is one of the main responsible for diffuse launching of contaminants in superficial reservoirs. Most of the damage, caused to water bodies is due to the lack of minimum investments on urban infrastructure works, basic sanitation and drainage.

The Rio Doce watershed with 387.80 km2, occupies about 0.7% of the Rio Grande do Norte state territory. It extends from the semi-arid to the coastline. Even near the coast zone the water deficit is elevated, mainly between September and February. The Extremoz Lake, main reservoir of this watershed (Figure 1), is located to the north of the municipality of Natal, northeastern region of Brazil, and supplies approximately 300,000 inhabitants. In the surrounding area of the Lake, since the seventies, a process of landscape demeaning happens because of the installation of an industrial pole and human settlements. In turn, this process has not been accompanied by the infrastructure needed to lessening the possible impacts to the Lake (Melo et al., 2014, 2012). These lake, is a water body located in the urban center and has been object of research in other studies, which have pointed out the decrease in the quality of its water (Barbosa et al., 2010; Jerônimo e Souza, 2013).

Figure 1 – Map of the location of the study area, drainage area, paved roads, municipalities and sampling stations



As well as other reservoirs, the Extremoz Lake is the destination of potentially harmful substances, including metals such as Cd, Cr, Pb, Ni, Zn and Cu. These metals may have natural origin, from the weathering of the crystalline basement; however,

when the concentration of these metals exceeds the expected for the local geological context, it is probable that human activities will be responsible for this increment (Callender, 2003; Sindern et al., 2007). In this way, several sources can be quoted, such as inappropriate disposal of household, sanitary, industrial effluents, as well as the wear of equipment used in industry, automobile tires and household waste accumulated in irregular form (Förstner, 1981).

The ecosystems have the natural capacity to assimilate the input of possible contaminants. When the quantity of contaminants exceeds the threshold of this capacity, problems such as reducing the recruitment rate of populations can be verified. However biological processes occurring in aquatic ecosystems are difficult to observe, which makes necessary the monitoring of aquatic ecosystem compartments, to prevent the occurrence of critical moments (Zagatto et al., 2014). Among these compartments the sediment has long been neglected, but it is the testimony of the physical, chemical and biological processes of what happens in the water column and nearby the hydric body (Tundisi e Tundisi, 2012).

Amphipods species such as *H. azteca*, are widely used to determine acute (lethal effect) or chronic (non-lethal effect) toxicity

of freshwater and brackish water sediment samples. These amphipods present epibentonic lifestyle, sexual reproduction, and feed on organic matter in suspension, filamentous algae, diatoms, parts of aquatic macrophytes and bacterioplankton. These organisms also serve as food for larger predators like other crustaceans, insects, fishes and birds. Hence a decrease in the recruitment rate of these organisms, may cause the imbalance in the whole biological chain of the affected ecosystem (Burton Jr et al., 2002).

Water bodies such as the Extremoz Lake, located in industrial areas, with accelerated urban growth and maintaining agricultural activities, are subjected to contamination by metals from various sources, as expected. The multipurpose of water and soil near reservoirs implies in more complex damage and conflicts between users; to solve these events, larger and more complex series of data are required. The aim of this study was to investigate the surface sediments of the Extremoz Lake, through the study of the chemical composition, granulometrical characteristics and the response to toxicity tests, with monthly frequency, for a period of nine months, between March and November 2014. This ecotoxicological monitoring of sediments of the Extremoz Lake, pioneer for your duration, sought information on the spatial and temporal variations of the concentration of heavy metals, and its relationship with the results obtained in ecotoxicological tests.

2 MATERIAL AND METHODS

2.1 Study Area

The Extremoz Lake is the largest reservoir of freshwater in the Doce river watershed. It is located among the municipalities of Extremoz, São Gonçalo do Amarante and Natal (Figure 1) about six kilometers away from the South Atlantic Ocean. The annual average precipitation ranges between 1300 mm to 2000 mm. The prevailing climate in the region, is the equatorial of savanna type with dry summer (As) (Kottek et al., 2006).

2.2 Sampling and samples preservation

The sampling occurred monthly in the early hours of the morning. Decontaminated and nontoxic plastic recipients, with lid, were used for sediment sampling. In the field, the samples were divided into two aliquots, one for physical and chemical analyses and one for ecotoxicological tests; the second aliquot required to be refrigerated until the moment of analyses and tests.

The choice of sampling locations was performed after visits to the area and preliminary ecotoxicological tests; the intention of this survey was to choose three stations in locations with different types of occupation. The T1 sampling station (245014.00 mE e 9368196.00 mS) is located in the north of Extremoz Lake, in an area used for leisure; less than 20m away from the margin, the presence of several provisional installations of bars and restaurants was verified; In addition, there are several spots of rainwater flow and the native vegetation has been replaced by exotic vegetation (grasses and medium-sized trees). In the south of the Lake, the station T2 (247155.00 mE e 9367468.00 mS) is located in an area of private farms; at 300m away from the collection site, in the east direction, there is an active railway; in approximately 350m there is an alcoholic beverage industry and, at 400m in the southwest direction, a water collection station for public supply in the region (70% of the water used for approximately 300,000 inhabitants are captured in this system). The station T3 in Guajiru river (242520.00 mE e 9365637.00 mS) is inserted into a typically rural landscape; the waters at that point drain slowly and much of the surface water is covered by aquatics macrophytes. In June, the increase in the water level and consequent loss of safety conditions disabled the collection at this sampling station.

2.3 Analyses of seasonality and soil occupation

In the database of the National Institute for Spatial Research (INPE), information was obtained regarding the average accumulated precipitation and satellite imagery of the drainage area of the Extremoz Lake. The average accumulated precipitation is important to compare the monthly precipitation during the studied period (2014) in relation to the accumulated monthly average precipitation history (1960 and 2013) and to the classification of Koppen & Geiger (Kottek et al., 2006)). With software ArcGIS 10.0, satellite images of the Extremoz Lake in 2014, were classified regarding the colors, in an unsupervised procedure for categorizing the pixels.

2.4 Physical and chemical analyses of sediment

For determining the organic matter content (OM) the method described in Lima et al. (2006) was used. In this method 10g of samples were placed in ceramic crucibles and taken to muffle furnace for 5 hours at $600\ ^{o}C$. The lost value is the amount of incinerated organic matter, leaving only the minerals present in the sediment.

The carbonate content ($CaCO_3$) was obtained from digestion with acetic acid (CH_3COOH) to 4% in 10g of sediment. At the end of this procedure the lost mass corresponds to the acid-digested carbonate (Herrmann, 1975).

The extraction of metals in sediment samples was carried out by mixed solution ($HCl\ 0{,}5N + H_2SO_4\ 0{,}025N$). The concentration of metals was analyzed by flame atomic absorption spectrophotometry (AAS), and measured the contents of Fe, Zn, Cu, Ni, Cd and Cr, in the fraction < 2mm, in approximately 5g of dry sample. For comparison parameter the maximum and minimum levels (ISQL, PEL e TEL) adopted by the CMME Canadian Environment Ministerial Council were used.

The particle size characterization consisted of sieving 30g of sediment samples, on automatic agitator for 15 minutes. Stainless steel sieves (Tyler series) were used to split the sediment into gravel (> 2mm), coarse sand (> 0,6mm), medium sand (0,3mm), fine and very fine sand (> 0,063mm) and silt-clay (< 0,063mm). The weight retained in each sieve is measured and the fraction with the highest mass indicates the particle size dominance of the sediment.

2.5 Ecotoxicological tests of sediment

To evaluate the toxicity of sediment samples, the *H. azteca* microcrustacean was used, cultivated in decontaminated plastic containers (aquariums) with maximum volume of 12 L. Commercial water was used as culture water and control water, the substrate used in the culture was dehydrated banana leaves. The composition of the culture water is shown in Table 1. The array aquariums contained 300 adult or juvenile organisms, without pairing. To maintain control of the organisms' ages, the newborns have been removed from the aquariums every seven days and relocated to other containers for maturing, until they had the suitable age for the tests. The renovations and cleaning of aquariums have occurred every 14 days. The organisms were fed daily with 10 mL of mixture containing fish food extract, vitamin complex and primrose oil.

In the bioassays, four replicates were prepared for each sediment sample; those consisted of 1 L flasks, containing 100 mL of sediment sample and 200 mL of cultivation water. The semi static chronic test regime was adopted and 10 newborns (7 to 14 days) per replica were exposed. The renovations occurred in 72 hours, 144h and 192h, when 120mL de water was withdrawn from the container; then the same amount of the cultivation water was restored; the organisms were fed with 3ml of the mixture. At the end of the test (240h) the total of survivors was counted and their length was measured. The test was considered valid when the survival rate in the control was equal to or greater than 80%.

Table 1 – Measured background concentrations of elements in the culture and control water

Parameter	Mean±SD		
pH		μS/cm	0.17±6.01
Eletrical Condutivity			66.70±12.87
Barium	(Ba)	mg.L^{-1}	0.05±0.03
Calcium	(Ca)		1.07±0.65
Potassium	(K)		2.88±1.53
Sulfate	(		0.71±0.08
Fluoride	(F)		<0.01
Nitrate	(NO_3)		1.42±0
Strontium	(Sr)		0.01±0
Magnesium	(Mg)		1.17±0.65
Sodium	(Na)		7.78±0.02
Bicarbonate	(HCO_3)		9.42±8.05
Chloride	(Cl)		11.80±1.53
Bromide	(Br)		0.03±0

2.6 Environmental risk assessment

One-way Anova followed by the Tukey test were used for comparison among the average of the physical and chemical variables of the sediment, in different locations and sampling periods, through the R statistical software. The Spearman Ranked Correlation in the Software Statistic 7 was used to assess correlations among the values obtained from the physical, chemical and ecotoxicological analyses of water and bottom sediment ($P < 0{,}005$).

Toxstat software 3.3 was used to assess the toxicity of samples from data obtained in the chronic ecotoxicological tests. First it was evaluated the normality and homocedasticity of the data, by Qui-Square (x^2) and Barttlett tests, respectively. If these conditions were met, the Tukey parametric statistical method was used, in order to analyze the significant difference between the control and each group of samples; if difference was established the sample was considered toxic and marked with an (*). When the data observed in the ecotoxicological tests did not meet the assumptions of normality or homocedasticity, the Kruskal Wallis test was used; with significant difference between the control and the group of samples, those were considered toxic (Zagatto et al., 2014).

The enrichment Factor (EF) (Yongming et al., 2006), calculated from the formula (1), was used to evaluate the degree of anthropic influence in the environment, from the classification shown in Table 2.

Table 2 – Classification of the Enrichment factor (EF)

	Classification of the Enrichment
FE < 2	Enrichment deficiency
FE = 2−5	Moderate enrichment $*$
FE = 5−20	Significant enrichment $**$
FE = 20−40	High enrichment $***$
FE > 40	Extremely high enrichment $****$

$$EF = \left(\frac{\left(\frac{Cm}{Cvrl}\right) Sample}{\left(\frac{Bm}{Bvrl}\right) VRL} \right. \quad (1)$$

where:

- *Cm* - Concentration of a particular element in the place of interest;
- *Cvrl* - Concentration of the reference element in place of interest;
- *Bm* - Reference value of the study element (background);
- *Bvrl* - Local reference value of the reference element (background).

Iron was chosen as reference element, as it presents the smallest geochemical mobility among the other analyzed metallic ions (Holland et al., 2005). The background reference values were established as average of the analytical results obtained in two samples reported in the Jundiai-Potengi river watershed (P01 and P02), by Sindern et al. (2007); these locations are subjected to the same climatic and geological regime of the Extremoz Lake and present little or no influence anthropic.

3 RESULTS

3.1 Accumulated monthly precipitation

The accumulated monthly precipitation between the years of 1960 and 2013 (historical trends) and accumulated monthly precipitation in 2014 was presented in Figure 2; the comparison between this two information was helpful to define which of the studied months were rainy or not. The months of sampling were divided into two groups: the rainy with precipitation higher than 100ml (March, May, June, July and September) and the dry months, with accumulated monthly rainfall less than 100ml (April, August, October, and November).

The soil use and occupation along the Extremoz Lake, displayed in Figure 3, showed that an intense occupational process happened in the North Arm (T2) of the Lake. Between 2004 and 2014 urban occupation begins to expand towards T3 (west) station. The urban area located near the T1 station has also become more evident over the years. In 2014 part of the margins of the Lake still presented typical configurations of rural areas, with development of agricultural activities.

Figure 2 – Monthly precipitation in the region of Extremoz Lake. Bars represents studied period (2014) and line is the historical trends (1960-2013)

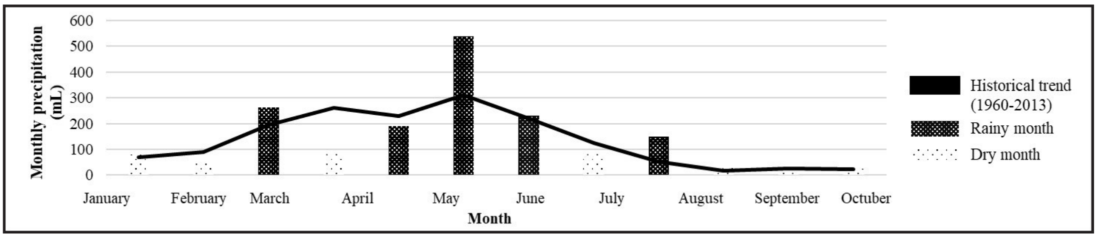
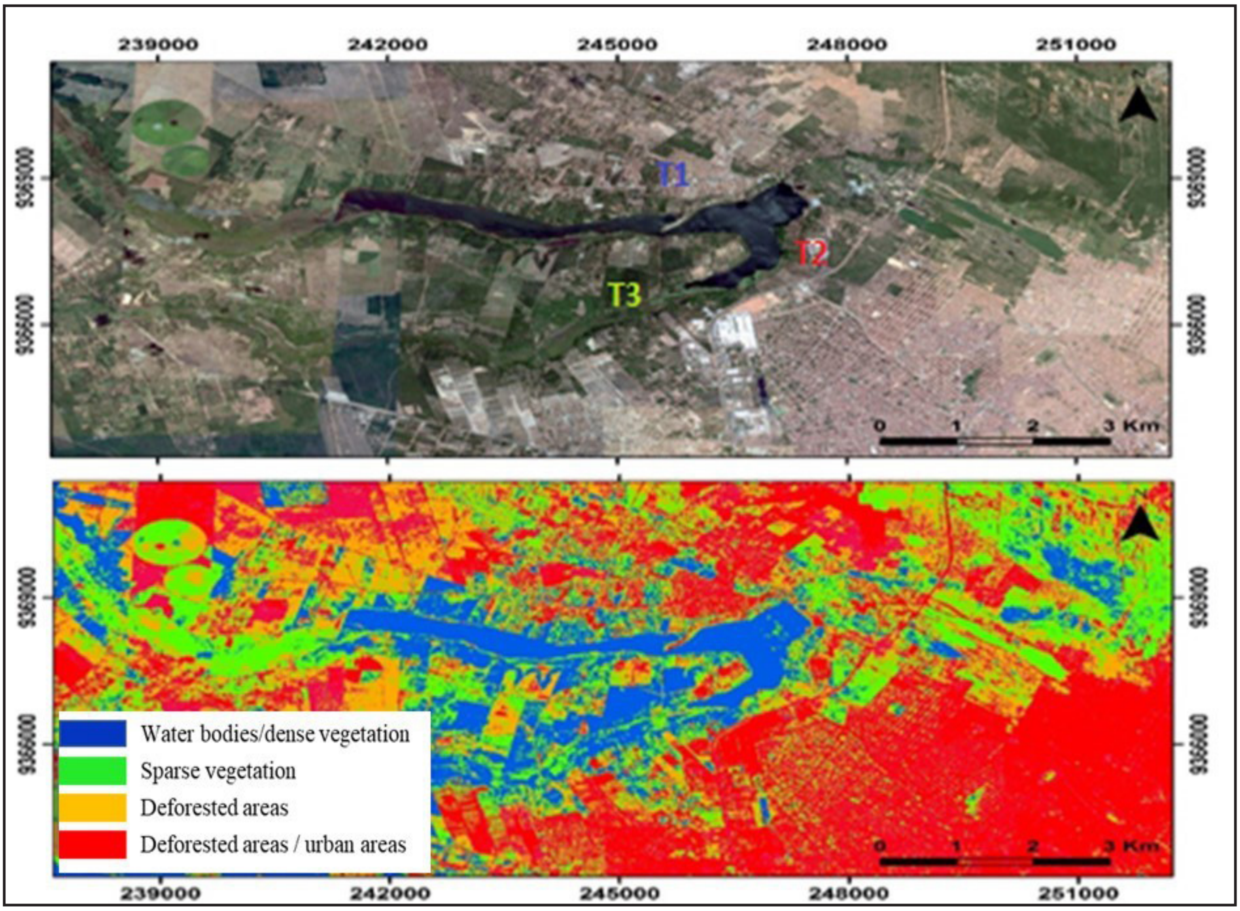


Figure 3 – Comparison of urban occupation between the periods of 1984, 2004 and 201

Source: Google Earth

The Figure 4 shows how the surroundings of the Extremoz Lake area were occupied until December 2014. The blue color corresponds to the water bodies and areas of dense vegetation, the green corresponds to the sparse vegetation, the orange color to the deforested areas and the red color to deforested and urban areas. From the analysis of these images, the presence of three distinct groups was considered; one with a predominance of residences; another with denser urban occupation, including the occupation of small and medium-sized industries (beverage and food industry, textile company and distribution of liquefied petroleum gas); the last grouping corresponds to rural areas (livestock farming and agricultural cultivation); this subdivision corresponds to the sampling stations T1, T2 and T3 respectively.

Figure 4 – a) Satellite image and sampling stations T1, T2 and T3. B) image with the colors of the pixels classified



Source: Google Earth

3.2 Physical and chemical analysis of the sediment

The Table 3 presents the results of the analyses of the organic matter content (OM), carbonate ($CaCO_3$), particle size and concentration of metallic ions (chromium, iron, nickel, copper, zinc, cadmium). In analyses of the OM content the highest value was 1.51% (T2 July) and the smallest 0.06% (T1 April); the maximum $CaCO_3$ content was 0.64% (T1 June) and the minimum of 0.001% (T1 November).

The maximum values recorded for Fe, Zn, Cu, Ni, Cd and Cr were respectively: 208.4 $mg.kg^{-1}$ (T3 May), 2.93 $mg.kg^{-1}$ (T1Jun), 0.72 $mg.kg^{-1}$ (T3 Nov), 0.42 $mg.kg^{-1}$ (T1 Jun), 0.05 $mg.kg^{-1}$ (T1 Mar, Jul and Set; T2 Mar, Apr, Jul, Aug and Set; and T3 Mar, Apr and May) and 0.5 $mg.kg^{-1}$ (T3 Nov). and the smallest values respectively 6.15 $mg.kg^{-1}$ (T2, Apr), 0.68 $mg.kg^{-1}$ (T2 Apr), 0.04 $mg.kg^{-1}$ (T2 Apr), 0.05 $mg.kg^{-1}$ (T2 set), 0.68 $mg.kg^{-1}$ (T2 Apr), 0.05 $mg.kg^{-1}$ (T1 Mar, Jul and Set; T2 Mar, Apr, Jul, Aug and Set; T3 Mar, Apr and May) and 0.05$mg.kg^{-1}$ (T1 Mar, Apr and Jul; T3 Jul).

The behavior of the variables analyzed in the sediment samples collected in the rainy period in T1, T2 and T3 was statistically similar to the average obtained in the dry months, that is, student's T analysis for parametric data (or Wilcoxon Rank Sum for non-parametric data), failed to reject the null hypothesis (HO). This indicated that in the period and in the studied locations, the behavior of the data was not influenced by precipitation. From that, it was possible to check the behavior of variables in each sampling site, that is, an analysis restricted to the differences in the averages of the variables between the sampling stations T1, T2 and T3. Table

4 displays the variables that show average values with significant differences among T1, T2 and T3, namely $CaCO_3$, Fe, Cu, Zn, gravel, coarse sand, medium sand and fine sand. The last column of this table summarizes the statistical analysis, which indicates the differences among the averages in the studied locations.

Table 3 – Physical and chemical analyses in sediment of the Extremoz Lake (T1 e T2) and Guajiru river (T3) between the months of March and November 2014

Month and Sampling station		O.M.	$CaCO_3$	Fe	Zn	Cu	Ni	Cd	Cr	Gravel	Coarse sand	Medium sand	Fine sand	Silt-clay
		(%)		mg.Kg^{-1}						(%)				
March	T1	0.06	0.16	12.79	1.12	0.16	0.15	0.05	0.05	4.02	6.13	16.81	71.87	1.17
	T2	0.4	0.31	6.68	0.84	0.14	0.32	0.05	0.12	1.86	18.1	36.34	41.44	2.26
	T3	0.66	0.61	125.7	2.21	0.35	0.32	0.05	0.25	11.84	20.03	24.16	39.47	4.5
April	T1	0.06	0.09	25.96	1.2	0.08	0.27	N/D	0.05	0.42	32.9	35.55	30.79	0.34
	T2	0.31	0.23	6.15	0.68	0.04	0.22	0.05	0.12	1.61	23.93	34.77	38.18	1.5
	T3	0.24	0.22	66.63	1.08	0.13	0.22	0.05	0.1	58.91	36.29	2.96	1.47	0.38
May	T1	0.35	0.23	81.9	1.87	0.34	0.17	N/D	0.15	6.16	24.19	27.16	40.5	1.99
	T2	0.29	0.25	50.37	0.8	0.11	0.2	N/D	0.25	0.58	20.73	46.81	30	1.87
	T3	0.62	0.4	208.4	2.2	0.56	0.22	0.05	0.2	36.06	25.03	12.99	24.2	1.74
June	T1	1.48	0.64	110.85	2.93	0.52	0.42	N/D	0.25	2.07	19.03	28.19	48	2.72
	T2	1.14	0.29	36.27	0.83	0.1	0.17	N/D	0.12	0.68	9.64	33.05	55.05	1.58
July	T1	0.48	0.26	42.03	1.53	0.22	0.27	0.05	0.05	4.93	18.72	42.46	33.42	0.46
	T2	1.51	0.54	99.35	0.93	0.11	0.15	0.05	0.3	3.01	20.06	26.75	47.37	2.8
	T3	0.63	0.43	93.92	1.08	0.06	0.22	N/D	0.05	4.35	29	40.63	25.19	0.83
August	T1	1.2	0.37	135.92	2.87	0.55	0.25	0	0.17	4.52	17.86	21.86	51.99	3.77
	T2	0.8	0.24	65.72	0.84	0.17	0.17	0.05	0.12	3.65	19.16	36.29	39.83	1.07
	T3	0.95	0.39	112.75	1.32	0.19	0.2	N/D	0.07	7.18	21.69	26.33	42.78	2.02
September	T1	0.4	0.28	51.55	1.46	0.26	0.2	0.05	0.1	2.46	7.96	28.16	60.36	1.06
	T2	0.75	0.24	56.55	0.83	0.17	0.2	0.05	0.1	2.45	9.08	23.96	62.85	1.66
	T3	0.74	0.5	129.77	1.28	0.21	0.05	N/D	0.2	13.11	28.21	29.27	28.06	1.35
October	T1	0.67	0.28	36.61	0.84	0.21	0.1	N/D	0.12	5.24	21.95	27.91	43.59	1.31
	T2	0.93	0.39	110.4	0.91	0.17	0.17	N/D	0.35	0.52	9.81	30.28	57.52	1.87
	T3	0.73	0.42	133.4	1.49	0.25	0.17	N/D	0.17	11.92	36.41	26.81	24.54	0.32
November	T1	0.37	0.001	27.2	0.84	0.11	N/D	N/D	0.07	0.93	10.83	20.49	67.04	0.71
	T2	0.64	0.1	34.6	0.77	0.12	N/D	N/D	0.2	0.5	11.94	38.76	48.64	0.16
	T3	0.86	0.45	150.85	2.68	0.72	N/D	N/D	0.5	6.54	15.66	18.51	56.16	3.14

O.M. (organic matter); CaCO3 (carbonate); (N/D) Not detected. (n = 26)

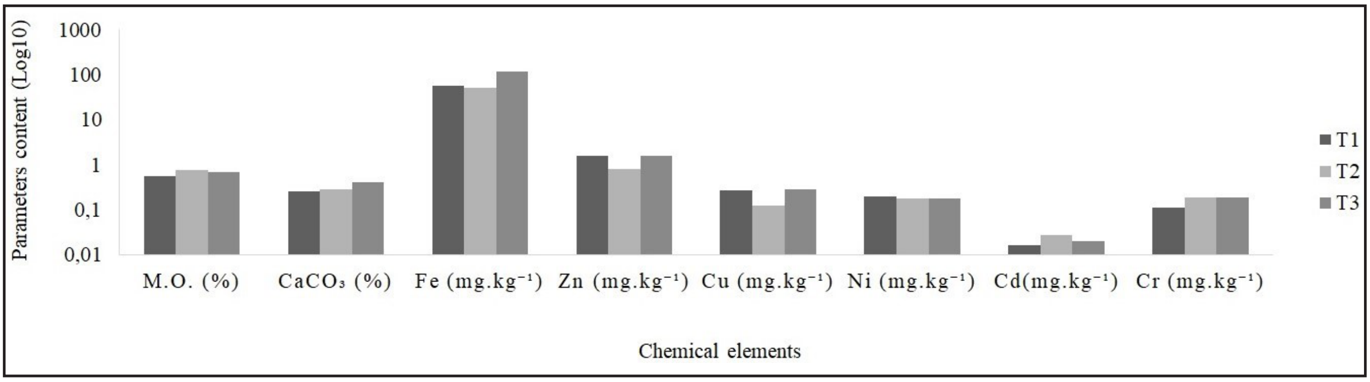
In Figure 5, the average concentration of the chemical variables analyzed in T1, T2 and T3 makes it possible to verify these differences in a more didactic way and to conclude that the T3 sampling station is distinct from the other sampling locations, for the indicated variables. For the grain size data of the sediment samples, fine sand is the most abundant particle size fraction in 69% of the samples, followed by medium sand (in 16%), gravel (7%) and coarse sand (4%) (Figure 6). In Table 4 it was verified that the average values of gravel, coarse sand, medium sand and fine sand, in the group of samples collected at T3, showed significant differences compared to the observations made in T1 and T2; these relationships were exposed in Figure 5. This trend was similar to the tendencies presented by the parameters $CaCO_3$, Fe, Cu and Zn, which indicates that the concentration of the chemical elements, was related to the particle size predominant in the samples.

Table 4 – Averages of the variables in T1, T2 and T3. Values of F with 2 degrees of liberty in the numerator and 23 degrees of liberty in the denominator. Tukey's HSD with confidence interval of 95%

		T1	T2	T3	ANOVA	Tukey's HSD
			Mean		F2,23	Conf. 95%
CaCO3	(%)	0.26	0.29	0.43	3.39; ($p < 0.1$)	T3 > T1
Fe		58.31	51.79	127.70	9.15; ($p < 0.001$)	T3 > T1 e T2
Cu	($mg.L^{-1}$)	0.27	0.12	0.31	3.16; ($p < 0.1$)	T3 > T2
Zn		1.63	0.82	1.67	5.974; ($p < 0.001$)	T3 > T2
Gravel		3.42	1.651	18.74	6.56; ($p < 0.001$)	T3 > T1 e T2
Coarse sand		17.73	15.83	26.54	5.14; ($p < 0.01$)	T3 > T1 e T2
Medium sand	(%)	27.62	34.11	22.71	3.67; ($p < 0.01$)	T2 > T3
Fine sand		49.73	46.76	30.23	4.82; ($p < 0.01$)	T1 e T2 > T3

Conf.: Confidence level of the interval

Figure 5 – Mean content of chemical elements analyzed in T1, T2 and T3



The enrichment factor (EF) was used to complement student's T (or Wilcoxon Rank Sum) and ANOVA One Way statistical tests, whose results were presented in the previous topic. With the EF it was possible to evaluate the behavior of metals in the sediment samples, relative to the concentration expected in an area with less human influence. The resulting values from the employment of the equation was presented in Table 5; these values show a discrepant order of magnitude among the metals analyzed. To circumvent this, a numeric sub classification was assigned to the nominal classification of the EF (Table 2), represented by asterisks (*): absence of an asterisk for enrichment deficiency and up to four asterisks for extremely high enrichment. With this graduation the maximum enrichment of a metal in a sample was standardized (****). In this way it was possible to verify the behavior of enrichment occurrences not only between metals, but also comparisons between months and sampling stations. For these analyses, the degree of enrichment of the metal was added to the sample and divided up as much as possible. This standardization allowed even to compare samples with different grain sizes.

In Figure 7, it can be observed that Zn and *Pb* were the elements that showed greater enrichment (respectively 0.60 and 0.57), followed by the elements *Cu* (0.48), *Cd* (0.42), Ni (0.33) and *Cr* (0.13). By checking the enrichment of each metal in the sampling stations, it is understood that most of the major proportions were obtained in the areas of greater occupation of the soil (T2 and T1). Moreover, the enrichment proportions of Cu, *Pb* and Cd in the sampling stations were predominantly greater in the rainy months. Ni concentrations in T1 and T3 follow this prognosis, while the enrichment in T2 the dry months (0.63) was slightly higher than the verified in the rainy months (0.60). The proportions of Cr enrichment in T1 and T2, presented greater values in the dry months. The proportions were equal at the sampling station T3.

Figure 6 – Retained weight percentage as a function of the grain sizes in T1, T2 and T3

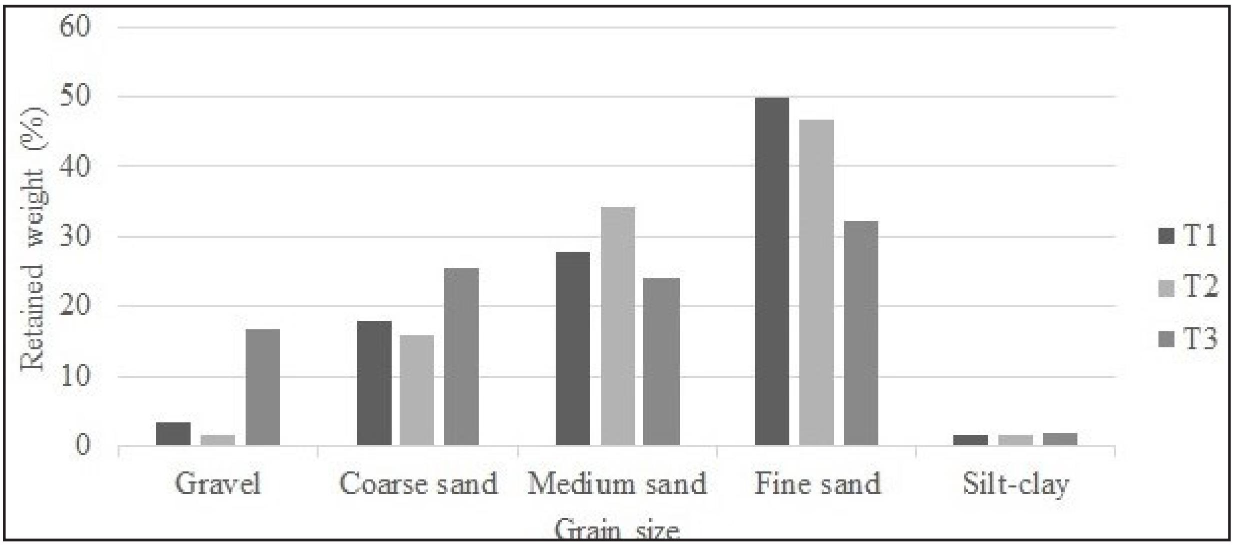
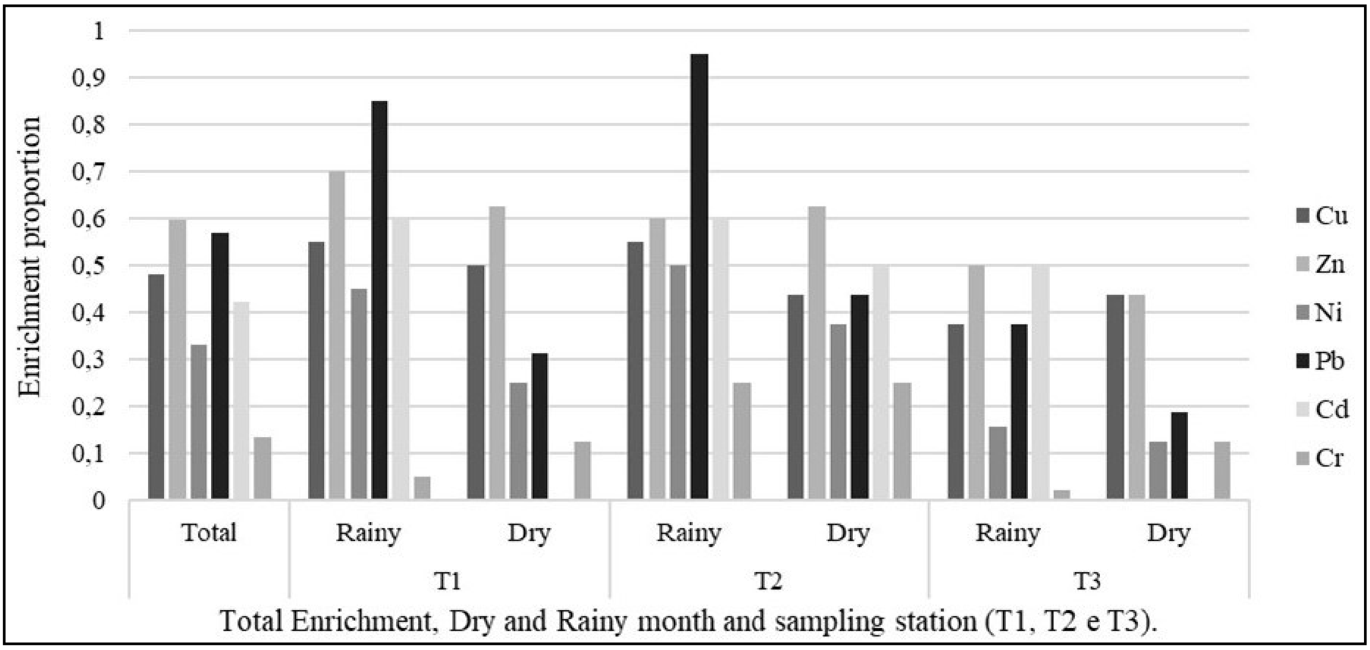


Figure 7 – Total Enrichment of metals and enrichment proportion in dry and rainy month in each sampling station



3.3 Ecotoxicological tests

H. azteca was used in the bioassay as organism tests, to assess the influence of sediment samples under other benthic organisms with similar sensitivity. From the comparison of the results observed in T1, T2 and T3, and controls, it was determined which samples are toxic (indicated with *) and non-toxic. A sample is considered toxic when there is significant difference between the survival and/or reproduction values relative to the control ($H_1 = \pi c \geq \pi t$). The ecotoxicological analysis was presented in Figure 8. The samples that showed acute toxic effect to the survival of *H. azteca* were T1 (March, April and July), T2 (March, April, May, June and October) and T3 (April). In the rainy months, five samples were verified with toxic effect and in the dry months, four samples.

4 DISCUSSION

The concentration of metals and the result of the ecotoxicological tests indicated acute degradation processes at the sampling station with higher urban densification, industries and traffic of vehicles, mainly during the rainy months. Lake sediment present natural Table 5: Metal enrichment factor of Cu, Zn, Ni, Pb, Cd and Cr at the sampling stations T1, T2 and T3 in the period between March and November 2014. enrichment of metals (Förstner, 1981; Watts e Tell, 2004); however, several studies point out that human activities developed primarily in urban areas, increase the concentration of metals to the extent that impacts on the biota of these ecosystems are perceived (dos Santos *et al*., 2011; Peluso *et al*., 2013; Liu *et al*., 2014; Wang *et al*., 2014).

Table 5 – Metal enrichment factor of Cu, Zn, Ni, Pb, Cd and Cr at the sampling stations T1, T2 and T3 in the period between March and November 2014

Month and Sampling station		**Enrichment factor**					
		Cu	**Zn**	**Ni**	**Pb**	**Cd**	**Cr**
March	T1	37***	62****	15**	49****	1864****	3*
	T2	62****	90****	60****	299****	3570****	12**
	T3	8**	13**	3*	8**	189****	1
April	T1	9**	33***	13**	11**	0	1
	T2	19**	79****	45****	242****	3878****	13**
	T3	6**	12**	4*	21***	357****	1
May	T1	12**	16**	3*	17**	0	1
	T2	7**	11**	5**	46****	0	3*
	T3	8**	8**	1	6**	114****	1
June	T1	14**	19**	5**	27***	0	1
	T2	8**	16**	6**	96****	0	2*
July	T1	16**	26***	8**	48****	567****	1
	T2	3*	7**	2*	29***	240****	2*
	T3	2*	8**	3*	11**	0	0
August	T1	12**	15**	2*	21***	0	1
	T2	8**	9**	3*	40***	362****	1
	T3	5*	8**	2*	14**	0	0
September	T1	15**	20***	5**	55****	462****	1
	T2	9**	10**	4*	43****	421****	1
	T3	5*	7**	0	0	0	1
October	T1	17**	16**	3*	0	0	2*
	T2	5*	6**	2*	0	0	2*
	T3	6**	8**	2*	0	0	1
November	T1	12**	22***	0	0	0	2*
	T2	10**	16**	0	0	0	4*
	T3	14**	13**	0	0	0	2*

*Moderate enrichment; **Significant enrichment; ***Very high enrichment; ****Extremely high enrichment.

Figure 8 – Results of the ecotoxicological tests, survival of the test organisms (Control, sampling stations and sampling months)

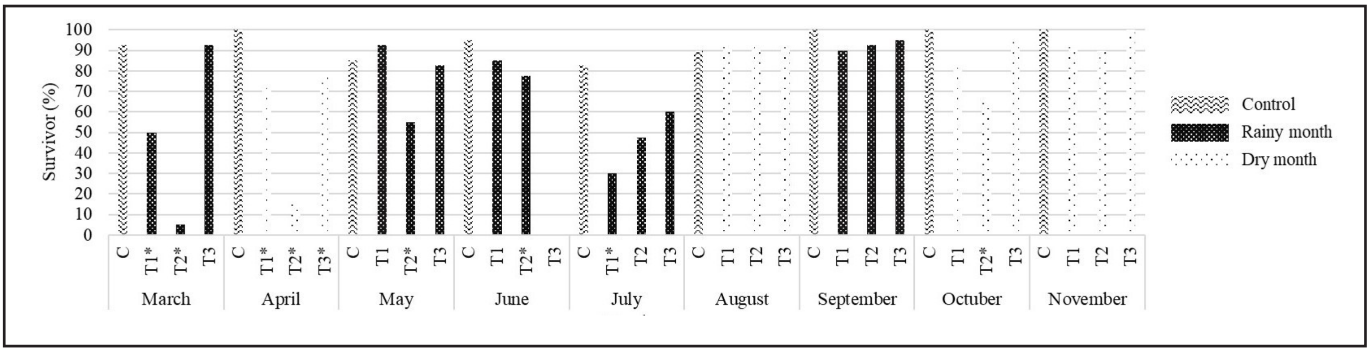


Table 6 shows the average concentration of metals in relation to the observed in the Doce river by Azevedo Filho et al. (2012). This river receives water from the Extremoz Lake, when the water level in the Lake is higher than the dam capacity. The population density along the path of the river is higher and the water is used in agriculture, the major economic activity developed. In addition to the metals in the river, the reference values adopted by the American Environmental Protection Agency (EPA) were presented to the Amphipod *H. azteca* and the sediment quality guidelines of the Canadian Environment Council (CCME).

The concentrations of metals in T1, T2 and T3 was similar to the observed by Sindern et al. (2007) in a nearby hydrographic basin, was lower than the values obtained by Azevedo Filho et al. (2012) in Doce river, and lower than the values of the regulatory environmental agencies. In the first case, the Doce river is a lotic environment, while the Lake is a lentic environment; both water bodies are subject to same climatic and geological conditions. Furthermore, the methodologies used by researchers to assess the metals in the sediment were similar, both the acid digestion capacity of the sample digesting solution, and the fact that the sediment samples were analyzed as collected in the field. The values obtained by Azevedo Filho et al. (2012)and the guidelines of the environmental agencies, take into consideration the concentrations of metals measured in the silt-clay portion of the sediment (< 0.063 mm), which explains the fact that the concentration of metals in our study is not similar to those values. This is partly due to the fact that in the fraction of the sediment composed by grains smaller than 0.063 mm, the concentrations of the elements tend to be larger than in the fine, medium, coarse sand and gravel, as silt and clay present specific surface area significantly higher relative to the other granulometries (Murray et al., 1999), which allows for a larger quantity of adsorption sites for chemical species in contact with the sediment. Thus, the grade of metals leached from a given sediment tends to be higher as smaller is the particle size of the analyzed fraction (Lima et al., 2006).

The effect of the grain size in the availability of metals was evidenced in Table 7, which corresponds to the correlation matrix among the survival data of the ecotoxicological tests, OM content, $CaCO_3$ content, concentration of metals (Fe, Cu, Cr, Pb, Zn, Cd and Ni) and the particle size distribution of sediment samples collected in three sampling stations with heterogenous human occupancy levels (T1, T2 and T3). Positive correlations were observed among OM (0.491), $CaCO_3$ (0.450), Fe (0.434), Cu (0.414) and Cr (0.575), with silt and clay; the contents of OM, $CaCO_3$, Fe, Cu and Cr, are expected to be higher than those obtained.

Moreover, other variables should be considered to evaluate the concentration of metals, as the level of anthropic influence on the surroundings of the water body, which can also be verified by the increment of organic matter in the ecosystems. In this study, the $CaCO_3$(0.557), Fe (0.569), Pb (0.710), and Cr (4.89) are positively correlated with the organic matter content in the samples. Sindern et al. (2007) showed that the elements Cu, Zn, Mo, Cd, Sn, Hg, Ag and Pb presented positive correlations and clear affinity with organic matter, which indicated their possible origin in anthropogenic sources. Another explanation for the significant positive correlation among Fe, Pb, Cr, OM and $CaCO_3$ is that these metals in aerobic and neutral pH conditions form complexes with organic ligands, oxides and clay. These metals may also precipitate with hydrated Fe and deposit in the sediment of rivers as a result of changes in conditions which naturally occur in urban or intermittent rivers (Persaud et al., 1993).

The metals Zn, Cu, Pb and Ni are known as anthropogenic contaminants. The correlation of the data points out to a possible availability of these elements for the benthic invertebrates. The main path of input of these elements is probably by surface sediments being washed during the rainy events. Due to the lack of drainage infrastructure, water flows freely, through the roads, patios of manufactures and the residences. Another decisive factor for this is due to the fact that the area near the T2 sampling station is formed primarily by clay soils, on an area with declivity between 3% and 7%, providing greater potential for superficial runoff. To Brown e Peake (2006) the enrichment of these metals is also related to the wear of the infrastructure used in industrial installations.

In certain concentrations these metals produce harmful effects to the aquatic biota, which ultimately decrease the rate of recruitment of organisms with high ecological importance, as is the case of amphipods of the *H. azteca* species. The relationship between the toxicity of the ecotoxicological tests and the concentration of metals was secondarily verified. Increased occurrence of toxicity events occurred in the same place and period. It is important to know that in addition to the concentration of metals itself, other variables may influence the toxicity of a certain compound, even in concentrations that are not expected to be toxic effects. As pointed out by Borgmann et al. (2005), many metals, in waters with hardness less than 60 $mg.L^{-1}\ CaCO_3$, are potentially more toxic. The pH is another variable that commonly affects the toxicity of metals, as well as hardness, that may interfere with the toxicity of metals that depend on the pH, as is the case of Zn, Cd and Cu (Wang et al., 2016).

Table 6 – Average concentration and standard deviation of the metals Fe, Zn, Cu, Ni, Pb, Cd and Cr in T1, T2 and T3 (Extremoz Lake), Doce river and Jundiaí-Potengi river. Concentration of effect in sediment (SEC) to the Amphipod H. *azteca*. Species Sensitivity Distribution (SSD), adopted by the Canadian Council of Ministers of the Environment (CCME), the Interim Sediment Quality Guideline (ISQG) and the Probable Effect Level (PEL)

		Extremoz Lake			Doce river	Jundiaí-Potengi river			
Parameter		**T1**	**T2**	**T3**	**Azevedo Filho et al. (2012)1**	**Sindern et al. (2007)2**	**EPA3**	**CCME5**	
			Mean±SD				**SEC4**	**SQG6**	**PEL7**
Fe		8.31±39.73	51.79±34.27	127.68±39.02	61376.97±70464.60	0.833*	18.84*	-	-
Zn		1.63±0.75	0.83±0.07	1.67±0.57	27.38±10.66	1.70	98.00	123	315
Cu		0.27±0.16	0.13±0.04	0.31±0.21	10.59±3.64	0.60	28.01	35.7	197
Ni	$mg.Kg^{-1}$	0.20±0.11	0.18±0.08	0.18±0.10	45.70±29.02	0.90	18.51	18	35.9
Pb		0.51±0.42	0.67±0.40	0.28±0.23	51.61±21.51	0.70	37.00	35	91.3
Cd		0.02±0.02	0.03±0.02	0.02±0.02	0.62±1.00	0.01	0.6	0.6	3.5
Cr		0.11±0.06	0.19±0.09	0.19±0.53	19.11±15.17	1.90	36.23	37.3	90

SD: Standard deviation. 1Azevedo Filho et al. (2012), silt-clay fraction (<0.063mm), acid digestion by the solution HCl-HNO3, analysis by Atomic Absorption Spectrometer (AAS); 2Sindern et al. (2007), 5g of the sieved fraction of sediment <0.5mm, HCl-HNO3 digestion, analysis by ICP-AES and graphite furnace ASS; 3Environmental Protection Agency; 4Sediment Effect Concentrations for H. azteca (EPA, 1996); 5Canadian Council of Ministers of the Environment; 6Interim Sediment Quality Guideline; 7Probable Effect Level; * Fe content (%).

Table 7 – Correlation coefficients of the results of ecotoxicological analyses (survival of H. *azteca*), organic matter (O.M.), carbonate (CaCO3), metals (Zn, Fe, Cu, Cr, Ni, Pb and Cd) and weight percentage in the separation of sediment in gravel, coarse sand, medium sand, fine sand, silt and clay

	Survivor of H. *asteca*	**M.O**	**$CaCO^3$**	**Fe**	**Zn**	**Cu**	**Ni**	**Pb**	**Cd**	**Cr**	**Gravel**	**Coarse sand**	**Medium sand**	**Fine sand**	**Silt-clay**
Survivor of H. *asteca*	1														
OM	0.358	1													
$CaCO_3$	0.308	0.557	1												
Fe	0.581	0.569	0.586	1											
Zn	0.389	0.213	0.412	0.695	1										
Cu	0.565	0.334	0.336	0.696	0.8	1									
Ni	0.01	-0.139	0.122	-0.061	0.187	0.078	1								
Pb	0.271	0.71	0.404	0.198	-0.04	0.172	-0.097	1							
Cd	0.372	0.249	0.213	0.202	0.078	0.005	0.026	0.353	1						
Cr	0.234	0.489	0.421	0.538	0.172	0.389	-0.001	0.469	0.193	1					
Gravel	0.427	0.084	0.274	0.646	0.589	0.593	-0.203	-0.2	-0.171	0.045	1				
Coarse sand	0.031	-0.206	0.022	0.272	0.164	-0.067	-0.128	-0.375	0.151	-0.008	0.463	1			
Medium sand	-0.42	-0.083	-0.23	-0.417	-0.415	-0.438	0.024	0.029	0.171	-0.071	-0.543	0.067	1		
Fine sand	0.116	0.278	0.043	-0.231	-0.119	0.06	0.04	0.437	0.057	0.009	-0.442	-0.899	-0.234	1	
Silt-clay	0.083	0.491	0.45	0.434	0.342	0.414	0.038	0.186	-0.016	0.575	0.124	-0.191	-0.252	0.231	1

5 CONCLUSION

There is a gradual increase in the contamination of water and sediment from the Extremoz Lake. As expected, the chemical composition of these compartments is closely associated with climatic conditions and the use and occupation of the territory in its proximity. In the rainy months and in the sampling station with greater anthropic influence (T2), the enrichment factor of metals in the sediment was higher than observed in the months of lower precipitation and in areas with less anthropic influence. This same behavior was observed for the results of the ecotoxicological tests, as the largest number of toxic samples was verified at the T2 sampling station and in the rainy months.

Toxicity events seem to be not directly related to one of the analyzed elements. However, several indications have been provided that the metals analyzed may adversely affect the rate of recruitment of organisms associated with the sediment, especially in areas with proven contamination. Therefore, it is important that additional studies are conducted looking for information on point-of-pollution sources and possible persistent organic contaminants. Because of the characteristics of the various activities along the Extremoz Lake, the presence and influence of these elements cannot be discarded.

It is necessary that the actions of management and water supply from the Extremoz Lake are intertwined to activities of water, sediment and biota monitoring, seeking interdisciplinary approaches to the possible risks to the health of the ecosystem and of the consumers, since acute toxicity was observed to *H. azteca*, in different samples.

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