METAL LEVELS IN WATER AND THE MUSCLE TISSUE OF FISHES IN THE CACHORROS RIVER, SÃO LUÍS ISLAND, STATE OF MARANHÃO, BRAZIL

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Abstract. The present study evaluates physicochemical variables, perform a microbiological analysis as well as determine heavy metal concentrations in the water and muscle tissue of fishes from the Cachorros River. The analysis of metal concentrations in the estuarine water revealed high levels of Fe and Al, with values above the limits established by Brazilian legislation. The concentration of total coliforms and thermotolerant coliforms tended to be higher in the rainy season. Regarding metal concentrations in the muscle tissue of fishes, high levels of Pb and Cd were found, especially in carnivorous and detritivorous species, with values above the limits established by national legislation. Two-factor analysis of variance revealed that only Pb demonstrated a significant interaction between seasonality and the feeding habits of the fish fauna, with higher concentrations of this metal in the dry season among detritivorous species in comparison to herbivorous species. The present findings demonstrate the contamination pattern that has been occurring in the Cachorros River, as evidenced by the concentration of trace metals in both the water and muscle tissue of fishes, which are an important source of protein as well as an economic resource for the population of this river basin.
Keywords: *estuarine fish, metal levels, microbiological contamination, physicochemical variables, health risk*

Introduction

A large portion of the world's population lives in urban centers near estuarine coastlines, which places anthropogenic pressure on water resources due to the demands and wastes of such populations (Cunha et al., 2005; Carmo et al., 2011; Barbosa, 2006). It is estimated that approximately 75% of the world's population will be living within 160 km of a coastline by the year 2025 (Moura, 2009). Thus, urbanization and

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industrialization have made the issue of the contamination of aquatic environments increasingly critical (Simões, 2007; Curcho, 2009). In Brazil, disorderly urbanization, the increase in the population and the indiscriminate construction over of natural areas have led to progressive environment degradation, which is directly associated with the health status of populations that reside around affected areas (Sanches Filho et al., 2013).

Estuaries and coastal regions are often used for the disposal of urban and industrial effluents, leading to the contamination of freshwater and marine life by diverse pollutants. From the public health standpoint, the degree of contamination of these ecosystems poses a risk to the river communities that use such water for the purposes of aquaculture, recreational fishing and leisure activities (Castro, 2009).

Pollution by trace metals constitutes a major source of contamination in aquatic environments. These elements are non-biodegradable and tend to accumulate in the tissues of living organisms, the consequences of which include the decimation of the biota and the intoxication of living beings throughout the food chain, which can eventually reach humans (Castro et al., 1999). Trace metals are chemically highly reactive, which explains the difficulty in finding these metals in their pure state in nature, since concentrations are normally very low and associated with other chemical elements.

Estuarine pollution can be evaluated by an analysis of trace metals found in the water, sediment and aquatic organisms. The characteristics of teleost fish, such as the rate at which they accumulate metals, abundance, sampling ease and adequate size for tissue analysis, make these organisms effective indicators of pollution in estuarine environments.

According to Lins et al. (2010), the close relationship between water quality and numerous illnesses that affect populations is widely known. It is therefore necessary to determine the source, mechanism of action and consequences of contaminants in aquatic environments. Exposure to industrial waste can affect human health in the form of headaches, nausea, skin irritation and lung irritation as well as serious reductions in neurological and hepatic functions (Moraes and Jordão, 2002). Thus, there is a need for research directed at the acquisition of knowledge regarding the environmental transformations that stem from the contamination of aquatic ecosystems as well as implications regarding the health of surrounding communities to assist in decision-making processes and the formulation of effective management policies.

The aims of the present study were to evaluate physicochemical variables, perform a microbiological analysis and determine heavy metal concentrations in the water and muscle tissue of fishes from the Cachorros River in the city of São Luís, state of Maranhão, Brazil.

Materials and methods

A large part of the watersheds on São Luis island concentrate industrial activities developed in the city of São Luís, state capital of Maranhão in northeastern Brazil. The Cachorros River is the largest estuarine river basin in the southeastern portion of the island, covering an area of 63.7 km² (*Fig. 1*), most of which is in the rural zone and near large industrial enterprises, such as the Itaqui Port Complex, ENESA and VALE, as well as smaller enterprises, such as slaughterhouses and mining activities. The river empties into the Coqueiro Strait in front of the ALUMAR port, where materials used in

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the production of aluminum (bauxite, coke, tar, coal and lye) are unloaded and alumina loading operations occur (Castro, 2009).

The estuary of the river is considered extremely important to the populations of the villages Parnauaçu, Cajueiro, Porto Grande, Limoeiro and Taim that conduct fishing activities and use the estuary for other purposes, such as navigation and recreation (Carvalho, 2011). The watershed also has mineral reserves for use in civil construction, such as sand and clay, which supply the internal market. However, mineral extractions are in need of greater control in terms of public power, as these raw materials are removed without planning or authorization (Castro and Pereira, 2012).

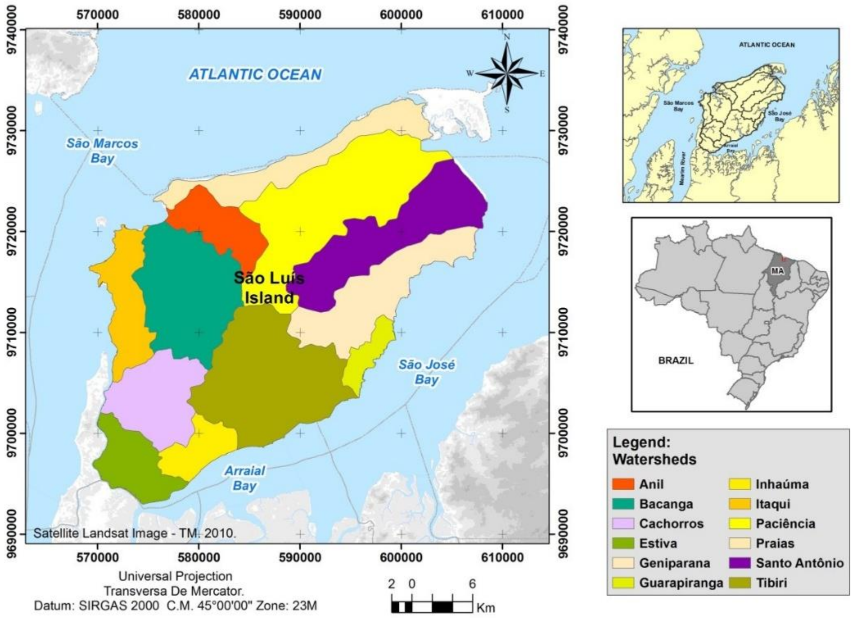

Figure 1. *Map of watersheds on São Luís Island, Maranhão, Brazil*

The evaluation of water quality involved the determination of physicochemical variables, a microbiological analysis and the determination of concentrations of heavy metals (Al, Fe, Zn, Cu, Cd and Pb). Four sampling campaigns were conducted in the rainy and dry seasons. Sampling was performed bimonthly from April to November 2014 at three points spatially distributed along the salinity gradient (*Fig. 2*). The coordinates of the sampling points were determined using of a Global Positioning System: Point 1 – 0571470/9704228; Point 2 – 0574746/9704786; and Point 3 – 0576841/9704838.

At each sampling point, water temperature (°C), pH, dissolved oxygen ($mg.L^{-1}$) and conductivity ($mS.cm^{-1}$) were recorded using a HANNA HI 9828 multi-parameter kit. Turbidity (UNT) was determined using a HANNA HI 93703 turbidimeter and water transparency (cm) was determined using a SECCHI disc. Water samples were also collected 50 cm from the surface in duly labeled 500-mL glass recipients for the subsequent determination of dissolved metals. Nitric acid (1 mL) was added to each sample to maintain the physicochemical properties of the water (APHA, 2012). The samples were placed in a cooler with ice and transported to the lab for storage under refrigeration. For the subsequent analyses, an aliquot (100 mL) was removed from each sample and placed on a hot plate for chemical digestion until reaching a volume of

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20 mL. Five mL of nitric acid were added every 15 min to facilitate the digestion. The samples were diluted to 100 mL with de-ionized water and filtered with a fiberglass filter with a porosity of 0.45 µm. Readings were performed in an atomic absorption spectrophotometer with a graphite furnace (SpectrAA24OZ). Other water quality variables were determined following the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). The collection methods and preservation of the samples followed the ABNT NBR 9897 and ABNT NBR 9898 guidelines, which stipulate the conditions for the sampling of domestic and industrial liquid effluents, sediments and water samples from receiving bodies of water.

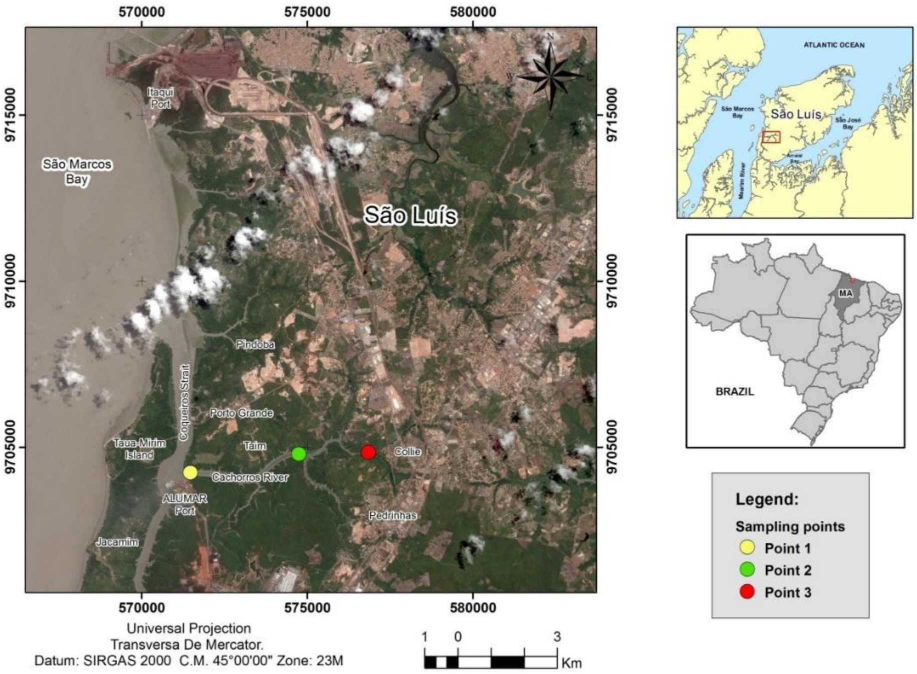

Figure 2. *Map of sampling points*

For the microbiological analysis, water samples were collected approximately 50 cm from the surface, placed in sterilized beakers with a capacity of 250 mL and transferred in coolers to the Microbiology Lab of the Federal University of Maranhão (Brazil). The multiple tube method was used for the determination of the most probable number (MPN) of total and thermotolerant coliforms as well as the identification of *Escherichia coli* following the methods established by the American Public Health Association (APHA, 2005).

Fish species were acquired from local fishermen, stored in plastic packages and transported under refrigeration to the lab. The muscle tissue was removed and weighed to form five replicates of approximately 4 g each. The samples were dehydrated for 24 h at 105 °C and digested with nitric acid + water ($HNO_3 + H_2O$) (1:1) and 150 µL of octanol ($C_8H_{18}O$) following the recommendations proposed by Carvalho et al. (2000). The extracts were analyzed at the Soil Lab of Maranhão State University (Brazil) using atomic absorption spectrophotometry for the trace metals Al, Fe, Zn, Cu, Cd and Pb. The determination of the elemental concentrations was performed through analyses of the ash obtained by dry digestion after complete decomposition of the muscle tissue, as

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adapted from Jones and Case (1990) and Perkin-Elmer (1973). The solutions were analyzed in an inductively coupled plasma atomic emission spectrophotometer (model 720-ES, VARIAN). Specific calibration curves were used for each element and all analyses were conducted in triplicate. The blank reading was performed ten times to calculate the quantification limit (LQ) and the detection limit (LD) of the equipment, which were calculated considering the mean white signal plus 10 times the standard deviation (SD) for LQ and the mean white signal plus 3 times standard deviation (SD) to LD,, according to definition and criteria established by the IUPAC (1997). The entire analytical procedure was tested for both measurement precision and accuracy in order to assess the degree of reliability which can be allocated to the data generated by this investigation. The precision of the method was established by a calculation of the between assay variation coefficients from data of ten independent analyses.

One-way analysis of variance (ANOVA) was used for the spatiotemporal comparison of the variables. In cases of significant values, Tukey’s test was used for the association between sampling months and points. Levene’s test was used to assess the equality of variances. Feeding habits of the species were defined based on information in Fishbase (Froese and Pauly, 2009) and two-way ANOVA was employed to determine possible associations between the concentration of metals in the muscle tissue of the fishes and both local seasonality and trophic level.

Results and discussion

The distribution of dissolved metals in estuarine environments depends on the behavior of physicochemical variables and their controlling mechanisms. Many studies have demonstrated that the partition of trace metals between particulate and dissolved phases depends on factors such as pH, salinity, temperature, redox potential, dissolved organic carbon and the composition of suspended particulate matter (Hatje et al., 2003). The samples from the Cachorros River demonstrated quite homogenous physicochemical aspects on the spatial scale, with no statistically significant differences among the sampling points (ANOVA; $p > 0.05$). On the temporal scale, a differentiation pattern was found for nearly all physicochemical variables ($p < 0.05$). These differences mainly occurred when comparing the rainy months (April and June) to the dry months (September and November). *Tables 1* and *2* display descriptive statistics and the results of Tukey’s test for the physicochemical variables in the study area on both temporal and spatial scales, respectively.

Table 1. *Mean and standard deviation (± SD) values and coefficient of variation (CV) for physicochemical variables in Cachorros River according to sampling month*

Variable	April		June		September		November	
	Mean ± SD	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**
Water temperature (°C)	29.9 ± 0.17^{a}	0.58	29.3 ± 0.15^{b}	0.52	28.7 ± 0.2^{c}	0.69	28.2 ± 0.51^{c}	1.02
pH	7.9 ± 0.16^{abc}	2.09	7.9 ± 0.11^{abc}	1.44	8.2 ± 0.21^{b}	2.53	7.8 ± 0.29^{c}	0.74
Conductivity (mS/cm)	29.0 ± 2.71^{a}	9.35	19.3 ± 1.01^{b}	5.26	43.1 ± 3.49^{c}	7.98	49.1 ± 0.06^{c}	3.60
Salinity	17.9 ± 1.91^{a}	10.71	29.1 ± 1.31^{b}	4.5	27.6 ± 2.48^{b}	8.99	32.3 ± 1.27^{b}	6.19
Total dissolved solids (mg/L)	14.6 ± 1.46^{a}	10.00	40.2 ± 29.8^{a}	74.07	21.7 ± 1.83^{a}	8.46	31.4 ± 2.89^{a}	3.69
Transparency (cm)	58.0 ± 11.26^{a}	19.43	13.3 ± 7.57^{b}	56.79	$51{,}0 \pm 10.53^{a}$	20.66	32 ± 1.15^{ab}	39.03
Dissolved oxygen (mg/L)	5.2 ± 0.44^{abc}	8.46	5.4 ± 0.35^{abc}	6.57	4.3 ± 1.03^{b}	23.75	6.6 ± 12.49^{c}	9.10

Same letters on same line indicate statistical equality and different letters indicate significant differences

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Table 2. *Mean and standard deviation (± SD) values and coefficient of variation (CV) for physicochemical variables in Cachorros River according to sampling point*

Variable	POINT 1		POINT 2		POINT 3	
	Mean ± SD	**CV**	**Mean ± SD**	**CV**	**Mean ± SD**	**CV**
Water temperature (°C)	29.1 ± 0.70 [a]	2.42	29.1 ± 0.75 [a]	2.57	28.9 ± 0.79 [a]	2.74
pH	8.1 ± 0.21 [a]	2.53	8.0 ± 0.22 [a]	2.74	7.9 ± 0.09 [a]	1.21
Conductivity (mS/cm)	37.2 ± 13.70 [a]	36.82	35.3 ± 14.05 [a]	39.73	32.8 ± 12.85 [a]	39.11
Salinity	28.3 ± 5.79 [a]	20.45	26.8 ± 6.52 [a]	24.29	24.9 ± 6.36 [a]	25.51
Total dissolved solids (mg/L)	35.6 ± 24.48 [a]	68.71	20.0 ± 9.18 [a]	45.79	25.3 ± 11.04 [a]	43.65
Transparency (cm)	35.5 ± 26.75 [a]	75.36	43.0 ± 25.47 [a]	59.23	37.2 ± 10.37 [a]	27.84
Dissolved oxygen (mg/L)	5.9 ± 0.81 [a]	13.6	5.4 ± 0.98 [a]	18.08	4.8 ± 1.07 [a]	22.37

In the water column, concentrations of dissolved trace metals tend to vary by several orders of magnitude due to the large number of variables involved in the dynamics of the process, such as daily and seasonal variations in water flow, local discharges of urban and industrial effluents, variations in pH and redox conditions, detergent concentrations, salinity and temperature (Förstner and Wittmann, 1983). Despite these variations, the determination of concentrations of dissolved metals in water is a useful tool for the evaluation of the degree of contamination of a given ecosystem that has been employed by researchers throughout the world (Garbarino et al., 1995; Hurley et al., 1995, 1998; Marjanovic et al., 1995; Watras et al., 1995; Smith et al., 1996; Mastrine et al., 1999).

The analysis of metals in the estuarine water of Cachorros River revealed Fe and Al with values higher than the limits established by Brazilian legislation throughout all sampling months and points. The concentration of Cu was high at all sampling points only in September. Concentrations of Pb, Cr and Cd remained below the detection limit throughout the study (*Table 3*).

Table 3. *Concentrations of heavy metals (mg.L^{-1}) in Cachorros River (limits established by CONAMA Resolution 357/05 in bold)*

Month	Sampling point	Heavy metals (mg.L^{-1})						
		Pb	**Cr**	**Cd**	**Fe**	**Zn**	**Al**	**Cu**
April	**P1**	< 0.01	< 0.01	< 0.005	0.108	< 0.01	0.409	< 0.005
	P2	< 0.01	< 0.01	< 0.005	0.11	< 0.01	0.386	< 0.005
	P3	< 0.01	< 0.01	< 0.005	0.158	< 0.01	0.487	< 0.005
June	**P1**	< 0.01	< 0.01	< 0.005	0.807	< 0.01	1.51	< 0.005
	P2	< 0.01	< 0.01	< 0.005	0.365	< 0.01	0.585	< 0.005
	P3	< 0.01	< 0.01	< 0.005	0.433	< 0.01	1.05	< 0.005
September	**P1**	< 0.01	< 0.01	< 0.005	0.0409	0.0105	0.0775	0.0276
	P2	< 0.01	< 0.01	< 0.005	0.429	< 0.01	0.371	0.0222
	P3	< 0.01	< 0.01	< 0.005	0.114	< 0.01	0.109	0.0192
November	**P1**	< 0.01	< 0.01	< 0.005	0.819	< 0.01	0.967	< 0.005
	P2	< 0.01	< 0.01	< 0.005	0.349	< 0.01	0.633	< 0.005
	P3	< 0.01	< 0.01	< 0.005	0.732	< 0.01	1.2	< 0.005
Limit - CONAMA Resolution 357/05		**0.21**	**1.1**	**0.04**	**0.3**	**0.12**	**0.1**	**0.005**

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Total and thermotolerant coliforms ranged from 23 to 2400 MPN.100 mL^{-1}, with the highest values in June (rainy season). *E. coli* was also detected in June, extending through to September at all sampling points. *E. coli* was only detected at Point 3 in November and was absent from all sampling points in April (*Table 4*).

Table 4. *Most probable number (MPN) of total and thermotolerant coliforms and identification of E. coli throughout sampling months and points*

Sampling month/points	MPN/100 ml of total coliforms	MPN/100 ml of thermotolerant coliforms	*Escherichia coli*
April			
1	9.3×10^1	9.3×10^1	Absent
2	2.4×10^2	2.4×10^2	Absent
3	1.1×10^3	1.1×10^3	Absent
June			
1	2.4×10^3	2.4×10^3	Present
2	2.4×10^3	2.4×10^3	Present
3	2.4×10^3	2.4×10^3	Present
September			
1	9.3×10^1	9.3×10^1	Present
2	2.3×10^1	2.3×10^1	Present
3	2.4×10^2	2.4×10^2	Present
November			
1	2.3×10^1	2.3×10^1	Absent
2	2.3×10^1	2.3×10^1	Absent
3	4.6×10^2	4.6×10^2	Present

According to Schenone et al. (2014), fishes are good indicators of pollution by trace metals and can be used for the identification of the potential risk for human consumption. Fishes living in polluted waters can accumulate toxic trace metals through the food chain to levels that can compromise human health.

The determination of the concentration of metals in the muscle tissue of fishes caught in Cachorros River revealed Mn and Cd only in the dry season, occurring in three species. Mn was only detected in *M. curema*, whereas concentrations of Cd were found in the muscle tissue of both *C. acoupa* and *S. herzbergii* at levels above the limit established by Collegiate Board Resolution 42/13, which imposes greater restrictions in comparison to other Brazilian resolutions. Levels of Zn and Cu were higher, but below the limits established by Brazilian legislation. Zn was found in all species sampled and Cu was found in all except *G. luteus* and *M. curema*. Higher concentrations of these elements were found in the rainy season. Pb was the only metal analyzed with values above the limits established by all Brazilian legislation, with significantly higher values in the dry season for all species.

Although there are no national laws regulating the concentrations of Fe or Al in the muscle tissue of fishes, high values were found in both the rainy and dry seasons. Fe levels were highest in *G. luteus* and *C. microlepidotus* in the rainy season, whereas the highest level in the dry season was found in *M. curema*, with concentrations twofold higher in comparison to the other species. It was not possible to analyze Al in the rainy season, but the results were notably high in the dry season, especially for *M. curema* and *C. microlepidotus*, both of which had concentrations greater than 180.0 mg.kg^{-1} (*Table 5*).

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Table 5. *Concentrations of trace metals in different fish species caught in Cachorros River in rainy and wet seasons (limits established by Brazilian legislation in bold); Feeding habit: D – detritivorous; C – carnivorous; H – herbivorous*

Season	**Species**	**Concentration of metals mg.kg^{-1}**							
		Mn	**Zn**	**Pb**	**Fe**	**Al**	**Cu**	**Cd**	**Feeding habit**
Rainy	*P. nodosu*	0	3.6	0.7	4.7	-	6.3	0	D
	S. herzbergii	0	9.5	0.0	9.3	-	1.9	0	D
	G. luteus	0	17.5	0.0	27.1	-	0	0	D
	C. microlepidotus	0	7.3	0.0	28.6	-	2.6	0	C
	M. curema	0	10.3	0.0	4.9	-	0	0	H
Dry	*C. acoupa*	0	5.7	6.4	6.2	69.2	0.04	0.09	C
	C. microlepidotus	0	3.6	8.4	5.1	185.8	0.56	0	C
	S. herzbergii	0	8.5	10.2	5.2	22.2	0.38	0.06	D
	M. curema	0.04	5.0	5.7	14.4	361.9	4.1	0	H
Legislated limit (mg.kg^{-1})		**-**	**50.0[b]**	**2.0[a.b]**	**-**	**-**	**30.0[b]**	**1.0[a.b]**	
				0.30[c]				**0.05[c]**	

[a]ANVISA, Ordinance n° 685, August 27, 1998
[b]BRASIL, Decree n° 55.871, March 26, 1965
[c]Collegiate Board Resolution Nº 42 from 2013

Two-way ANOVA was used to determine significant differences in metal concentrations in the muscle tissue of fishes as a function of season (dry and rainy) and feeding habit (detritivorous, carnivorous and herbivorous). The only significant interaction between factors regarded Pb, as concentrations of this metal were higher in the dry season in detritivorous species in comparison to herbivorous species (*Table 6*).

Table 6. *Results of two-factor ANOVA for evaluation of concentration of Pb as function of season and feeding habit*

	Pb (mg.kg^{-1})				
	SumSqrs	**df**	**MeanSqr**	**F**	**p**
Season	126.2	1	126.2	162.7	0.00104
Feeding habit	9.41	2	4.705	6.066	0.08827
Interaction	23.48	2	11.74	15.14	0.02707
Within	2.327	3	0.7756		
Total	139	8			

Knowledge on seasonal variability in the concentration of trace metals in the water and muscle tissue of fishes in estuarine environments is extremely useful to understanding the effects of human actions on transitional ecosystems and the toxicological impact on populations that depend on these fishing resources. Marine fishes constitute an important source of protein and are a representative component of the human diet for a multitude of people who live in the rural zones of coastal cities (Mziray and Kimirei, 2016). The gradual increase in industrialization and urban expansion of the Industrial District of the city of São Luís, which potentiates the

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introduction of undesirable amounts of pollutants in the aquatic environment, underscores the need for studies on the determination and quantification of trace metals in different compartments and trophic levels of the aquatic biota.

Besides industrial expansion, the estuarine region of Cachorros River is submitted to other forms of environmental pressure, such as domestic sewage, effluents from agricultural activities and livestock farming and the extraction of sand. However, according to Coimbra (2003), these factors have less importance when compared to the potential risks of contamination due to industrial activities, which affect the quality of aquatic ecosystems, posing potential risks to organisms and an important portion of the local human population that depends on fishing resources as a source of income and food.

Conclusion

The physicochemical variables of the water (temperature, pH, salinity and dissolved oxygen) only demonstrated temporal differences, thereby confirming seasonality as the determinant factor of such variations.

Concentrations of Al and Fe were high in both the water and muscle tissue of the fishes analyzed, although such concentrations could be attributed to natural processes, such as the breakdown of rocks and the transport of soils.

Concentrations of Cu and Pb were high in the water and muscle tissue of fishes, respectively, which raises concerns regarding the potential for the biomagnification of these elements and serious public health risks.

The interaction of season and feeding habits was significant with regard to the concentration of Pb in detritivorous species.

Escherichia coli in the water in June, September and November reveals contamination of a fecal origin due to the absence of adequate sanitation in the rural communities surrounding the Cachorros River.

Besides, further studies are needed to evaluate the extent of heavy metal contamination in sediment and other structures such as gills and liver, as well as whether feeding behavior, habitat preferences and life history patterns could influence heavy metal in fish. This study suggests a contamination of water resource due to the anthropogenic sources and a competent surveillance and monitoring program becomes extremely necessary to any attempt of managing the coastal areas in urbanized regions.

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