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Assessment of potentially toxic metals in water, sediment, and the tissues of seven important fish species from neotropical brazilian river

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Fish are often used as bioindicators of the presence of potentially toxic metals in aquatic ecosystems. The objective of these study was to quantify the levels of Cd, Cr, Cu, Hg, Pb and Zn in water and sediment samples from the Sorocaba River and musculature and gill samples of seven species of fish (*Hoplosternum littorale*, *Pterygoplichthys ambrosettii*, *Hypostomus ancistroides*, *Geophagus iporangensis*, *Prochilodus lineatus*, *Psalidodon* cf. *fasciatus,* and *Rhamdia quelen*. In addition, the Bioaccumulation factor and Bioconcentration factor were obtained. The water and sediment analysis indicates average concentrations of metals below the maximum limit allowed by Brazilian legislation. Results above the legislation were found for chromium, in 18 samples: eight gills and 10 muscles. The trophic group that presented the highest contamination was the iliophages, followed by insectivores. There were no significant differences between the trophic groups in the absorption of the analyzed metals except for Zn and Hg in fish gills. The bioaccumulation factor in the gills and muscles showed that Hg and Zn had the highest values for the sediment in most species studied. Future research is needed to broaden the assessment as fish are consumed and water collection for supply has recently started downstream of the studied area.

Keywords: Bioaccumulation factor, Bioconcentration factor, Environmental contamination, Fish fauna, Sediment contamination.

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Peixes são frequentemente usados como bioindicadores da presença de metais potencialmente tóxicos em ecossistemas aquáticos. O objetivo deste estudo foi quantificar as concentrações de Cd, Cr, Cu, Hg, Pb e Zn em amostras de água e sedimento do rio Sorocaba e em amostras de musculatura e brânquias de sete espécies de peixes (*Hoplosternum littorale*, *Pterygoplichthys ambrosettii*, *Hypostomus ancistroides*, *Geophagus iporangensis*, *Prochilodus lineatus*, *Psalidodon* cf. *fasciatus* e *Rhamdia quelen*), pertencentes a diferentes níveis tróficos. Além disso, foi obtido o fator de bioacumulação e o fator de bioconcentração. A análise de água e sedimento indicaram concentrações médias de metais abaixo do limite máximo permitido pela legislação brasileira. Foram encontrados valores acima da legislação principalmente para o cromo: oito brânquias e 10 músculos. O grupo trófico que apresentou maior contaminação foi o iliófago, seguido pelos insetívoros. Não foi verificado diferenças significativas entre os grupos tróficos na absorção dos metais analisados, exceto para zinco e mercúrio em brânquias de peixes. O fator de bioacumulação nas brânquias e músculos mostrou que Hg e Zn apresentaram os maiores valores para o sedimento na maioria das espécies estudadas. Pesquisas futuras são necessárias para ampliar a avaliação à medida que os peixes são consumidos e a coleta de água para abastecimento começou recentemente a jusante da área de estudo.

Palavras-chave: Contaminação ambiental, Contaminação do sedimento, Fator de bioacumulação, Fator de bioconcentração, Ictiofauna.

INTRODUCTION

Potentially toxic metals, despite occurring naturally in the environment through rock weathering, are also introduced by anthropogenic activities such as the emission of domestic and untreated industrial effluents, as well as by deposition of agricultural waste, being generally the aquatic ecosystems their final destination (Luz, 2016). Highly polluted environments may cause genetic mutations to their wildlife, with the consequent occurrence of tumors and the death of both cells and tissues. In particular, potentially toxic metals may cause deleterious effects to biota such as reproductive inhibition, consequently reducing the diversity of species in the affected environment (Viana *et al*., 2018). These effects occur due to the high toxicity and accumulation of metals when their concentration rates surpass the established normative levels (Niencheski *et al*., 2014).

The metals are classified as cumulative chemical contaminants; when the chemical agent enters the organism and its elimination rate is less than its entry rate, occurs the bioaccumulation process (directly, through contaminated sediment and water exposition, or progressively, through the trophic chain) (Voigt, 2016). Due to this ability of bioconcentrating, potentially toxic metals, many species of fishes have been used in the recent years to monitor pollution levels in aquatic environments (Gomes, Sato, 2011). On fishes, this process occurs through absorption at the surface of the integument (skin and scales), into the respiratory tract (gills and integument) and also through feeding (Antonio *et al*., 2014). The metal waste released into the river flow is partitioned between water and suspended materials, with part of these contaminants following the river course and the rest of them being deposited on the sediment layer, becoming source of contamination to organisms in contact with the riverbed (Souza *et al*., 2015).

Surveys carry out in the region (Smith *et al*., 2014) prove the great diversity in species and in number of fishes that can be found in this area, characterizing the river fishing potential, being amateur fishing and the consequent human consumption common habits alongside the river's course (Smith *et al*., 2003, 2009). The objective of this work was to quantify the concentration levels of potentially toxic metals in the selected species of fishes, water and sediment from the Sorocaba river, aiming to verify if they are above the limit levels established by the Conselho Nacional de Meio Ambiente (CONAMA) for the maintenance of the ecological balance, as well as for the human consumption, established by the Agência Nacional de Vigilância Sanitária (Anvisa) and identify potential bioaccumulation and bioconcentration of metals in gills and muscle tissues of the studied fish species.

MATERIAL AND METHODS

The studied specimens were collected from the Sorocaba River, in the section that crosses the city of the same name. The Sorocaba River drainage basin is located in the State of São Paulo, Brazil, and has a total area of 5,269 km^2, covering eighteen municipalities (Smith, 2014). Specimens of seven main species of fish, assigned to different trophic levels, were selected: insectivore-omnivore (*Psalidodon* cf. *fasciatus*; five specimens), insectivore (*Geophagus iporangensis*; three specimens), iliophage (*Hoplosternum littorale*, *Hypostomus ancistroides*, *Prochilodus lineatus* and *Pterygoplichthys ambrosettii*; five specimens each), insectivore-piscivore (*Rhamdia quelen*, three specimens). Fish were sampled from February to March 2019 at five sites: 1- (23º28'04"S 47º26'43"W), 2- (23º28'10"S 47º27'24"W), 3- (23º28'36"S 47º26'28"W), 4- (23º28'17"S 47º26'30"W), and 5- (23º29'25"S 47º26'24"W) (Fig. 1). Fish were captured using gillnets (10 m length, mesh sizes ranging from 3 to 12 cm between opposite knots), which were deployed for 12 h. Vouchers of all species were deposited in the fish collection of the Departamento de Zoologia e Botânica do Instituto de Biociências, Letras e Ciências Exatas (DZSJRP 19760, DZSJRP 21424, DZSJRP 21419, DZSJRP 22631) and Museu de Zoologia da Universidade de São Paulo (MZUSP 115244, MZUSP 115267, MZUSP 115270).

The water and sediment samples were collected at three points of the studied section: 2- (23º28'10"S 47º27'24"W), 3- (23º28'36"S 47º26'28"W), and 4- (23º28'17"S 47º26'30"W). The water samples at three different depths in the water column (surface, middle and bottom). The water with the aid of a 5-liter Van Dorn sampler and immediately poured into a container for mixing the three layers. The collected samples were placed in 250 mL plastic bottles, kept on ice at 4º C until transport to the laboratory. Sediment samples were collected at the same points with a 50 to 60 cm deep dredger, stored in 1 kg plastic pots and kept on ice at 4º C transport to the laboratory (CETESB, 2011).

The metals analyzed were Cd, Cu, Pb, Cr, Hg and Zn. The analyzes were performed by Hidrolabor Quality Control Laboratory LTDA., responsible for consulting and analysis on water, food and effluents in partnership with the Laboratório de Ecologia Estrutural e Funcional da Universidade Paulista (UNIP), Sorocaba Campus. The fish specimens were submitted to Eugenol (clove oil) anesthetic for euthanasia, then muscle and gills samples were extracted. The muscular samples were taken from the left dorsal region; all gill arches were used. These were then packed in polyethylene films and stored in a freezer at -20º C. Subsequently all the samples were oven dried at 600º C and ground.

About 1g of the sample was transferred to borosilicate test tubes. Then 10mL of concentrated nitric acid and 2.5 mL of concentrated perchloric acid were added, allowing to react for 24 h. The sample was taken to a digester at 600º C and heated to near dryness; it was then cooled and re-solved in 10mL of Milli-Q water. Then 1mL of concentrated HCl was added, followed by an additional volume of Milli-Q water, enough to fill a 25 mL volumetric flask. The procedure was performed in triplicate, as well as the measurements, using flame atomic absorption spectrophotometry (air-acetylene torch) (GBC 932 plus), as employed by Lima *et al.* (2015).

The analysis of metals in water samples was performed according to the methodology 3120 B, established by Rice *et al.* (2017), using an Inductively Coupled Plasma Mass Spectrometer (ICP-MS), which consists of a frequency-field-ionized argon stream, oscillating at 27.1MHz. The samples were then subjected to a temperature of approximately 8000º K, resulting in the dissociation of its molecules and producing ion emission spectra. For the analysis of Hg in water, the methodology 7473 (EPA, 2007) was applied, which consists of drying and decomposing the sample by controlled heating in an oxygenated decomposition furnace, releasing the mercury. The products of this heating are directed to an amalgamator, responsible for the mercury capture, and then heated so the metal vapor is absorbed into the spectrophotometer; its absorbance was measured at 253.7 nm.

Metal sediment analyses were conducted according to the 6010 C methodology established by EPA (2007), using an inductively coupled plasma atomic emission spectrometry equipment (ICP-AES), which measures emission spectra. The samples were oven dried at 40ºC and ground, then nebulized, and their product directed to the equipment plasma, where the spectra are produced and their emission lines monitored. For mercury analysis, we used the same methodology applied in water analysis (EPA, 2007). The results obtained were compared to the quality standards established by Agência Nacional de Vigilância Sanitária (Anvisa, 1998, 2013), with Brazilian limits (Freshwater Class II, CONAMA 2005 and sediment, CONAMA 454/2012) and international limits set for chronic and acute exposure of aquatic life (USEPA, 2017).

Bioaccumulation factor (BAF) and bioconcentration fator (BCF), were calculated to evaluate the concentration of metals in fish tissues in relation to those of sediments and water, respectively (Barron, 1995). BAF assesses the tendency of a given metal to accumulate in the body due to exposure to sediment or consumption of contaminated food resources (Voigt *et al*., 2015). The BCF indicates the degree of affinity of contaminants to living organisms and water (Voigt *et al*., 2016). They were calculated following the formulas: BAF = Mtissue / Msediment and BCF = Mtissue / Mwater, where: Mtissue concentration of a given metal in biological sample; Msediment concentration of a given metal in the sediments; Mwater concentration of a given metal in the water.

A nonparametric Mann-Whitney test (5% significance level) was applied to compare metal concentrations between the Insectivore group (*Psalidodon* cf. *fasciatus*, *Geophagus*

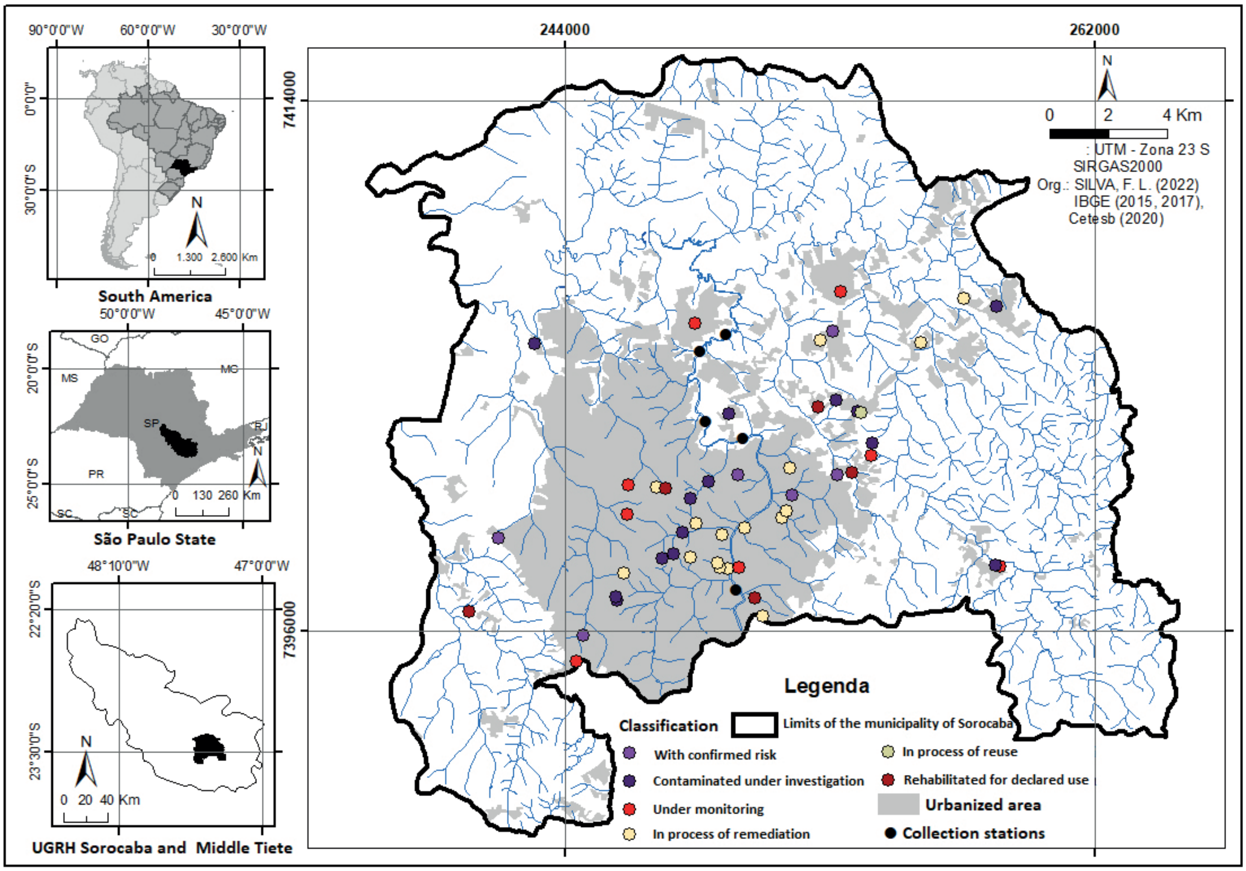

FIGURE 1 | Location of the study area in the Sorocaba River drainage, São Paulo State, Brazil, indicating the Sorocaba river, the fish collection points, in addition to the urban area and areas of contamination.

iporangensis, and *Rhamdia quelen*) and the Iliophage group (*Hoplosternum littorale*, *Hypostomus ancistroides*, *Prochilodus lineatus*, and *Pterygoplichthys ambrosettii*). The null hypothesis (H_0) adopted for the interpretation of the test establishes that there are no significant differences between the groups (Insectivores and Iliophages) regarding the absorption of the analyzed metals, while the alternative hypothesis (H_1) considers the opposite.

RESULTS

The water and sediment analysis indicates average concentrations of metals below the maximum limit allowed by Brazilian legislation (Resolution CONAMA, 2005 and CONAMA, 2012), limits established by the World Health Organization (WHO, 2011) and USEPA (2017) (Tab. 1). Considering the values for each sample, metals found in water had only zinc quantified (0.03 mg/kg), but the result is below the MAC indicated by the legislation (CONAMA, 2005). Analysis of sediments presented results above the Level 1 MAC (Maximum Allowable Concentration) only for cadmium, on three collection points, according to resolutions.

TABLE 1 | Average concentration of metals in water (mg/L⁻¹) and sediment (mg/Kg⁻¹) (mean±SD) of the Sorocaba River. *Only one sample reached the quantification limit but was not above the MAC.

Metal	Water	Sediment	CONAMA (2005) Water	WHO (2011) Water	USEPA (2017) Water (chronic exposure)	USEPA (2017) Water (acute exposure)	CONAMA (2012) Sediment Nível 1	CONAMA (2012) Sediment Nível 2
Cd	<LQ	0.726 ± 0.085	0.001	0.003	0.072	0.018	0.6	3.5
Cr	<LQ	10.11 ± 1.65	0.050	0.050	0.074	0.57	37.3	90
Cu	<LQ	9.546 ± 2.74	0.009	2.000	0.009	0.013	35.7	197
Pb	<LQ	5.05 ± 0.66	0.010	0.010	0.025	0.065	35	91.3
Zn	<LQ	54.82 ± 32.3	0.010	0.010	0.12	0.12	123	315
Hg	0.03*	0.0446 ± 0.018	0.180	3.000	0.077	0.014	0.17	0.486

Results above the MAC were found mainly for chromium, in 18 samples: eight gills and 10 muscles. The other metals analyzed also presented samples above MAC but less frequently, being five samples for cadmium, three for zinc, two for mercury and one for copper and lead. Except for *Prochilodus lineatus*, the other species analyzed presented samples above MAC. *Hypostomus ancistroides* presented values above MAC for two gill samples (Tab. 2) and for all metals (except lead) in the muscle samples (Tab. 3), as established Resolution 42/2013 (Anvisa, 2013) and by the Ordinance nº685/1998 (Anvisa, 1998).

TABLE 2 | Concentration (mg/100g) of the metals considered in the study, for fish gill samples of the Sorocaba River. N: Number of analyzed samples; LQ: Limit of Quantification (Cd- 0.0250 mg/100 g. Cr-0.025 mg/100 g. Cu- 0.025 mg/100 g. Hg- 0.001 mg/100 g. Pb- 0.025 mg/100 g. Zn- 0.025 mg/100 g); 1- CONAMA, Resolution - RDC No. 42 of August 29, 2013; 2- Anvisa Ordinance No. 685, of August 27, 1998; *Only one sample reached the quantification limit but was not above the MAC; **Only one sample reached the quantification limit and it was above the MAC.

Species	N	Trophic group	Cd (0.005mg/100g)[1]	Cr (0.01mg/100g)[2]	Cu (3mg/100g)[2]	Hg (0.05mg/100g)[1]	Pb (0.03mg/100g)[1]	Zn (5mg/100g)[2]
P. cf. *fasciatus*	5	Insectivore-onivore	0.179**	0.051*	0.231*	0.017 ± 0.013	<LQ	4.20 ± 0.625
G. iporangensis	3	Insectivore	0.027**	0.122 ± 0.057	1.07 ± 1.4	0.005 ± 0.001	<LQ	3.99 ± 0.996
H. littorale	5	Iliophage	<LQ	0.062**	0.13 ± 0.026	0.005 ± 0.002	<LQ	1.75 ± 0.203
H. ancistroides	5	Iliophage	0.1**	<LQ	0.171 ± 0.073	0.014 ± 0.007	0.008*	4.79 ± 3.71
P. lineatus	5	Iliophage	<LQ	<LQ	<LQ	0.128 ± 0.014	<LQ	1.94 ± 0.294
P. ambrosettii	5	Iliophage	<LQ	0.091 ±0.049	0.164 ± 0.073	0.019 ± 0.016	<LQ	2.72 ± 0.381
R. quelen	3	Insectivore-piscivore	<LQ	0.097 ± 0.04	0.841 ± 0.610	0.004 ± 0.0004	<LQ	2.53 ± 0.380

TABLE 3 | Concentration (mg/100g) of metals considered in the study, for fish muscle samples from the Sorocaba River. N: Number of analyzed samples; LQ: Limit of Quantification (Cd- 0.0250 mg/100 g. Cr-0.025 mg/100 g. Cu- 0.025 mg/100 g. Hg- 0.001 mg/100 g. Pb- 0.025 mg/100 g. Zn- 0.025 mg/100 g); 1- CONAMA, Resolution - RDC No. 42 of August 29, 2013; 2- Anvisa Ordinance No. 685, of August 27, 1998; *Only one sample reached the quantification limit but was not above the MAC; **Only one sample reached the quantification limit and it was above the MAC.

Species	N	Trophic group	Cd (0.005mg/100g)[1]	Cr (0.01mg/100g)[2]	Cu (3mg/100g)[2]	Hg (0.05mg/100g)[1]	Pb (0.03mg/100g)[1]	Zn (5mg/100g)[2]
P. cf. *fasciatus*	5	Insectivore - omnivore	0.018**	<LQ	0.069*	0.020 ± 0.014	<LQ	2.06 ± 0.615
G. iporangensis	3	Insectivore	<LQ	0.57 ± 0.56	0.759	0.006 ± 0.001	0.22**	3.12 ± 0.986
H. littorale	5	Iliophage	<LQ	0.057**	0.075 ± 0.015	0.005 ± 0.002	<LQ	1.17 ± 0.766
H. ancistroides	5	Iliophage	0.011**	0.087 ± 0.023	0.06*	0.028 ± 0.031	<LQ	4.11 ± 1.02
P. lineatus	5	Iliophage	<LQ	<LQ	<LQ	0.005 ± 0.0004	<LQ	2.80 ± 0.373
P. ambrosettii	5	Iliophage	<LQ	0.057**	0.086*	0.016 ± 0.011	<LQ	0.881 ± 0.151
R. quelen	3	Insectivore - piscivore	<LQ	0.074**	0.299 ± 0.204	0.011 ± 0.002	<LQ	1.55 ± 0.376

Gill samples of all species presented 16 samples above MAC, while musculature samples presented 15 results above MAC. *Pterygoplichthys ambrosettii* presented four samples above the MAC for gill and one for musculature; *H. ancistroides* presented two samples for gill and seven for musculature; *H. littorale* and *R. quelen* presented one sample above MAC for each material; *P.* cf. *fasciatus* presented three samples for gill and 1 for musculature, *G. iporangensis* presented five samples for gill and four for musculature. Seven species of fish of different trophic levels were collected: insectivore – omnivore (*P.* cf. *fasciatus*), insectivore (*G. iporangensis*), iliophage (*H. littorale*, *H. ancistroides*, *P. lineatus*, and *P. ambrosettii*) and insectivore – piscivore (*R. quelen*).

The trophic group that presented the highest contamination was the iliophage, with 16 samples above the MAC, followed by insectivores with nine samples above MAC, insectivore – omnivore with four samples and Insectivore – piscivore with two samples above MAC. The Mann-Whitney statistical test ($\alpha = 0.05$), performed for the gill and musculature samples, did not indicate significant differences between the trophic groups (insectivore and iliophage) in the absorption of the analyzed metals, except for zinc and mercury.

The bioaccumulation factor in gills and muscles of the fish sample is shown in Tab. 4. The result showed that Hg have higher bioaccumulation factor respectively in muscles and gills for sediment in most species studied, compared to the other metals. Besides, bioaccumulation factor was higher in the gills than in the muscles, showing that bioaccumulation occurs more intensely in the gills, starting from the sediment. The BCF order obtained from the gills and muscles in relation to the sediment show the relevance of Hg and Zn (Tab. 5).

TABLE 4 | Bioaccumulation factor (BAF) and bioconcentration factor (BCF) obtained from gill and muscle of studied species.

Parameter	Cd	Cr	Cu	Hg	Pb	Zn
Gills/Water *P.* cf. *fasciatus*	-	-	-	-	-	-
Gills/Sediment *P.* cf. *fasciatus*	2.45	0.05	0.24	3.8	-	0.77
Muscles/Water *P.* cf. *fasciatus*	-	-	-	-	-	-
Muscles/Sediment *P.* cf. *fasciatus*	0.25	-	0.07	4.44	-	0.38
Gills/Water *G. iporangensis*	-	-	-	-	-	-
Gills/Sediment *G. iporangensis*	0.37	1.21	1.12	1.11	-	0.73
Muscles/Water *G. iporangensis*	-	-	-	-	-	-
Muscles/Sediment *G. iporangensis*	-	0.56	0.80	1.33	0.44	0.57
Gills/Water *H. littorale*	-	-	-	-	-	-
Gills/Sediment *H. littorale*	-	0.061	0.14	1.11	-	0.32
Muscles/Water *H. littorale*	-	-	-	-	-	-
Muscles/Sediment *H. littorale*	-	0.05	0.08	1.11	-	0.21
Gills/Water *H. ancistroides*	-	-	-	-	-	-
Gills/Sediment *H. ancistroides*	1.37	-	0.18	3.11	0.016	0.87
Muscles/Water *H. ancistroides*	-	-	-	-	-	-
Muscles/Sediment *H. ancistroides*	0.15	0.086	0.063	6.22	-	0.75
Gills/Water *P. lineatus*	-	-	-	-	-	-
Gills/Sediment *P. lineatus*	-	-	-	28.4	-	0.35
Muscles/Water *P. lineatus*	-	-	-	-	-	-
Muscles/Sediment *P. lineatus*	-	-	-	1.11	-	0.51
Gills/Water *P. ambrosettii*	-	-	-	-	-	-
Gills/Sediment *P. ambrosettii*	-	2.88	0.17	4.22	-	0.50
Muscles/Water *P. ambrosettii*	-	-	-	-	-	-
Muscles/Sediment *P. ambrosettii*	-	0.056	0.09	3.55		
Gills/Water *R. quelen*	-	-	-	-	-	-
Gills/Sediment *R. quelen*	-	0.096	0.88	0.89	-	0.46
Muscles/Water *R. quelen*	-	-	-	-	-	-
Muscles/Sediment *R. quelen*	-	0.073	0.31	2.44	-	0.28

TABLE 5 | The bioconcentration factor order obtained for the studied species.

	BCF order
Gills/Sediment *P.* cf. *fasciatus*	Hg > Cd > Zn > Cu > Cr
Muscles/Sediment *P.* cf. *fasciatus*	Hg > Zn > Cd > Cu
Gills/Sediment *G. iporangensis*	Cr > Hg > Cu > Zn > Cd
Muscles/Sediment *G. iporangensis*	Hg > Cu > Zn > Cr > Pb
Gills/Sediment *H. littorale*	Hg > Zn > Cu > Cr
Muscles/Sediment *H. littorale*	Hg > Zn > Cu > Cr
Gills/Sediment *H. ancistroides*	Hg > Cd > Zn > Cu > Pb
Muscles/Sediment *H. ancistroides*	Hg > Zn > Cd > Cr > Cu
Gills/Sediment *P. lineatus*	Hg > Zn
Muscles/Sediment *P. ambrosettii*	Hg > Zn
Gills/Sediment *P. ambrosettii*	Hg > Cr > Zn > Cu
Muscles/Sediment *P. ambrosettii*	Hg > Cr > Cu
Gills/Sediment *R. quelen*	Hg > Cu > Zn > Cr
Muscles/Sediment *R. quelen*	Hg > Cu > Zn > Cr

DISCUSSION

The results obtained in the present study indicate the bioaccumulation of metals in the sediments and fish are within the acceptable limits established by the current legislation (CONAMA and ANVISA). Souza *et al.* (2015) state that metals released into the water body are partitioned between water and suspended materials, with the latter being deposited in the sediment layer. However, even if the sediment has been eventually contaminated by potentially toxic metals, it may not necessarily imply in immediate harm to the environment, once its consequences in terms of bioavailability depend on the chemical form of each metal that may characterize it as a contaminant. The bioavailability of metals occurs in five phases along the sediment line, namely: a) exchangeable phase, b) leachable phase, c) reducible phase, d) oxidizable phase, e) pseudo-residual phase. In the last three phases, metals tend to be strongly bonded to the compounds present in the sediment, making them unavailable for biota absorption.

The sediment's metal accumulation capacity is determined by complex dynamic factors, making predictions about the long-term consequences difficult, so it is not possible to affirm that the contamination by these metals occurred before or after the depollution process of the Sorocaba River, implemented by the municipality in 2000 (SAAE, 2016). According to Santos, Jesus (2014), potentially toxic metals are stable and persistent environmental contaminants, as their distribution along the water column depends on physical and chemical phenomena (complexation, adsorption, desorption, precipitation and redissolution) and other aquatic ecosystem conditions, such as pH and the presence of organic matter. These characteristics help to explain the absence of metals above the quantification limit obtained in water analyses of the studied river. Besides, how report Alloway (2013) and Doria *et al.* (2017) the metal distribution in sediments provides a record of the spatial and temporal pollution history of an ecosystem, this signifies that, although there is environmental licensing and the Sorocaba River is going through a depollution process, it has been suffering from the consequences of human activity for a considerable amount of time.

No previous studies were found regarding the types of sediment present along the Sorocaba River, but during the collections it was identified the presence of sandy and muddy sediments. No studies were found regarding which types of sediments are most likely to accumulate contaminants. According to Souza *et al*. (2015), physical and chemical conditions of the water-sediment interaction determine the absorption of metals by the organisms and the sediment itself. Conditions involve the ionic composition of water, changes in pH, organic matter content, temperature and others. Rainfall is also a determining factor in the metal to water concentration ratio, as simultaneously to surface runoff and surface metal loading, which are more intense during the rainy season, there is also an increase in the discharge of watercourses, contributing to the dilution and transport of these metals (Magalhães *et al*., 2016). Considering that the analyzed water collections were carried out in rainy periods, the metal concentrations tend to be reduced, which helps to explain the absence of results above the MAC for water samples.

In the present work the sediment presented results above the MAC rate for cadmium, indicating that this metal is undergoing chemical changes that allow us to classify it as a contaminant of greater bioavailability and consequently of greater absorption by the biota. This is confirmed by the gill and muscle analyses, which show cadmium as the second metal with more results above the MAC rate. In aquatic organisms, the absorption of cadmium is favored by the increase in water temperature and reduced by the increase in salinity and water hardness. The bioaccumulation of this metal can affect the growth and cell division of the exposed organisms, particularly in fishes, it can difficult the absorption of calcium, causing hypocalcemia.

The organisms in embrionary and larval stages are the most affected by the exposure to cadmium, which in large concentrations may cause bone malformation with permanent consequences. Biochemical alterations may also occur, such as changes in glycogen reserves and glucose levels caused by the carbohydrate and protein metabolisms' decrease. For Sampaio (2003), the alterations that occur in the human organism when exposed to excessive cadmium concentration levels are similar to those observed in aquatic organisms. Cadmium is highly toxic and may be released in nature by zinc refineries, metallurgical and pigmentation industries (all present in the studied area) or by the flow of fertilizers used in agriculture (Vieira *et al*., 2015).

The large number of gill and musculature samples with higher levels of chromium contamination is shows this metal might has already gone through its storage period in the sediment layer, having suffered the chemical reactions that turned it into its contaminating chemical form, easily absorbed by fish and consequently bioaccumulated along the trophic chain. The toxicity of the metal depends not only on its concentrations in the medium and on its specimens, but mainly on the physical and chemical conditions of the water-sediment system to which they are exposed (Amado, Chaves-Filho, 2015). The chromium toxicity is higher when it is observed in its hexavalent form. Its effects may vary according to the species, the amount of metal absorbed and the exposure period.

The possible physiological effects in fishes include corrosion of the mucous membrane, as well as respiratory and hematological alterations (Petersen, 2016). Histological changes were also observed, including hyperplasia and fusion of branchial lamellae, as well as abnormal increases in the number of mucus-secreting cells (Fujimoto, 2018). Still according to Fujimoto (2018), the low oxygen concentrations in polluted aquatic environments favor the transformation of trivalent chromium into hexavalent chromium by oxide-reduction reactions, facilitating its passage through cell membranes and allowing its interaction with proteins and nucleic acids in the organism, characterizing the carcinogenic and mutagenic potential of this metal.

Copper, being an essential element, has an easier absorption and body regulation, which may explain why the results obtained for this metal were less significant, since only one sample was above the MAC. Another justification lies on the fact that copper levels tend to be higher in liver and gonad samples, not analyzed in the present study, causing effects on the respiratory system, behavior, growth and reproduction of these organisms (Tajiri *et al*., 2011).

Zinc is also an essential metal for the organism, related to the activities of cell division, growth and reproduction process; in fish, it presents a higher concentration index in its gonads, which may be the reason why the results above the MAC are of little significance in the present study, as it did not analyze this organ (Lima *et al*., 2015). Regarding mercury, studies show that the highest concentrations of this metal occur in carnivorous fish, while species of other trophic levels such as iliophages and omnivores have lower accumulation rates, once their diet retains few amounts of this metal (Kasper *et al*., 2012). As the present study analyzed a larger number of Iliophage organisms, the low mercury concentration in the results might be related to the feeding habits of these organisms.

The result obtained for *Hypostomus ancistroides* is due to the Iliophage character of the species, considering that metals tend to be found in greater abundance in the sediment, where the species feeds (Pereira *et al*., 2019). This is also why it was the trophic group with the higher MAC. Regarding *Prochilodus lineatus*, also being classified as an Iliophage, it did not present abnormal values for any of the metals analyzed, probably for being migratory despite species that doesn't feed in the studied area for an extensive period (Ribeiro, 2013). Silva *et al.* (2021) mention that species with low dispersion are highly exposed to deleterious effects related to polluting activities, unlike species with greater mobility such as migratory ones. These authors cite *Psalidodon* cf. *fasciatus* as a migratory species, with greater mobility and they attribute to this characteristic the lower concentration of metals found in this species, which can be used as an explanation for the species *Prochilodus lineatus* in the present study.

The fact that the insectivorous trophic group presented the second largest number of samples above the MAC, followed by the insectivorous-omnivore group, is due to the bioaccumulation process along the trophic chain. Species belonging to these trophic groups often feed on invertebrates such as insects, larvae and small mollusks that tend to accumulate these metals through their diet and also through superficial contact with the sediment layer, due to their lack of mobility (Pereira *et al*., 2019). The smaller number of samples above MAC for Insectivorous-piscivorous may be explained by the reduced size of the sample, since only three individuals of the *Rhamdia quelen* species were found during the collections. This contradicts the expected results, since fishes at a higher trophic level tend to show higher metal levels, due to bioaccumulation. The Mann-Whitney tests ($\alpha = 0.05$) applied indicate that was rejection of the null hypothesis only for the Zinc and Mercury gill analysis, indicating that there are significant differences in the absorption of these metals by the studied groups, reinforcing the literature that indicates the tendency of a greater absorption in a given trophic group compared to the others.

In the evaluated stretch of the Sorocaba River, high metal concentrations in the riverbed sediments influenced the bioaccumulation and bioconcentration of metals in the studied fish species. For all species, bioaccumulation factor (BAF) and bioconcentration factor (BCF) obtained were more related to the exposure to contaminated sediments than contaminated water. For example, *Rhamdia quelen*, *Hoplosternum littorale*, *Hypostomus ancistroides*, *Prochilodus lineatus*, and *Pterygoplichthys ambrosettii* depend on benthic food sources, having a lot of contact with contaminated sediments. Benthophagic species tend to bioaccumulate and bioconcentrate metals due to their exposure to contaminated sediments and/or their consumption on benthic preys (Silva *et al*., 2021). These same authors reported that *Geophagus iporangensis*, the well-known benthic consumer that exploits invertebrates and sediments, fits into the same situation.

It is likely that the high degree of bioaccumulation of Zn verified in the present study is linked to the diet of the studied species, since according to Bordajandi *et al.* (2003), eating habits play a marked role in bioconcentration for some metals, in addition to their metabolic function (Maceda-Veiga *et al*., 2012). This metal is involved in the functions of several enzymes and are considered essential metals (Silva *et al*., 2021), in addition to regulating enzymatic detoxification processes (Agtas *et al*., 2007).

Based on the results obtained from the BCA, it was verified that the muscle tissue has a lower affinity for the bioaccumulation of metals when compared to the gills, and these results are in agreement with the studies carried out by Bajc *et al.* (2005), Shukla *et al.* (2007) and Nwani *et al.* (2010). This is to be expected, as the gonads, liver, kidneys and gills are the target organs for metal accumulation, as they are metabolically active (Yilmaz, 2003). The bioaccumulation factor (BAF), calculated from fish caught in the field, is more ecologically relevant because it is responsible for dietary, respiratory and dermal exposures (Costanza *et al*., 2012), which demonstrates the relevance of this study.

This is the first study conducted in the Sorocaba River in Brazil, and although the river has been recovered for more than two decades, concentrations of potentially toxic metals in Sediment and fish are of concern. The results obtained in the present study indicate bioaccumulation of metals in the sediment and fishes of the Sorocaba River, in relation to the acceptable limits established by current legislation (CONAMA and Anvisa), which considers the health and wellbeing of humans as well as the ecological rock. Therefore, the human consumption of these fishes should be limited, and any interventions aimed at depolluting the sediment layer should be carried out with care, to avoid the contamination of the final disposal site. Periodic monitoring of these potentially toxic metals is recommended, both in fish and in the studied river, to ensure the continued safety of the population and to assess the stage of environmental contamination, especially after the implementation of the collection and treatment of water from the Sorocaba River downstream of the studied excerpts. Thus, as argued by Singh *et al*. (2014) that regular monitoring of heavy metal contamination in fish species that thrive in contaminated water should be carried out to investigate the issue of food safety (Canli, Atli, 2003; Mansilla-Rivera, Rodríguez-Sierra, 2011). Our findings address an important aspect of biomonitoring using appropriate tools and have greater implications for the development of fish consumption advice and public awareness of the consequences of eating contaminated fish. This initiative will be an important step in taking steps to remove heavy metals from river water as needed in the environmental and food safety vision.

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AUTHORS' CONTRIBUTION

Karla Fernanda Sanches Rodrigues: Conceptualization, Investigation, Writing-original draft.

Welber Senteio Smith: Conceptualization, Supervision, Writing-original draft, Writing-review and editing.

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COMPETING INTERESTS

The authors declare no competing interests.

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