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Bioaccumulation of mercury, cadmium, zinc, chromium, and lead in muscle, liver, and spleen tissues of a large commercially valuable catfish species from Brazil

FÁBIO P. ARANTES[1], LOURENÇO A. SAVASSI[1], HÉLIO B. SANTOS[2], MARCOS V.T. GOMES[3] and NILO BAZZOLI[1]

[1]Programa de Pós-Graduação em Zoologia de Vertebrados, Pontifícia Universidade Católica de Minas Gerais, Av. Dom José Gaspar, 500, 30535-901 Belo Horizonte, MG, Brasil
[2]Laboratório de Patologia Experimental, Universidade Federal de São João Del Rei, Campus Ciências e Saúde, Av. Sebastião Gonçalves Coelho, 400, Chanadour, 35501-296 Divinópolis, MG, Brasil
[3]Centro Integrado de Recursos Pesqueiros e Aquicultura de Três Marias/CODEVASF, Estrada Piscicultura, s/n, 39205-000 Três Marias, MG, Brasil

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ABSTRACT

The increasing amounts of heavy metals entering aquatic environments can result in high accumulation levels of these contaminants in fish and their consumers, which pose a serious risk to ecosystems and human health. We investigated the concentrations of mercury (Hg), cadmium (Cd), zinc (Zn), chromium (Cr), and lead (Pb) in muscle, liver, and spleen tissues of *Pseudoplatystoma corruscans* specimens collected from two sites on the Paraopeba River, Brazil. The level of heavy metals concentrations in the tissues was often higher in viscera (i.e. liver and spleen) than in muscle, and thus, the viscera should not be considered for human consumption. Correlations between metal concentrations and fish size were not significant. Although the levels of muscle bioaccumulation of Hg, Cd, Zn, Cr, and Pb, generally do not exceed the safe levels for human consumption, the constant presence of heavy metals in concentrations near those limits considered safe for human consumption, is a reason for concern, and populations who constantly consume fish from polluted rivers should be warned. Our findings also indicate that in a river network where certain areas are connected to other areas with high rates of environmental pollutants, people should be cautious about the regular consumption of fish, even when the fish consumed are caught in stretches of the basin where contamination levels are considered low, since many of the freshwater fish with high commercial value, such as the catfish surubim, are migratory.

Key words: contamination, freshwater fish, heavy metal, human health.

INTRODUCTION

In the last decades, the rapid development of industry and agriculture has resulted in increased heavy metals pollution, which is a significant environmental hazard for invertebrates, fish, and humans (Uluturhan and Kucuksezgin 2007). Among the contaminants, heavy metals should be highlighted due to the consequences of their bioaccumulation in aquatic ecosystems (Nadmitov et al. 2014, Marques et al. 2009, Sloman 2007). Significant quantities of these metals are discharged into rivers, where they can be strongly accumulated and biomagnified in water, sediment, and the aquatic food chain, resulting in both sub-lethal effects or death in local fish populations (Megeer et al. 2000, Protano et al. 2014, Xie et al. 2014, Xu et al. 2004).

Correspondence to: Nilo Bazzoli
E-mail: bazzoli@pucminas.br

The toxicity of trace metals has long been a concern, considering they are not removed from aquatic ecosystems by self-purification (Harikumar and Nasir 2010) and accumulate in suspended particulates and sediments (De Jonge et al. 2012), thereby potentially threatening human health and ecosystems via the food web (Eslami et al. 2011). Consequently, evaluating the ecological risk from trace elements has become a hot topic (Mario et al. 2012).

Heavy metals enter the aquatic environment from both natural pathways and a variety of anthropogenic sources (Youn-Joo 2003), and they can have a negative impact on aquatic ecosystems, the food chain, and human health. The concentration of heavy metals in biological compartments, such as fish muscle, is a complex combination of biological and ecological variables (Barletta et al. 2012). In fish, these elements can cause disturbances in growth and reproduction, as well as histopathological alterations in the skin, gills, liver, spleen, and kidneys (Vitek et al. 2007). In addition, some metals may decrease the plasticity of the cardiorespiratory responses, reducing the survival chances of fish under hypoxic conditions, which has been frequently observed in their wild habitats (Monteiro et al. 2013). In humans, heavy metals accumulation has hazardous effects on the brain, liver, kidneys, lungs, and muscles (Petera and Viraraghavanb 2005, Youn-Joo 2003). For these reasons, evaluation of heavy metal levels in commercially important fish is important from a toxicological perspective, verifying whether there is a significant health risk arising from fish consumption.

Currently, some physiological and histopathological biomarkers of fish have been extensively used to document and quantify the effects of pollutants in aquatic environments, such as follicular atresia, heat shock proteins, apoptosis, metallothionein, vitellogenin in males, hormonal disturbances, melanomacrophagic centres (MMCs), fibrosis, and steatosis in teleosts (Santos et al. 2005, Prado et al. 2011). Many studies suggest that the general function of the MMC is the centralisation of destruction, detoxification, or recycling of endogenous and exogenous materials (Vogelbein et al. 1987, Ellis et al. 1976). An increase of MMC numbers and size has often been associated with degraded environmental conditions (Agius and Roberts 2003, Ribeiro et al. 2011).

The surubim catfish (*Pseudoplatystoma corruscans*) is one of the most important high-value species in Brazil (Roubach et al. 2003) reaching 180 cm in length and 100 kg. The surubim is a nocturnal piscivorous fish in any habitats, seasons, or sizes (Agostinho et al. 2004). Demand for the species as a food, sport and ornamental fish, together with the effects of anthropic actions, has caused the decline of the natural stocks (Campos 2005). Published studies have focused on surubim biology (Mateus and Petrere Jr 2004), reproduction (Brito and Bazzoli 2003), and nutrition (Martino et al. 2005). However, there are few studies about surubim meat quality and the contamination levels of these fish in nature.The São Francisco River basin is one of the most important in Brazil, with drainage areas in five states, and crossing three biomes. One of its main tributaries is the Paraopeba River, located in the state of Minas Gerais crossing 35 municipalities (IGAM 2005).

We investigated accumulation level of mercury (Hg), cadmium (Cd), zinc (Zn), chromium (Cr) and lead (Pb) in the muscle, liver and spleen tissues of *P. corruscans*, to determine whether specific elements exceeded the maximum acceptable concentrations for human consumption (ANVISA 1998).

MATERIALS AND METHODS

This study was conducted in accordance with the Brazilian legislation (Law #11.794, October 8, 2008). All capture, holding, and experimental techniques were performed in accordance with the Committee of Ethics in Animal Experimentation's (CEUA) regulations.

The analyzed fish were captured at two sites on the Paraopeba River (Fig. 1): Section A - just downstream of the Igarapé thermoelectric power plant (19° 57’ 50.24” S, 44° 16’ 52.42” W), and Section B - just downstream of the Retiro Baixo hydroelectric power plant (18° 52’ 28.64” S, 44° 46’ 50.27” W). Section A, is characterised as a highly impacted site, with its water contaminated by waste from the electroplating and steel industries, agriculture, and sewage, with contaminants exceeding safe levels for human health (IGAM 2012). On the other hand, section B, has much better water quality (IGAM 2012). Samples were collected from 32 specimens of *P. corruscans* (11 males and 21 females), from December 2010 to December 2012. The body length of the fish ranged from 55.0 to 90.0 cm for males and from 60.0 to 124.0 cm for females, and the body weight ranged from 1.1 to 8.88 kg for males and 1.62 to 18.54 kg for females.

For heavy metals analyses, fragments of the right dorsal muscle, spleen, and liver were collected and stored at -20 ºC prior to analysis. The analytical method used for determining the concentration of total mercury (Hg) in fish samples was based on thermal decomposition with detection by atomic absorption spectrometry, following the recommendations of the U.S. Environmental Protection Agency - EPA 7473 (1998). Data were expressed in $\mu g/g^{-1}$ for wet

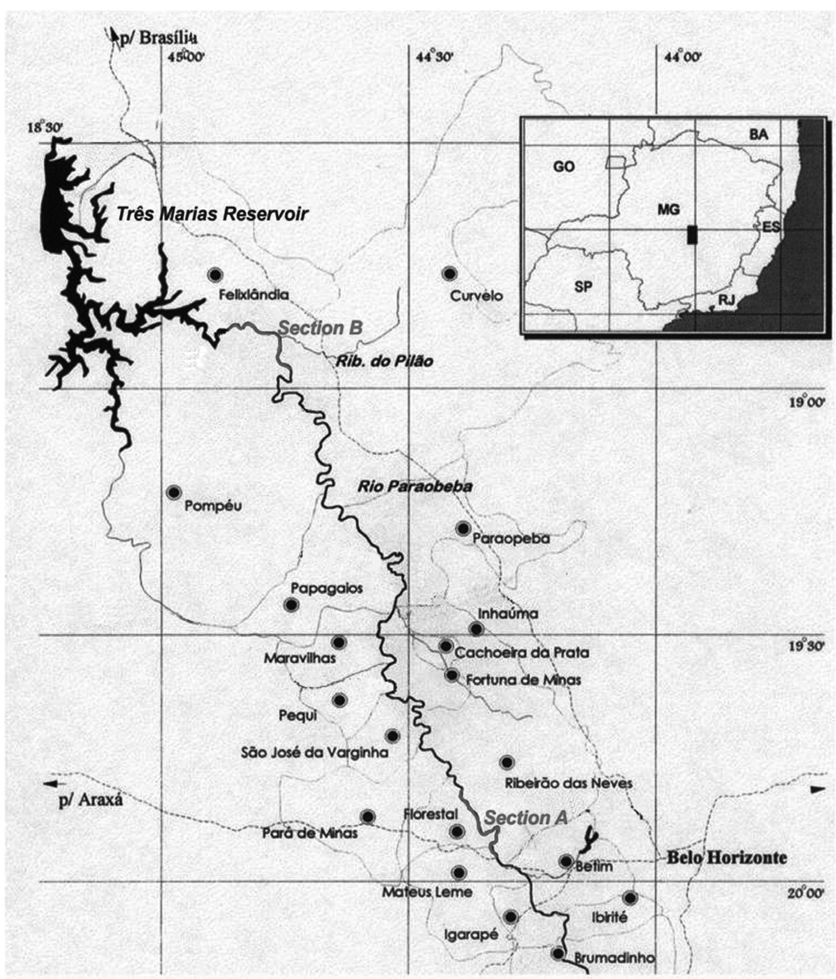

Figure 1 - Map of the Paraopeba River showing the location of sampling sites for the current study: Section A and Section B. (See the colors in the online version.)

weight. The determination of the concentration of Cd, Zn, Cr, and Pb in the samples was based on acid decomposition in a closed vessel device using microwaves (U.S. EPA-3052, 1996), and detection by atomic absorption spectrometry, performed in an atomic absorption spectrophotometer with flame and graphite furnace, model iCE 3500, Thermo Fisher Scientific brand. To certify the accuracy of the analysis, measurements of heavy metals for two reference materials were performed, where the recovery rates (average) were 99.2% and 95.5%.

Fragments of the liver and spleen of all fish caught were removed and fixed in Bouin's fluid for 8-12 h at room temperature, stored in 80% alcohol, and embedded in paraffin. At least 3 sections of each fragment (3-5 µm thickness) were stained with haematoxylin-eosin or Gomori's trichrome. Morphological analyses were performed to detect MMCs. The morphometry was performed under a light microscope coupled to a 5.0 megapixel digital camera and computer using the Motic Images Plus 2.0 image analyzer software. We calculated the total area of each histological section, area of each individual melanomacrophage centre, total area of the MMCs, and performed a count of the MMCs.

We also analyzed the relationship between the levels of contamination in *P. corruscans* with fish size, sex, and sampling site (section A and B). The relationship between the levels of contamination by Cd, Pb, Cr, Hg, and Zn from the liver, spleen and muscle were analyzed. Descriptive statistics for biological variables were performed using GraphPad InStat (Software Inc., Version 3.05, San Diego, CA, USA), and the values were expressed as the means ± standard deviation (SD). The data were analyzed using the KS normality test. When the distribution was not normal, the data were analyzed by the nonparametric Kruskal-Wallis Test (Non Parametric ANOVA) with Dunn post-test to verify the existence of significant differences between the parameters ($P < 0.05$). When the distribution was normal, the data were analyzed by an unpaired T-test (when the standard deviation was equal) and unpaired T test with Welch correction, when the standard deviation was different. The confidence interval was 95%.

RESULTS

The analysis of heavy metals showed the presence of Hg, Cd, Zn, Cr, and Pb in the spleen, liver, and muscle of all analyzed samples (Fig. 2). The livers showed higher levels of contamination for Cd and Zn, while the spleens showed higher levels of Cr (Fig. 2). It is also important to highlight that levels of Hg and Pb were higher or similar in muscle tissue compared to the liver and spleen.

The statistical analysis showed significant differences between the levels of heavy metals in the surubim collected from the two sections from Paraopeba River (Table I). For muscles, a significant difference in Cd and Zn levels was observed for fish from section A as compared to those from section B. Furthermore, the livers and spleens, showed significant differences for Pb between the sampling sites. Lastly, the Cr concentration was high in the spleens of fish caught in section A.

A positive correlation between fish size and levels of contamination was registered only for some of the analyzed metals, such as Hg, which was positively correlated in males and females for all analyzed tissues (Table II). Furthermore, there was no positive correlation between fish size and the levels of contamination by Pb for any of the tissues analyzed.

The histological analysis showed MMCs in the livers (Fig. 3a) and spleens (Fig. 3b). These structures were formed by macrophage aggregates, which presented pigmented material varying from pink to red and light brown to dark brown (Fig. 3c). The MMCs were associated to blood vessels (Fig. 3c and d), presenting a spherical or irregular shape (Fig. 3a-f), and varying in their individual size (Table III). Moreover, different levels of fibroses were observed in the liver (Fig. 3e-f).

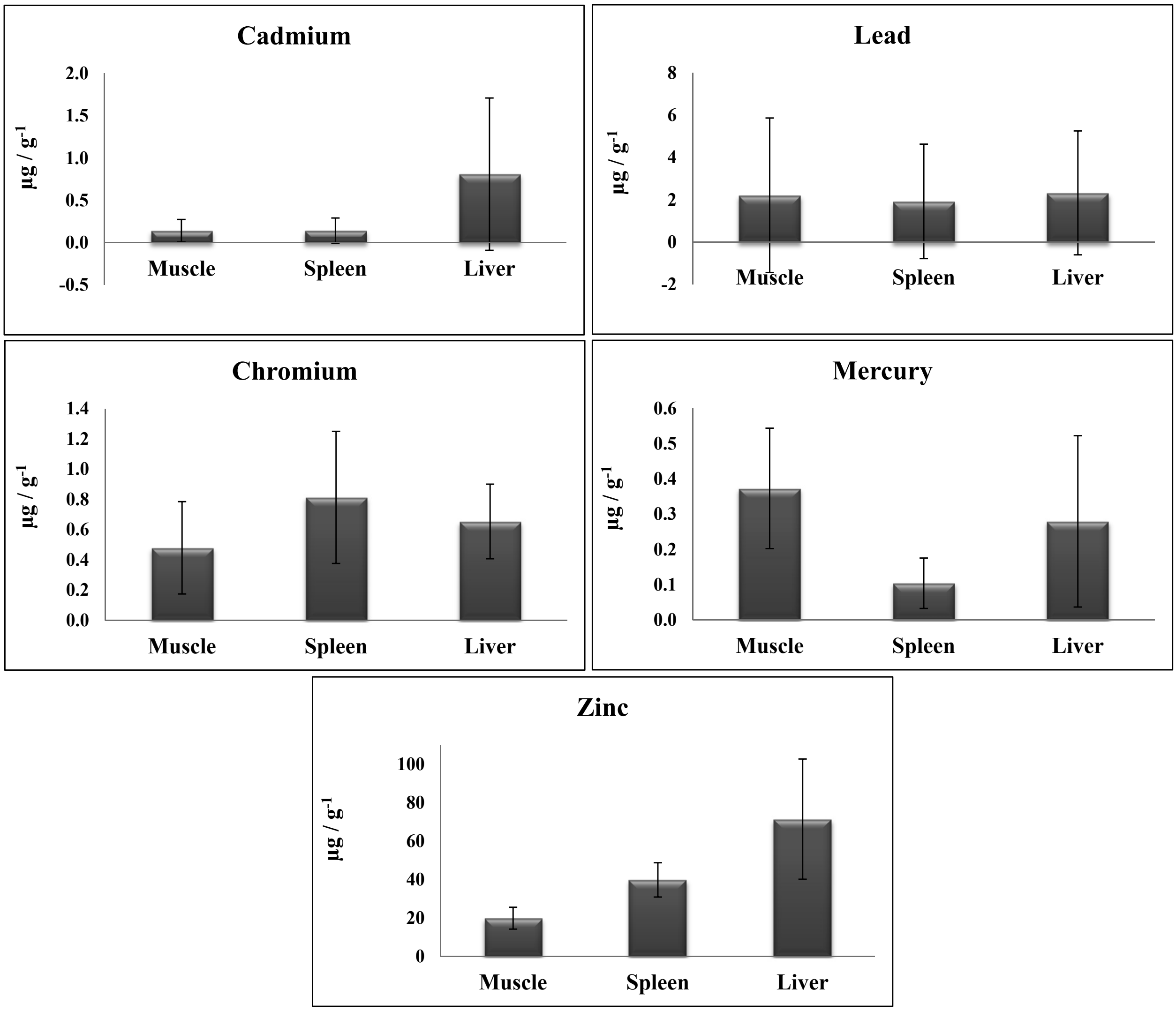

Figure 2 - Bioaccumulation (μg/g $^{-1}$ wet weight) of Cd, Pb, Cr, Hg and Zn in muscle, spleen and liver tissues of *P. corruscans*. Mean and standard deviation. The different letters mean statistically significant differences ($p < 0.05$) to the values in the same graph.

TABLE I

Bioaccumulation (μgg^{-1} wet weight) of cadmium, zinc, lead, chromium, and mercury in muscle, liver and spleen of *P. corruscans* from two sections of the Paraopeba River. Data expressed in mean ± standard deviation.

	Muscle			Liver			Spleen		
	Section A	Section B		Section A	Section B		Section A	Section B	
Cd	0.07 ± 0.06	0.19 ± 0.15	**s**	0.65 ± 0.61	0.97 ± 1.09	**ns**	0.08 ± 0.05	0.19 ± 0.18	**ns**
Zn	17.81 ± 4.23	21.71 ± 6.28	**s**	69.54 ± 28.07	72.45 ± 35.87	**ns**	43.35 ± 12.32	37.6 ± 4.91	**ns**
Pb	0.94 ± 0.98	3.31 ± 4.64	**ns**	1.03 ± 0.67	3.37 ± 3.66	**s**	0.67 ± 0.37	2.96 ± 3.35	**s**
Cr	0.54 ± 0.42	0.42 ± 0.32	**ns**	0.72 ± 0.23	0.59 ± 0.26	**ns**	1.04 ± 0.53	0.66 ± 0.28	**s**
Hg	0.41 ± 0.18	0.35 ± 0.17	**ns**	0.26 ± 0.01	0.31 ± 0.32	**ns**	0.11 ± 0.08	0.11 ± 0.06	**ns**

ns = not significant; s = significant.

TABLE II
Relationship between the levels of cadmium, chromium, lead, mercury and zinc contamination in liver, spleen and muscle and the body size of *P. corruscans*.

		Cd	Cr	Pb	Hg	Zn
Female	Liver	0.91	0.47	- 0.02	0.95	0.05
	Spleen	0.71	- 0.39	- 0.09	0.63	0.25
	Muscle	- 0.02	0.06	- 0.04	0.54	- 0.07
Male	Liver	- 0.05	0.07	- 0.01	0.59	- 0.45
	Spleen	- 0.20	-0.37	- 0.28	0.38	- 0.62
	Muscle	0.33	0.38	0.04	0.40	0.50

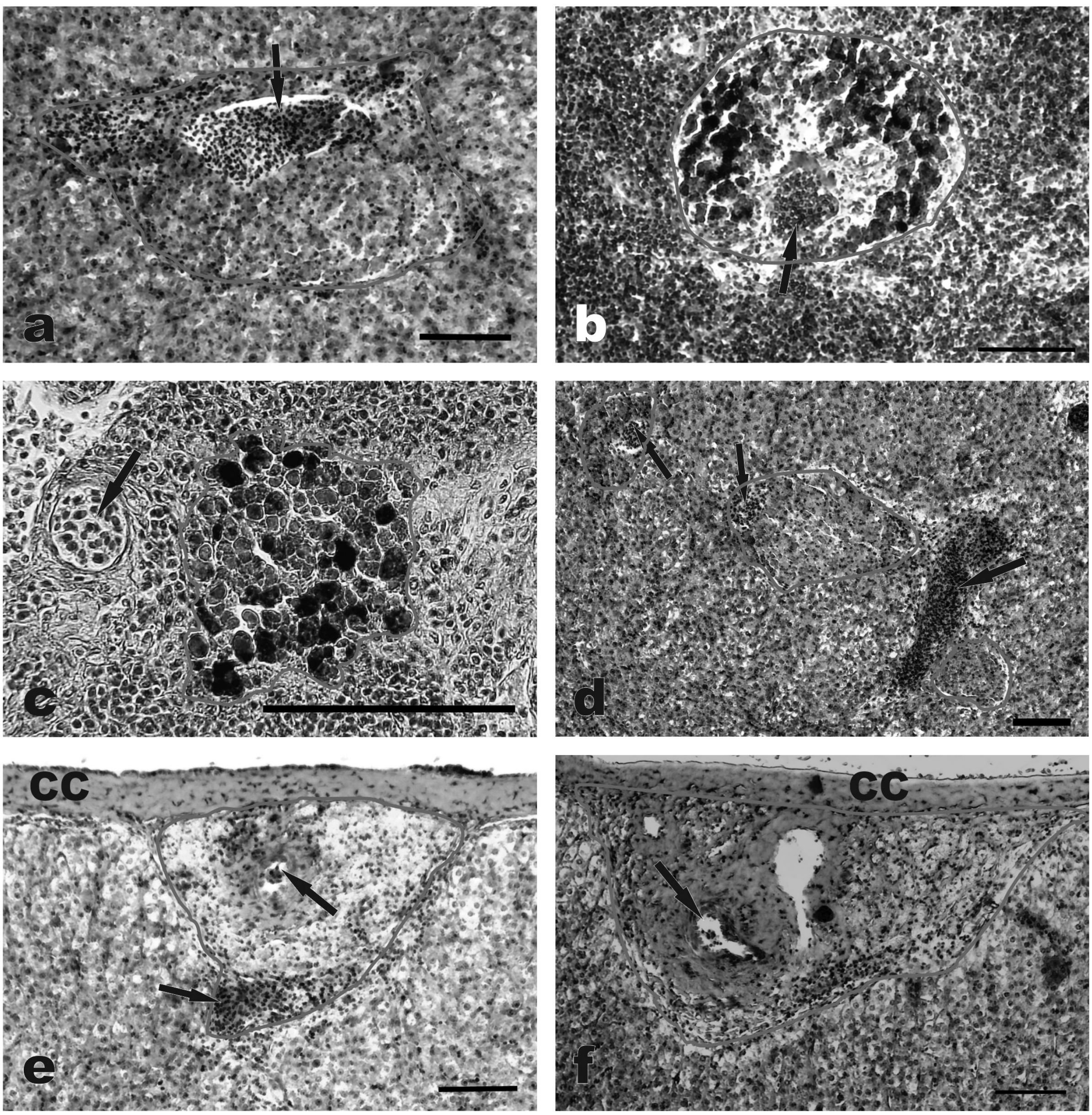

Figure 3 - Histological sections of liver (**a**, **d**, **e** and **f**) and spleen (**b** and **c**) of *P. corruscans* showing MMCs (inside the circles) in association with blood vessels (arrows). MMCs distributed throughout the parenchyma of the organs **a**-**d**, and adhered to the liver conjunctiva capsule (**e** and **f**). Gomori's trichrome stain. Scale bar corresponds to 100 µm. (See the colors in the online version.)

For the liver, melanomacrophagic centres (MMCs) occurred in 63% of fish collected in section A and 77% of those in Section B. In relation to the spleen, the presence of MMCs was detected in 100% of the fish from section A and in 92% of fish from section B. The largest total area occupied by MMCs (1.24% of the analyzed tissue), and the largest number of MMCs (555 centres) were recorded in the spleens. Meanwhile, the liver registered the highest area occupied by a single MMC, 5,467 μm^2 (Table III).

Statistical analyses showed no significant relationship between the presence or morphology of MMCs and the length/weight of the fish (Table III), nor any relationship to the sex of the individuals. However, correlations between the MMCs and levels of contamination by Pb, Cd, Cr, Hg and Zn were observed. We only observed a significant positive relationship between the number of MMCs and the area occupied by them in the livers to the concentration of Hg.

Considering the two length classes (63-75 and 76-88 cm) of the fish caught in the two sections sampled from Paraopeba River, we observed that the fish collected from section A, had a higher number of MMCs, both in the liver and in the spleen, and greater individual area occupied by MMCs in the livers, whereas fish collected from section B showed a larger area occupied by an individual MMC in the spleen. Fish from the section A, which has higher pollution levels and belong to the length class 76-88 cm showed larger total areas occupied by MMCs in the livers and spleens.

DISCUSSION

Fish are an important source of food and represent a major part of many natural food chains. Therefore, the levels of contaminants in fish are of particular interest because of the potential effects of these polluting substances on the fish themselves and on the organisms that consume them, including humans (Burger and Gochfeld 2005). Our results showed the presence of Hg, Cd, Zn, Cr, and Pb in all *P. corruscans* collected from the Paraopeba River. Moreover, the results indicated a variation between the heavy metals levels in the analyzed muscle, liver, and spleen tissues, as also registered by Taweel et al. (2013).

In general, the levels of heavy metals accumulation in *P. corruscans* were greater in the liver, spleen, and muscle, respectively. Sindayigaya et al. (1994) found higher levels of Zn and Cd in

TABLE III
Individual area, total area and number of MMCs per mm^2 in liver and spleen of *P. corruscans*. Data expressed as mean ± standard deviation and range, with *p* value (Mann-Whitney test).

			Male	Female	*p*
Individual area of MMC's (μm^2)	**Liver**	M±SD	716.7 ± 792.2	908.9 ± 1361.3	0.89
		Range	188.0 - 2 345.0	227.0 – 5 467.0	
	Spleen	M±SD	1 058.5 ± 1 157.7	653.76 ± 660.15	0.37
		Range	157.0 – 3 461.0	173.0 – 3 024.0	
Total area of MMC's (%)	**Liver**	M±SD	0.06 ± 0.1	0.10 ± 0.2	0.84
		Range	0 - 0.4	0 - 0.6	
	Spleen	M±SD	0.52 ± 0.4	0.33 ± 0.2	0.19
		Range	0 - 1.2	0.01 - 0.7	
Number of MMC's / 1000 mm^2	**Liver**	M±SD	1.6 ± 1.5	0.82 ± 0.6	0.09
		Range	0 - 5.1	0 - 1.8	
	Spleen	M±SD	10.5 ± 10.2	8.08 ± 6. 6	0.18
		Range	0.4 - 32.5	0 - 23.5	

fish livers in comparison to other organs. The liver is often used as a reference for analysis of tissue damage caused by environmental toxic compounds (Amaral et al. 2002).

Although there is a relationship between the levels of contamination of the aquatic environment with the bioaccumulation of heavy metals in fish, our results showed that only Cr in the spleen of *P. corruscans* was detected at significantly higher levels of contamination in section A (i.e., the section with higher levels of pollutants). This result could be related to the fact that migratory fish like *P. corruscans* can move between sites with higher levels of pollutants and less degraded places. Consequently, consumption of fish can be dangerous even when these fish are caught in areas considered to have low rates of contamination. Furthermore, in our findings, it was not possible to verify any relationship between the levels of heavy metals bioaccumulation to the sex of the analyzed fish, as has previously been reported by Kehrig et al. (2008) for other Brazilian commercially important fish.

In our study, concentrations of Hg and Cd increased in accordance to fish size, whilst no significant concentration-size relationship was found for the other metals, indicating that accumulation of Pb, Zn, and Cr in *P. corruscans* from the Paraopeba River is not necessarily related to age. Barone et al. (2013) also found a significant concentration-size relationship for Hg in livers of two fish species. Due to their cumulative potential, high levels of Hg in fish, present a significant risk in the aquatic ecosystem, as well as to human health (Dang and Wang 2012).

In the present study, the muscles exhibited high levels of Hg contamination, and the Hg levels were also positively correlated to body weight, confirming the theory of bioaccumulation from continuous exposure to the metal, as also recorded by Seixas et al. (2012). As reported for other fish (Sellanes et al. 2002), in our work, muscle samples with levels of Hg contamination above the safe limit for human consumption (ANVISA 1998) were not recorded. Despite this, we note that in all the fish analyzed we detected the presence of Hg, including some cases of levels near the safe limit for human consumption. Furthermore, the elevated levels of Pb contamination observed in the liver, spleen, and muscle of *P. corruscans* do exceed the safe limit for human consumption. *Astyanax* sp. from another Brazilian river presented high concentrations of lead in its muscles and viscera (Jordão et al. 1999). As in mammals, the principal effects of chronic Pb exposure on fish are presumably haematological (Schmitt et al. 2002), neurological (Noureddine et al. 2005) and renal (Patel et al. 2006) impairment. Although the presence of Cd in muscle, liver and spleen tissues was detected in all examined fish, Cd levels above those considered safe for human consumption were only registered in hepatic tissue, reinforcing the hypothesis that the liver would actually be a target organ for processing and bioaccumulation of heavy metals (Sindayigaya et al. 1994).

The melanomacrophagic centres (MMCs) act upon the centralisation of destruction and detoxification of endogenous and exogenous materials (Rabitto et al. 2005). In *P. corruscans*'s spleen, the MMCs occurred mainly close to ellipsoids, and such proximity is related to the fact that ellipsoids have a high filtration capacity and capture pathogens, which can subsequently be conducted to the MMC in the spleen (Danneving and Landsverk 1995). In *P. corruscans* liver, the MMCs were mainly observed near hepatic veins and arteries, indicating their interaction with the blood system. This result showed that the amount or area occupied by MMCs in the spleens and livers of *P. corruscans* is not directly correlated to the size and weight of the fish, unlike that observed for other teleosts (Haaparanta et al. 1996). Moreover, there was also no significant correlation between the MMCs and the sex of the individuals analyzed. Thus, our findings indicate that MMCs in *P. corruscans* (at least partly) are physiologically decreased and are more related to the level of environmental stress in which the fish are found at the moment, than to their size or sex, which therefore shows that MMCs are not cumulative throughout the life of the fish. An increased MMC presence in the spleen and liver has often been associated with degraded environmental conditions (Agius and Roberts 2003). Corroborating these authors, our results showed that the MMCs in fish captured from section A (high pollution levels) are larger than section B. A positive correlation between the quantity and the area occupied by the MMCs and bioaccumulation of heavy metal was only significant for Hg in the liver. This result contrasts with that registered by Rabitto et al. (2005), where Pb contamination in *Hoplias malabaricus* resulted in increased MMCs and a reduction of free macrophages.

In conclusion, the present study provides crucial information on the distribution of heavy metals in tissues of a fish with great economic importance, showing that the viscera (i.e., liver and spleen) usually contain high amounts of heavy metals, and should thus not be considered for human consumption. Although the levels of muscle bioaccumulation of Hg, Cd, Zn, Cr, and Pb in the analyzed samples of *P. corruscans* generally do not exceed the safe levels for human consumption (ANVISA 1998), the constant presence of heavy metals in concentrations near those considered safe for human consumption is a reason for warning populations who regularly consume fish from the Paraopeba River. Moreover, our results indicate that in a river network where there are connected areas with high rates of environmental pollutants, consumption of fish can be dangerous even when these fish are caught in areas considered to have low rates of contamination.

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RESUMO

O aumento da entrada de metais pesados nos ambientes aquáticos pode resultar em altos níveis de acumulação destes contaminantes nos peixes e seus consumidores, representando um risco para os ecossistemas e para a saúde humana. Investigamos as concentrações de mercúrio (Hg), cádmio (Cd), zinco (Zn), cromo (Cr) e chumbo (Pb) nos tecidos do músculo, fígado e baço de *Pseudoplatystoma corruscans*, coletado em dois trechos do rio Paraopeba, Brasil. Nossos resultados mostraram que as concentrações dos metais pesados foram mais elevadas nas vísceras, isto é fígado e baço, indicando que as vísceras de *P. corruscans* não devem ser utilizadas para consumo humano. As correlações entre as concentrações de metais pesados e o tamanho dos peixes não foram estatisticamente significativas. Embora os níveis de Hg, Cd, Zn, Cr e Pb no músculo geralmente não tenham ultrapassado os limites seguros para o consumo humano, a presença constante desses metais em concentrações próximas a estes limites é motivo de alerta para as populações que consomem regularmente peixes de rios poluídos como o rio Paraopeba. Nossos resultados também indicaram que em uma rede fluvial, onde existam áreas poluídas conectadas a ambientes de boa qualidade, o consumo regular de peixes, mesmo quando estes são capturados em trechos onde os níveis de contaminação forem considerados baixos, pode oferecer risco à saúde, uma vez que muitos peixes de água doce com alto valor comercial como *P. corruscans* são migradores.

Palavras-chave: contaminação, peixes de água doce, metal pesado, saúde humana.

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