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Full Paper

Interspecific Differentiation in Heavy Metals Concentration in Fishes of the Apa River, Upper Paraguay River Basin

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Abstract: The concentration of heavy metals in fishes is an important indicator of geomorphological or environmental integrity in aquatic habitats. Aiming to evaluate metal concentration in the muscles of fish native to the Apa River, Upper Paraguay River Basin (UPRB), we analyzed 124 individuals of eight fish species from the Cachoeira do Apa district (Porto Murtinho) from 2013 to 2014, including *Brycon hilarii*, *Leporinus friderici*, *Megaleporinus macrocephalus*, *Megaleporinus obtusidens*, *Piaractus mesopotamicus*, *Prochilodus lineatus*, *Pterodoras granulosus* and *Salminus brasiliensis*. The samples were frozen; in the laboratory, the musculature was removed, and the samples were digested using tri-acid etch solution. Later, the concentration of heavy metals was quantified in a flame atomic absorption spectrophotometer. A significant difference was observed in the concentration of metals in fish ($F=27.79$, $p<0.001$). Considering the multivariate comparison of studied species, we observed that *P. lineatus* had a much different pattern of metal concentration. *P. granulosus* also presented a pattern different from that of other species. No species presented a concentration of metals above the limit defined by Brazilian legislation; however, species differed in their metal concentrations according to position in the water column and feeding, suggesting that an integrated assessment of metal concentration may be an indicator of ecological differences in the obtaining of resources by species in aquatic communities.

Keywords: bioaccumulation; contamination; Fe; Cu; Zn

1. INTRODUCTION

Water resources are essential for the maintenance of all life on the planet, and they are important for carrying out industrial activities, as well as agriculture and livestock grazing. In addition, water resources are the final destination for virtually all waste generated by these activities. Natural concentrations of metals in the aquatic environment may be mainly defined by geomorphological characteristics; however, its concentration can be increased owing to human activities, such as agriculture [1], mining [2] and chemical pollution caused by household and industrial waste [3]. Therefore, contamination of aquatic ecosystems with organic or inorganic material is inevitable. Heavy metals are contaminants commonly found in aquatic ecosystems, and they can cause severe damage to aquatic biota [4]. In this context, fish are considered sources of minerals, proteins and vitamins. As such, it is necessary to assess the concentration of heavy metals in these individuals as a precaution against harming the health of the populations that use these fish as food [5].

However, studies on fish toxicity represent a new and growing field, and most studies are restricted to *in vitro* analysis of the effect of heavy metals on fish [6]. For the Pantanal region, the number of studies is even smaller, resulting in a corresponding and even smaller sampling of heavy metals in the water and sediment of the Cuiabá and São Lourenço Rivers [7]. No studies have reported on heavy metals in water, fish or sediments of the Apa River Basin. Therefore, this study aimed to determine the concentration of selected metals in the muscle of eight fish species from the Apa River, UPRB, and associate such metal concentration with fish feed.

2. MATERIAL AND METHODS

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The individuals were collected in the Apa River Basin (Figure 1), Cachoeira do Apa district, between 2013 and 2014. To collect samples, seining nets were used with different mesh sizes. In the sampled sections, the mean width was 100 m, the mean depth was 1.2 m, and the current velocity was 0.9 $m.s^{-1}$. A total of 124 individuals belonging to eight species were collected and analyzed, including *Brycon hilarii* (Piraputanga) 10 individuals, *Leporinus friderici* (Piau) 10 individuals, *Megaleporinus obtusidens* (Piavuçú) 10 individuals, *Megaleporinus macrocephalus* (Piavuçú) 6 individuals, *Piaractus mesopotamicus* (Pacu) 6 individuals, *Prochilodus lineatus* (Curimba) 47 individuals, *Pterodoras granulosus* (Armal) 5 individuals and *Salminus brasiliensis* (Dourado) 21 individuals.

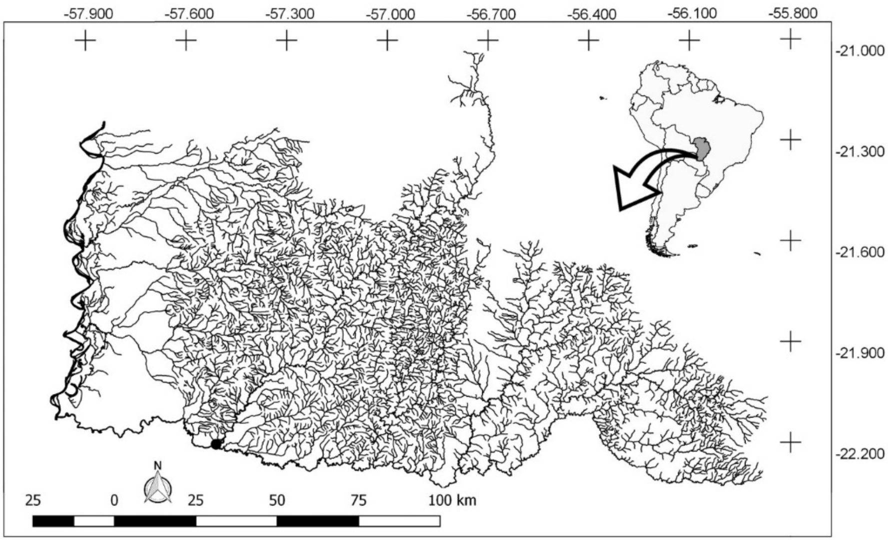

Figure 1. Location of the sampled points in the Apa River Basin, UPRB. Black circle indicates the sampling point at Cachoeira do Apa.

Musculature samples of the studied species were obtained in the field and frozen. In the laboratory, the samples were stored in polyethylene bottles and stored in a freezer at -6 ºC. Afterwards, they were dried in a greenhouse at 60 ºC and crushed. Afterwards, 1g of the dried sample was transferred to boron silicate test tubes. Then, 10 mL of concentrated nitric acid and 2.5 mL of concentrated perchloric acid were added and allowed to react for 24 hours. The sample was submitted to a digester at 600 °C and warmed to near dryness. The sample was cooled, redissolved in 10 mL of Milli-Q water (Millipore Corp.). Then, 1 mL of concentrated hydrochloric acid was added, and the volume was filled with Milli-Q water in a 25 mL volumetric flask. Subsequently, the material was transferred to polyethylene bottles, and these solutions, when necessary, were diluted to carry out the readings.

The measurements were also performed in triplicate, using flame atomic absorption (air-acetylene) spectrophotometry. The metals analyzed were Cr, Fe, Ni, Zn and Cu, selected by instrumental availability and by the absence of clear pollution source. Experimental conditions of analysis for each metal, using the flame atomic absorption spectrophotometer, are summarized in Table 1.

Table 1. Operating conditions used for the flame atomic absorption spectrophotometry measurements in the determination of the metals studied and the coefficient of determination (R^2) obtained using the external standard method.

Element	Linear range (mg L^{-1})	λ (nm)	Coefficient of Determination (R^2)
Cr	0.1 – 12.0	357.9	0.9998
Fe	0.2 – 9.0	248.3	0.9996
Ni	0.2 – 8.0	232.0	0.9992
Zn	0.1 – 6.0	213.9	0.9992
Cu	0.2 – 6.0	324.7	0.9994

The concentration of minerals present in the samples was calculated from the construction of specific calibration curves for each element with at least five points and having R^2 equal to, or greater than, 0.9992 (Table 1). The minimum limits of determination for each element were Cr (0.1 mg L^{-1}), Fe (0.2 mg L^{-1}), Ni (0.2 mg L^{-1}), Zn (0.1 mg L^{-1}) and Cu (0.2 mg L^{-1}).

Variation in the concentration of heavy metals (iron, zinc and copper) in each species of fish was quantified through a multivariate analysis of variance (MANOVA) using the "manova" function of the "vegan" package. To obtain an ordering of individuals analyzed in a multivariate space with the object of visualizing possible differences among the studied species, we performed a Principal Coordinate Analysis (PCoA), using Euclidean distance between individuals. This analysis was performed through the "betadisper" "vegan", and all analyses were made on the R platform (R Core Team 2017).

3. RESULTS AND DISCUSSION

All analyzed heavy metals had a concentration below tolerance limits, as defined by the Agência Nacional de Vigilância Sanitária that establishes tolerance limits for Cu (30 mg/kg) and Zn (50 mg/kg). Fe has no stipulated limit. Cr and Ni did not present detectable concentrations in any analyzed sample. The concentration of heavy metals in aquatic ecosystems is influenced by natural processes, such as weathering of rocks, sediment transport and amount of organic matter in water, as well as by anthropic activities, such as urbanization [8], industry and agriculture [2, 3]. The study area is located near the Cachoeira do Apa Municipal Natural Park, a conservation unit, and in the surroundings, the population density is still low.

The results of multivariate analysis of variance (MANOVA) showed that analyzed species have significant differences in metal concentration ($F=21.07$; $p<0.001$) and that all metals differed among species. However, iron had the greatest difference ($F=46.92$; $p<0.001$), followed by copper ($F=45.26$; $p<0.001$) and finally by zinc ($F=15.91$; $p<0.001$). Comparing analyzed species, *P. lineatus* had the highest iron concentration, while *L. friderici* had the lowest concentration. For copper concentration, *S. brasiliensis*, *L. friderici* and *M. macrocephalus* had high values, while *P. granulosus* and *P. lineatus* had low values. For zinc concentration, we observed that *M. obtusidens* had a high concentration, while *L. friderici* and *M. macrocephalus* had a low concentration (Figure 2).

Considering the multivariate comparison of studied species, we saw that *P. lineatus* had a much different pattern of metal concentration and that *P. granulosus* also presented a pattern different from that of other species. Using the second axis of PCoA, it could be seen that *M. friderici* and *M. macrocephalus* were located at the inferior portion of the axis, while *S. brasiliensis*, *B. hilarii*, *P. mesopotamicus* and *M. obtusidens* were located at the upper portion of the axis (Figure 3).

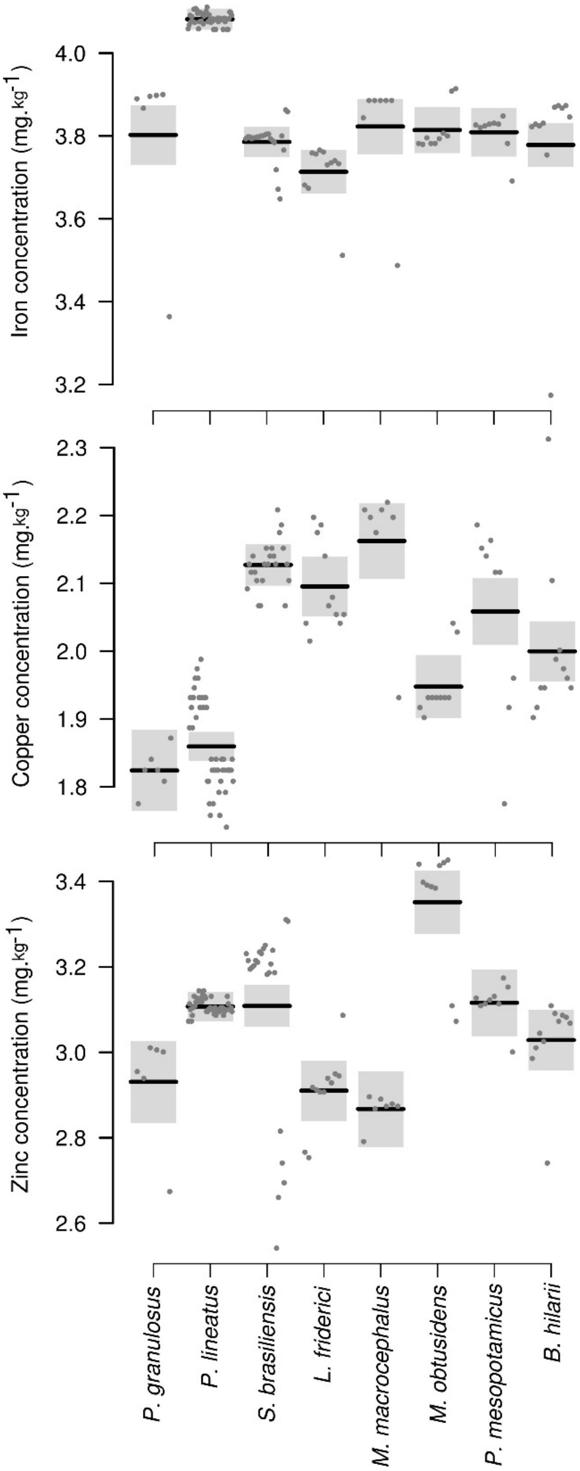

Figure 2. Mean and confidence intervals for metal concentration among studied fish species in the Apa River, UPRB, Brazil.

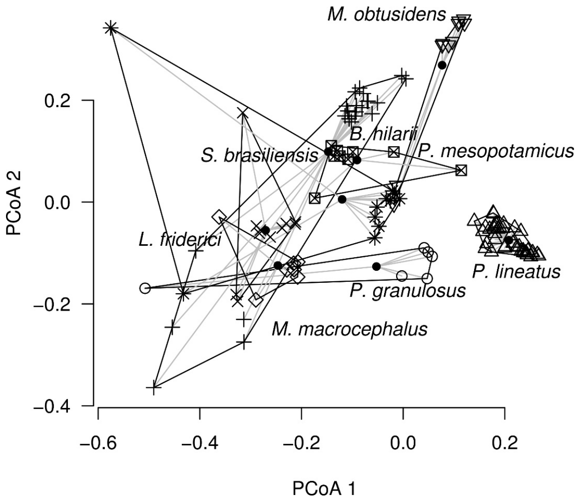

Figure 3. Principal Coordinates Analysis ordination for metal concentration in the musculature of eight studied fish species in the Apa River, UPRB, Brazil.

Prochilodus lineatus is a detritivorous species [9] of the Miranda River, having some anatomical characteristics adapted to feeding near sediment. In the Apa River, we observed a predominance of moluscs (bivalvia) in stomachs of *P. granulosus*, which associates this species with sediment. Therefore, the differentiation of *P. lineatus* and *P. granulosus* in a first axis suggests that this result can be attributed to their position near sediment and that metal absorption results from feeding and/or sediment, then these species can be a good indicator of environmental integrity [10].

A high concentration of heavy metals was also found in other benthopelagic species [11]. However, based on our data, two detected metals showed a similar pattern for these species with intermediate concentration of zinc and low concentration of copper, suggesting that each metal, depending on availability and solubility, can present a different absorption rate for each fish species.

For copper concentration, as well as zinc, the formation of two subgroups of *P. lineatus* individuals (Figure 2) was observed, suggesting that they occupy areas in the lower portion of the basin with different hydrogeochemical characteristics, irrespective of reproductive behavior. Under these circumstances, we cannot determine whether reproduction sites are the same for sampled individuals. However, based on metal concentration in muscle, we suggest that they occupy different areas of the floodplain during feeding periods.

Variations in metal concentration in fish with different feeding habitats are well documented [12, 13] and are associated with the different rate of metal incorporation in organisms in the food chain, as well as differences in feeding habitat, as has been evaluated for many analyzed species [9, 14-17]. Therefore, even though the metals analyzed do not present concentrations higher than those allowed by Brazilian laws, our results did show clear differences when comparing species with different trophic guild. It can be concluded that metal concentration can be used as a chemical signature of feeding resources and habitat used by the studied fish species.

4. CONCLUSION

While no species presented a concentration of metals above the limit of tolerance, as defined by Brazilian legislation, species differed in their metal concentrations according to their position in the water column and feeding, suggesting that an integrated assessment of metal concentration may be an indicator of ecological differences in the obtaining of resources by species in aquatic communities.

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