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Heavy metal bioaccumulation of the characiform *Brycon falcatus* Muller & Troschel, 1844 in the Teles Pires basin, Southern Amazon

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ABSTRACT. The Teles Pires River basin is located in an area of the Southern Amazon where discharge of tannery effluents and intense agricultural activity occurs. These activities increase the risk of contamination by runoff and leaching of pesticides and heavy metals. This study presents the concentration and bioaccumulation of heavy metals copper (Cu) and total chromium (Cr) in matrinxã (*Brycon falcatus)*, a species of fish highly consumed in the region. Liver and muscle tissue was analyzed from 41 samples of *B. falcatus* collected during the dry season in 2013 from the Teles Pires River basin. Considering that bioaccumulation is the progressive increase in the amount of a substance in an organism or part of an organism, copper bioaccumulation in liver samples from *B. falcatus* was verified. Cr and Cu concentrations were higher in the liver than in the muscle tissue of fish collected in all rivers. The highest concentrations of Cr in the liver were observed in fish collected from the Teles Pires River (1.87 $\mu g \cdot g^{-1}$) and the Celeste River (1.06 $\mu g \cdot g^{-1}$). The highest concentrations of Cu in the liver were observed in fish collected from the Cristalino River (44.20 $\mu g \cdot g^{-1}$) and the Teles Pires River (34.77 $\mu g \cdot g^{-1}$). The high concentration of Cu in the livers of fish collected from the Teles Pires river basin reflects the economic activities of mining and agriculture in the surrounding areas of this basin.

Keywords: agriculture, Cr, Cu, matrinxã, mining, Tapajós River Basin.

Bioacumulação de metais pesados no characiforme *Brycon falcatus* Muller & Troschel, 1844 na bacia do rio Teles Pires, Amazônia Meridional

RESUMO. Na Amazônia Meridional a bacia do rio Teles Pires está numa área onde existe despejo de efluentes de curtumes e intensa atividade agrícola, aumentando o risco de lixiviação e escoamento de defensivos agrícolas e metais pesados. Este estudo apresenta a concentração e bioacumulação de metais pesados, cobre (Cu) e cromo (Cr) total no matrinxã, *Brycon falcatus*, uma espécie de peixe muito consumida regionalmente. Analisamos o fígado e tecido muscular de 41 exemplares de *B. falcatus* coletados durante a estação seca de 2013 na bacia do rio Teles Pires. Considerando que bioacumulação é o aumento progressivo na quantidade de uma substância em um organismo ou parte deste, verificamos bioacumulação de cobre nas amostras de fígado de *B. falcatus*. As concentrações de Cr e Cu foram maiores no fígados do que no músculo dos peixes nos rios coletados. As maiores concentrações de Cr no fígado foram observadas nos peixes coletados no rio Teles Pires (1,87 $\mu g \cdot g^{-1}$) e no rio Celeste (1,06 $\mu g \cdot g^{-1}$). As maiores concentrações de Cu no fígado foram observadas nos peixes coletados no rio Cristalino (44,20 $\mu g \cdot g^{-1}$) e no rio Teles Pires (34,77 $\mu g \cdot g^{-1}$). As altas concentrações de Cu no fígado dos peixes coletados na bacia do rio Teles Pires refletem as atividades econômicas do garimpo e agricultura no entorno desta bacia.

Palavras-chave: agricultura, Cr, Cu, matrinxã, garimpo, bacia do rio Tapajós.

Introduction

Heavy metal contamination of fish is related to human activities, namely the discharge of effluent into water bodies due to population increase, urban development, industrialization, and agricultural practices (Giguere, Campbell, Hare, McDonald, & Rasmussen, 2004). Heavy metals that enter water bodies through effluent discharge or which are carried by rainwater accumulate in aquatic organisms and transfer to humans through feeding (Korn, Santos, Rosa, Teixeira, & Oliveira, 2010). There are organs which are important in helping to determine the bioaccumulation of metals in fish, such as the liver (Licata et al., 2005), which plays a vital role in metabolism, biotransformation and excretion of contaminants in fish, and is one of the largest organs (Figueiredo-Fernandes, Fontainhas-Fernandes, Monteiro, Reis-Henriques, & Rocha, 2006). Copper (Cu) is a heavy metal that can be found in nature in its elemental form and is widely used in galvanoplasty and in the formulation of insecticides and fungicides (Wuana & Okieimen, 2011). Chromium (Cr) is used in the production of metal alloys, anti-corrosive paints and in leather tanning (Oga, Camargo, & Batistuzzo, 2008). Cr is a dangerous heavy metal which can accumulate in many organisms thousands of times above the level found in the surrounding environment (Duffus, 1980).

The majority of studies of heavy metals in the Amazon basin have focused on mercury contamination of fish (Malm, Branches, Akagi, Castro, & Pfeiffer, 1995; Dorea, Moreira, East, & Barbosa, 1998; Kehrig, Howard, & Malm, 2008), water (Lacerda et al., 1991; Roulet, Lucotte, Guimarães & Rheault, 2000), river sediments (Mascarenhas et al., 2004; Siqueira & Aprile, 2012), and humans (Akagi et al., 1995; Malm et al., 1995; Lebel et al., 1998; Khoury et al., 2013). Studies on Cr and Cu contamination in the Amazon basin are scarce, with few studies focusing on sediments (Silva, Ramos, & Pinto, 1999), water (Pinto et al., 2009; Lima et al., 2015), and fish (Lima et al., 2015). Some studies on Cr and Cu contamination in the Tapajós River basin have been reported, with some of these studies focusing on humans (Rodrigues et al., 2009) and others on water (Miranda, Pereira, Alves, & Oliveira, 2009; Castilhos et al., 2010). The Teles Pires River is a tributary of the Tapajós River, located in an area in which the main economic activities include cattle raising and agriculture. This lack of knowledge has led to the compromised quality of water resources, particularly due to leaching, eutrophication, and loss of riparian vegetation (Goulding & Barthem, 1997). Within the Teles Pires River basin the Verde and Teles Pires rivers receive effluent discharge from intense agricultural activity (soya and cotton) and leather tanneries. Because Cu is used in agricultural inputs and Cr is discharged as part of tannery effluents, Cu and Cr may contaminate the water bodies of the Teles Pires River basin.

Brycon falcatus is one of the most commonly consumed fish present within the Teles Pires River basin due to the excellent quality of its meat (Sanches & Galetti, 2007), and has a high commercial importance in commercial extractive fishing and sport fishing. *B. falcatus* is an omnivorous (Albrecht, Caramaschi, & Horn, 2009; Matos & Carvalho, 2015) and migratory species, commonly known as matrinxã, that belongs to the subfamily Bryconinae. This species occurs in the hydrographic basins of the Guyanas, Suriname, Amazon, Orinoco, and Araguaia-Tocantins rivers (Lima, 2003). Considering the current scenario of possible heavy metal contamination of the Teles Pires River basin coupled with the high consumption rate of matrinxã that originate from this basin, the goal of this study was to determine total Cr and Cu concentration in the muscle and liver tissue of *B. falcatus,* and investigate the relationship between Cr and Cu bioaccumulation with length and weight of *B. falcatus*. In this study the concept of bioaccumulation as defined by the International Union of Pure and Applied Chemistry (1993) was used, which assumes that bioaccumulation is the increasing quantity of a substance in an organism or part of an organism that occurs due to the ingestion rate exceeding the body's ability to remove the substance from the body.

Material and methods

Study area

The Teles Pires River basin is located in the northern state of Mato Grosso and is one of the main tributaries of the Tapajós River basin. Sample collections were performed in the upstream to downstream direction in the following rivers: the Celeste River (agriculture), located in the Vera municipality, the Verde River (discharge of tannery effluents, agriculture, and cattle raising), located in the Sorriso municipality, the Teles Pires River (discharge of tannery effluents, agriculture, and cattle raising), located in the Sinop municipality, the Tapaiúna River (cattle raising), located in the Nova Canaã do Norte municipality, and the Cristalino River, located at a Conservation Unit in the Novo Mundo municipality (Figure 1). The Cristalino River is mostly located inside the Cristalino State Park (Parque Estadual do Cristalino - PEC), with the entire area surrounding the Cristalino basin legally protected against deforestation and human occupation. The fish collected at the PEC Conservation Unit were considered the control sample.

Fish collection and biometry

Fish collections were performed between April and July 2013. Captures were performed using fishing poles with rods and artificial bait. Following capture, the fish were anesthetized with Eugenol® (Vidal et al., 2008), euthanized, immersed in ice, and placed in plastic bags. In the laboratory, the total length (cm), standard length (cm), and weight (kg) were measured for all specimens.

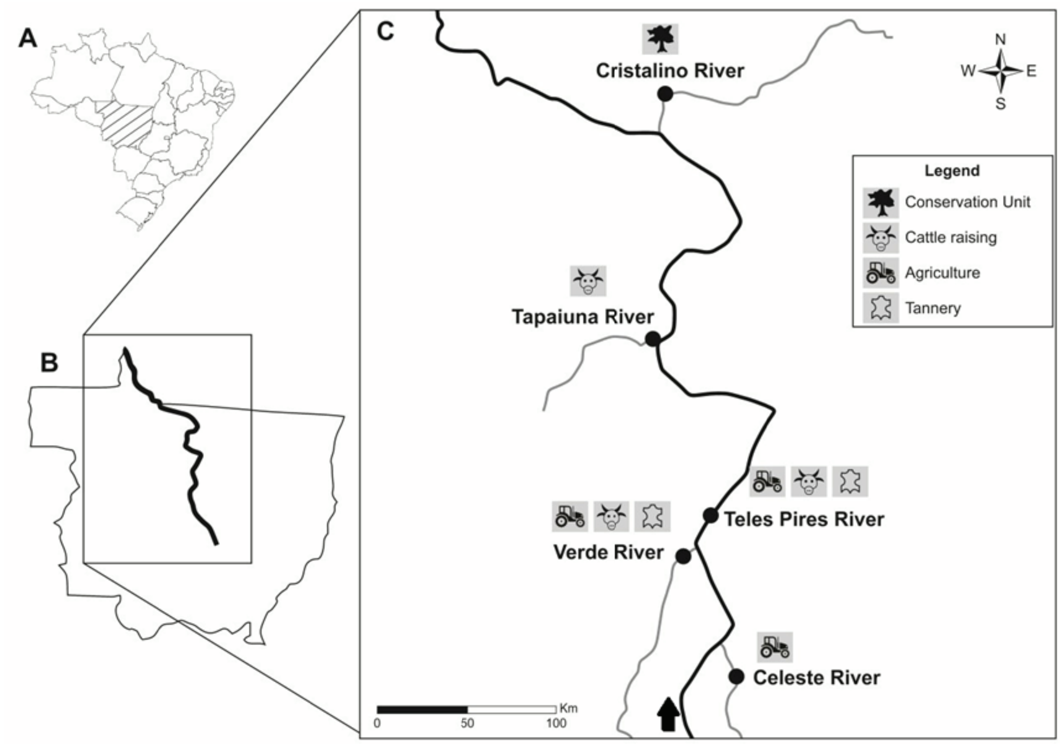

Figure 1. **A.** Map of Brazil. **B**. Map of Mato Grosso state highlighting the Teles Pires basin. **C.** Sample collection points of matrinxã (*Brycon falcatus*): Celeste River (12°24'56.00"S and 55°31'28.00"W), Verde River (11°4'1.99"S and 55°34'17.00"W), Teles Pires River (11°34'48.00"S and 55°39'5.00"W), Tapaiúna River (10°41°29.28"S and 55°56'51.11"W) and Cristalino River (9°32'47.00"S and 55°47'38.00"W), Mato Grosso State, Brazil.
Source: the authors

Liver and muscle tissue samples from the region under the dorsal fin and above the lateral line (~10 g) were collected using stainless steel surgical instruments, stored at –20°C, and used for the determination of Cr and Cu concentrations. Matrinxã control specimens were deposited at the museum of the *Universidade Estadual de Campinas* – UNICAMP deposit n° ZUEC 9190; and at the *Acervo Biológico da Amazônia Meridional* – ABAM of the *Universidade Federal de Mato Grosso* - UFMT.

Hepatosomatic index (HSI)

The HSI is most associated with exposure index for contaminants. The increase in liver size, and thus the HSI, can indicate exposure to heavy metals increasing the enzymatic detoxification process. The HSI may be a suitable biomarker for substances that are toxic to the liver (Haux & Larsson, 1984). This index can reflect both the demand of metabolic energy as the nutritional status in the short term, and can be regarded as a general indicator of health in fish sensitive to environmental contaminants (Everaarts, Shugart, Gustin, Hawkings, & Walker, 1993).

The hepatosomatic index (HSI) was calculated (Vazzoler, 1981):

$$HSI = 100 \times (\text{liver weight (g)} / \text{total body weight (g)})$$

Heavy metal analysis

Total Cr and Cu concentrations were determined through nitric acid and hydrogen peroxide wet digestion (Association of Official Analytical Chemists. [AOAC], 1990; Morgano et al., 2011). Following thawing, muscle and liver tissue samples (wet weight) were ground and placed in test tubes that had been previously washed with 10% nitric acid to avoid contamination. Four milliliters of concentrated nitric acid was added to each test tube, and the tubes were left to stand for 12 hours. The samples were then placed in a heating block at 80ºC for four hours; 1 ml of hydrogen peroxide was added, and the samples were heated at 110ºC for an additional six hours. After cooling, the samples were resuspended in distilled water in a 25-ml volumetric flask. All analyses were performed in triplicate. The total Cr and Cu concentrations were determined by atomic absorption spectroscopy (Spectrometer Varian AA 140) using an air-acetylene flame for Cu and a nitrous oxideacetylene flame for Cr. The detection limit of the Atomic Absorption equipment was Cr 75 ppb and Cu 30 ppb. The equipment was calibrated with a standard solution Cr and Cu traceable NIST-USA. The precision and accuracy of the method was tested by the use of known concentration samples prepared with the standard solution of each metal. Samples were periodically read and demonstrated the accuracy of the method, and it was found that the instrument remained calibrated during the course of the study. Blank samples were analyzed periodically to test if there were any memory effects.

Statistical analysis

Correlations between muscle and liver total Cr and Cu concentration ($\mu g \cdot g^{-1}$) with standard length, and correlations between HSI and total concentration of Cr and Cu in the muscle and liver of fish collected in rivers of the Teles Pires River basin were analyzed using general linear model analysis (GLM). The total Cr and Cu concentration in the muscle and liver, and HSI of *B. falcatus* were subjected to an analysis of variance (ANOVA) followed by a post-hoc Tukey's test to determine significant differences between the rivers of the Teles Pires basin; the adopted level of significance was $p < 0.05$. All analyses were performed using R v. 3.1.3 software (R Core Team, 2014).

Results

Forty-one *B. falcatus* individuals were captured in the Teles Pires River basin. The average and range for the measured lengths (total and standard), weight and HSI are presented in Table 1. In the present study, the HSI of collected matrinxãs was not significantly different between rivers ($p \geq 0.05$) by ANOVA, ranging from 0.166 (Cristalino River) to 0.390 (Teles Pires River). There was also no correlation between HSI and total Cr and Cu in muscle and liver tissue.

The GLM analysis, performed using the data from all matrinxã specimens collected in the Teles Pires River basin, revealed no significant correlation between Cu concentration in muscle and liver tissue, and no significant correlation between Cr concentration in muscle to the weight and/or standard length of the sampled fish. However, Cu concentration in liver tissue was significantly and positively correlated with the standard length ($p=0.00051$) and weight ($p = 0.00090$) of fish (Figure 2).

Table 1. Total length (TL), standard length (SL), weight (kg) and hepatosomatic index (HSI) of *Brycon falcatus* specimens collected in rivers of the Teles Pires basin during the dry season in 2013. The values represent the averages and ranges (between brackets).

Rivers	N	TL (cm)	SL (cm)	Weight (Kg)	HSI
Celeste	7	40.30 (37.00 - 45.50)	30.14 (20.50 - 37.50)	1.07 (0.25 - 2.09)	0.347[a]
Verde	4	24.10 (23.50 - 24.70)	22.00 (19.00 - 29.50)	0.41 (0.21 - 0.95)	0.331 [a]
Teles Pires	10	42.72 (29.50 - 51.00)	34.60 (23.50 - 41.00)	1.57 (0.43 - 2.83)	0,390 [a]
Tapaiúna	10	34.45 (28.50 - 39.50)	27.45 (22.50 - 31.00)	0.67 (0.35 - 0.95)	0.285 [a]
Cristalino	10	49.38 (42.00 - 53.00)	39.45 (32.50 - 43.00)	2.23 (1.12 - 3.01)	0.166 [a]

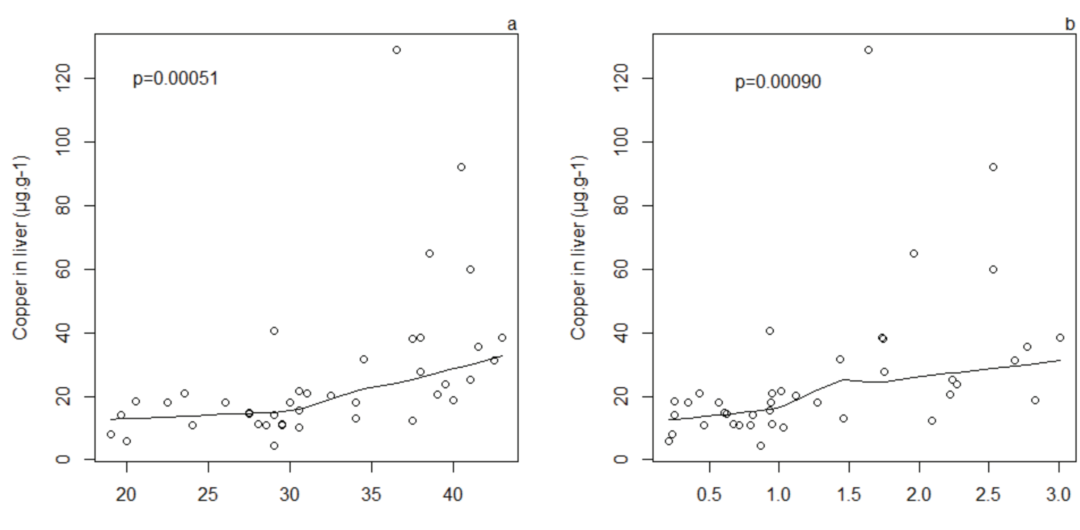

Figure 2. Scatter plot of Cu concentration in liver tissue ($\mu g \cdot g^{-1}$) (wet weight), versus **(a)** standard length (cm) and **(b)** weight (kg) of *Brycon falcatus* collected in rivers of the Teles Pires River basin during the dry season in 2013.
Source: the authors.

Cr and Cu concentration in liver tissue was higher than in muscle tissue of fish collected in all rivers (Table 2). Cr concentration in liver and muscle tissue was similar and not significantly different between the sampled rivers. The highest Cu concentrations in liver tissue were observed in fish collected in the Cristalino River (44.20 $\mu g \cdot g^{-1}$) and the Teles Pires River (34.77 $\mu g \cdot g^{-1}$), with the lowest concentrations observed in fish from the Verde River (9.89 $\mu g \cdot g^{-1}$).

Discussion

In this study the highest concentration of metals was found in the liver. A previous experiment in the Amazon with *Brycon amazonicus* fed to satiation exhibited a HSI of 1.31 (Tavares-Dias et al., 2008). Another previous experiment, in southeastern Brazil using *Brycon cephalus* exhibited a HSI of 0.96 (Monteiro, Almeida, Rantin, & Kalinin, 2006). Sampling of matrinxã was conducted in the dry season, which is the start of reproductive migration and there is a decreased availability of food. We conclude that the presence of heavy metals in the muscle and liver tissue of *B. falcatus* had not affected the HSI, with low HSI values in the range of 0.166-0.390 reflecting the low food supply of the dry season.

Different ecological and biological factors control heavy metal bioaccumulation in fish. Fish age and size are commonly used to explain bioaccumulation patterns. In mildly contaminated waters, metals enter fish mainly through feeding (Farkas, Salánki, & Apecziár, 2003). In addition to size and age, changes in diet composition may influence heavy metal bioaccumulation (McIntyre & Beauchamp, 2007). *Brycon* fish have an ontogenetic diet, where in the juvenile stage they are generally omnivorous with a tendency to carnivory (Ceccarelli & Senhorini, 1996), and in adulthood are omnivorous with a tendency to frugivory (Blanco-Parra & Bejarano-Rodríguez, 2006; Albrecht et al., 2009; Matos & Carvalho, 2015). Fish with carnivorous habits have a higher potential for bioaccumulation due to their position at the top of the aquatic food chain (Dorea & Barbosa, 2005). In this study, a positive correlation was observed between Cu concentration in liver tissue to the weight and standard length of matrinxã specimens collected in rivers from the Teles Pires River basin. Several other studies have also shown a positive correlation between metal concentration and fish body size (Mastala, Balogh, & Salansk, 1992; Farkas et al., 2003; Kasimoglu, 2014). Agricultural activity is intense in the vicinity of the rivers (Verde, Celeste, Tapaiúna, and Teles Pires) sampled in this study. Cu is heavily used in agriculture due to its fungicidal, bactericidal, and disinfectant properties that prevent putrefaction of stored seeds (Penteado, 2000; Sfredo, 2008). Even if pesticides are degraded before they leach into the water table, studies indicate that leaching is an important source of agricultural pollution (Moura, Franco & Matallo, 2008). The Cu used in agriculture may be contaminating the rivers of the Teles Pires River basin. However, the Cu contamination in the Cristalino River originates from another source - intense mining activity occurred along the Cristalino River in the 1980's and 1990's (Farid et al., 1992). Mining turns the soil, exposing it to erosion and redox reactions or acidification. These processes increase the availability of heavy metals naturally present in soil and rocks containing metals such as zinc, Cu, and lead. A high Cu concentration due to soil turning from mining activity was observed in rivers of the Paraguay River basin (Rodrigues-Filho & Maddock, 1997). In the 1970's, based on aerogeophysical surveys, a Brazilian company selected the Cristalino River as a new source of Cu deposit (Santos, 2002). Mining activity, together with higher dissolution due to water oxidation associated with low pH values, increased Cu availability in the Cristalino River.

Agriculture is the main economic activity within the region containing the Verde, Celeste, and Teles Pires rivers. This activity facilitates contamination of these water bodies through agricultural inputs, with rainfall leading to soil leaching.

Table 2. Mean ± SD of total chromium and copper concentration ($\mu g \cdot g^{-1}$) (wet weight) in muscle and liver tissue of *Brycon falcatus* collected in rivers of the Teles Pires basin during the dry season in 2013. Means followed by the same letter in each column were not significantly different ($p \geq 0.05$) by ANOVA.

Rivers	Heavy metal $\mu g \cdot g^{-1}$			
	Cr		Cu	
	Liver	Muscle	Liver	Muscle
Celeste (*n*=7)	1.06 ^{a} ± 1.34	0.01 ^{a} ± 0.04	13.40 ^{b} ± 5.46	0.28 ^{a} ± 0.07
Verde (*n*=4)	0.00 ^{a} ± 0.00	0.00 ^{a} ± 0.00	9.89 ^{bc} ± 3.67	0.31 ^{a} ± 0.05
Teles Pires (*n*=10)	1.87 ^{a} ± 3.81	0.03 ^{a} ± 0.06	34.77 ^{ab} ±33.93	0.33 ^{a} ± 0.10
Tapaiúna (*n*=10)	0.86 ^{a} ± 1.33	0.05 ^{a} ± 0.09	14.78 ^{b} ± 3.85	0.35 ^{a} ± 0.06
Cristalino (*n*=10)	0.00 ^{a} ± 0.00	0.00 ^{a} ± 0.00	44.20 ^{a} ± 21.88	0.27 ^{a} ± 0.08

Large amounts of Cu are used in agriculture, and the acceptable limit of Cu in food is 30.0 $\mu g \cdot g^{-1}$ according to Brazilian (Decree MS 55.871/65) (Decreto Nº 55.871, 1965) and international legislation (Food and Agriculture Organization / World Health Organization [FAO/WHO], 1997).

This limit was only exceeded in samples of liver tissue taken from fish collected in the Cristalino and Teles Pires rivers. For the Teles Pires River, this Cu contamination may be explained by the intense agricultural activity in close proximity to the margins of the Teles Pires, Verde, Celeste, and Tapaiúna rivers. However, the Cristalino River is located within the State Park of Cristalino, where there is no agricultural activity. The high Cu concentration in liver tissue of fish from the Cristalino River may therefore be due to the presence of Cu in the water. This site has been previously mapped as a natural Cu deposit, and mining activity together with water oxidation associated with low pH have made this metal available in the river. Water, food, and sediment are the main pathways of heavy metal absorption by fish (Kehrig et al., 2008).

Conclusion

The high concentration of Cu in the livers of fish collected from the Teles Pires river basin reflects the economic activities of mining and agriculture in the surrounding areas of this basin. Our results suggest that Cu may originate from the water; however, the total Cu concentration in food sources and the water in these rivers should be analyzed together with other organs of matrinxã such as the gills, kidneys, stomach, and intestines, to verify the bioaccumulation pathways.

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