Orbital: The Electronic Journal of Chemistry
journal homepage: www.orbital.ufms.br
e-ISSN 1984-6428

| Vol 10 | | No. 4 | | Special Issue June 2018 |

FULL PAPER

Zinc, Copper and Iron in Consumed Fish from Tapajós River Basin, PA, Brazil

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Article history: Received: 30 August 2017; revised: 07 November 2017; accepted: 11 November 2017. Available online: 10 June 2018. DOI: http://dx.doi.org/10.17807/orbital.v10i4.1066

Abstract:

Nowadays, a wide range of ecosystems are under anthropogenic disturbance. Aquatic organisms are exposed to a large number of toxic substances and can be used for environmental monitoring. Zinc, copper and iron are essential metals, however, in high concentrations have toxic effects. This study investigated the concentration of these elements in fish muscle from Tapajós river basin to estimate human exposure and its spatial variation along the Tapajós river. Fish (n = 129) from four areas along 400 km of the Tapajós river (Buburé, Itaituba, Alter do Chão and Santarém) were assessed. Metal concentrations were measured by flame atomic absorption spectrometry after acid mineralization. Zinc, copper and iron concentrations in fish ranged from 2 to 15 μg.g^{-1} wet weight (w.w.), 1 to 4 μg.g^{-1} w.w. and 5 to 286 μg.g^{-1} w.w., respectively. The concentrations of these elements were below the maximum residue level permitted in food established by the Brazilian legislations (ANVISA). The estimated daily ingestion values of the three elements did not exceed the reference value proposed by World Heath Organization based on a diet composed of these fish species. Zinc, copper and iron are essentials, therefore, there is also a recommendation of the minimum daily ingestion to avoid deficiency. Considering a daily consumption of 200 g of these fish, the population no reaches of the recommended minimum value of these elements.

Keywords: amazon; bioavailability; heavy metals; human consumption

1. Introduction

Nowadays, a wide range of ecosystems are under high degree of anthropogenic disturbance. Domestic and industry effluents are the main routes of heavy metals disposal into the majority of aquatic ecosystems [1]. The Amazon basin has a diverse array of aquatic systems in pristine state. Nevertheless, the demographic density has increased and have raised the anthropogenic pressure, which includes metals input to these aquatic systems. Domestic sewage, metal mining, oil exploration and refining are important sources of metals release to the aquatic environments in the Amazon [2]. On the other hand, these aquatic systems are important to Amazon people subsistence, including the artisanal fishing. This duality of different uses and its impacts has highlighted the importance on preservation of local habitats.

Fish is the main source of protein in the diet for this population and one of the most important economic activity for many local fishermen. The Amazon has the highest proportion of Brazilian people ≥18 years old who consume fish at least one day per week. This is 93.2% of the entire population, well above the national average (54.6%) and the north region average (77.2%) [3]. Besides the majority of the population consuming fish, the consumption per capita is one of the highest in the world, reaching around 200 g/inhabitant/day [4-6]. Fish are source rich in omega-3 fatty acids, which is linked to reduction in cholesterol levels and incidences of heart disease, stroke and premature labor [7, 8].

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The consumption of fish with concentration of metals above the limit recommended by the World Health Organization (WHO) may cause public health intoxication (e.g., 300 μg.kg^{-1}.day^{-1} for zinc, 500 μg.kg^{-1}.day^{-1} for copper and 800 μg.kg^{-1}.day^{-1} for iron). Although they perform essential biological functions and participate in several stages of human metabolism, some elements can cause serious damage to organisms when ingested in high concentration, as zinc, copper and iron.

The Tapajós river, an important tributary of Amazonas river, was intensely studied for mercury assessment [4, 6, 9-12]. Mercury was used by gold miners and is an element naturally present in the Amazon basin in high concentrations [13]. However, few studies assessed the concentrations of other heavy metals in fish in that region. Due to the increase of anthropogenic pressure on the Amazon systems that could contaminate the fish, this work evaluated the bioaccumulation of zinc, copper and iron by different species of fish collected in the Tapajós river and if these organisms are safe for consumption regarding these three metals

2. Results and Discussion

Zinc concentrations of the 23 species analyzed ranged from 2 μg.g^{-1} w.w. for *B. cepahlus* to 15 μg.g^{-1} w.w. for *P. rutiloides* (Table 1). No significant difference was observed among Zn concentrations in the non-carnivorous and carnivorous fish (Figure 1A). Zn is an essential element for animals, acting in the active center of more than 300 enzymes [14, 15].

Some authors suggest that the concentrations of this metal in fish for Tapajós is a natural bioconcentration process, absorbed independently of human action, due to the natural leaching of the soil, mainly during the rainy season, that loads Zn from soils to aquatic systems [16]. However, Buburé sampling site presented higher concentrations of this metal in fish muscles compared to fish from other three sites (Figure 2A). In this region (Itaituba and Buburé) it was observed concentrations of Zn in water above 0.180 mg.L^{-1} [17, 18], two orders of magnitude higher than those observed in the Santarém region, which had values below to 0.005 mg.L^{-1} [19]. These higher concentrations in Itaituba region were associated with natural erosive processes that frequently occur in this region and erosive processes increased by anthropic activities, such as deforestation [17, 18]. However, the concentrations of Zn were higher only in fish from Buburé (and not from Itaituba). The reasons for this pattern are not clear, but a presence of an important port in Buburé could increase the environmental impact of this locality. All materials arrive in the region by this port, and are transported to Itaituba by road (where the river is not navigable). Therefore, the Zn concentrations observed in the present study can be a result of natural and antropogenic processes that load this metal to the Tapajós river.

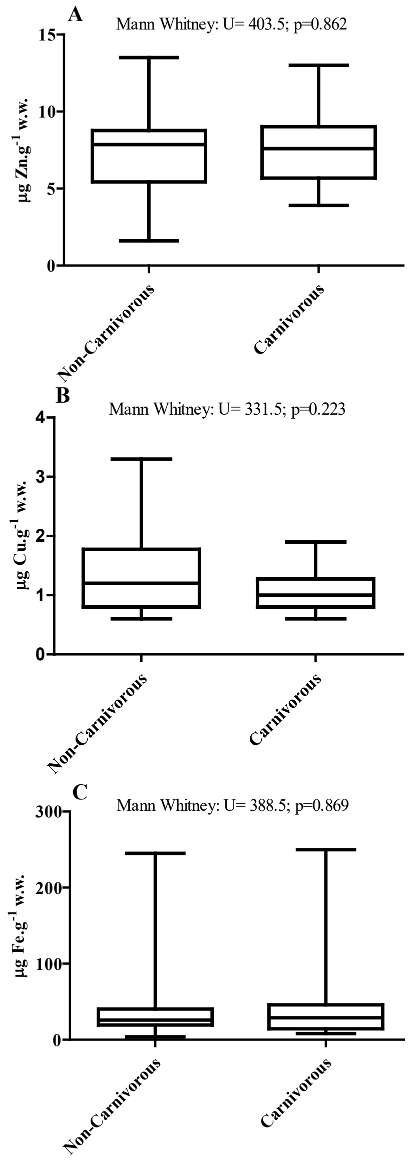

Figure 1. Zinc (A), copper (B) and iron (C) concentrations (μg.g^{-1} wet weight) in non-carnivorous and carnivorous fish from Tapajós river. Box Plot represents: (-) Median ([]) 25% - 75% (I) Maximum and Minimum.

Table 1. Mean and standard deviation of zinc, copper and iron concentrations in μg.g^{-1} wet weight in the muscle of carnivorous (C) and non-carnivorous (NC) fish species collected in four cities located along the Tapajós riverbank.

Fish species	Sampling site	n	Zn	Cu	Fe
Semaprochilodus insignis (NC)	BUBURÉ	15	9±3	1.6±0.4	55±23
Schizodon vittatus (NC)		4	10±3	1.3±0.4	52±20
Myleus schomburgki (NC)		4	7±2	0.7±0.1	8±5
Serrasalmus calmoni (C)		3	8±3	1.2±0.2	40±20
Leporinus friderici (NC)		3	5±1	1.5±0.2	13±2
Rhytiodus sp. (NC)		3	7±1	0.7±0.1	32±11
Ciclha monoculus (C)		5	11±1	1.1±0.4	203±26
Hemisorubim platyrhynchos (C)		3	13±1	1.0±0.2	16±4
Triportheus elongatus (NC)		5	8±2	1.4±0.1	34±12
Plagioscion squamosissimus(C)		3	9±1	1.0±0.2	250±36
Geophagus proximus (NC)		3	13±2	1.0±0.4	27±10
Plagioscion squamosissimus (C)	ITAITUBA	7	6±1	1.0±0.2	26±10
Psectrogaster rutiloides (NC)		4	11±4	1.1±0.4	50±24
Leiarius marmoratus (NC)		6	6±2	0.9±0.3	27±7
Hypophthalmus marginatus (NC)		6	7±2	0.7±0.1	22±8
Cichla monoculus (C)		7	7±2	1.2±0.5	28±19
Cichla pleizona (C)		3	5±1	0.6±0.2	128±37
Brycon cepahlus (NC)	ALTER DO CHÃO	4	8±1	2.5±0.5	25±2
Prochilodus nigricans (NC)		4	6±3	1.4±1.0	80±60
Hypophthalmus edentatus (NC)		4	7±1	1.0±0.3	19±1
Schizodon fasciatus (NC)	SANTARÉM	8	8±1	0.9±0.4	17±4
Piaractus brachypomus (NC)		4	6±2	1.0±0.1	22±3
Colossoma macropomum (NC)		4	7±3	1.5±0.4	24±5
Pseudoplatystoma fasciatum (C)		4	7±1	1.3±0.2	17±1
Semaprochilodus taeniurus (NC)		6	5±1	2.0±0.2	23±21
Brycon cepahlus (NC)		7	4±3	1.2±0.4	21±6

Copper is an essential metal, necessary for the synthesis of hemoglobin in the blood [20]. Cu concentrations of the 23 species analyzed ranged from 0.6 μg.g^{-1} w.w. for the *G. proximus* to 3.5 μg.g^{-1} w.w. for *B. cepahlus* (Table 2). No significant difference was observed among Cu concentrations of the non-carnivorous and carnivorous fish (Figure 1B). In the Cassiporé river basin, in the Northern region of Brazil, [21] also did not find different muscular Zn and Cu concentrations between fish species with different feeding habits. In Alter do Chão, it was observed higher concentrations of this element in the fish when compared to the other sites (Figure 2B). The Alter do Chão and Santarém regions have open dump, manure disposal [22], and many channels of domestic sewage [19]. The sewage and land use can increase copper concentrations of biota [1]. Therefore, the higher concentrations observed in fish from Alter do Chão could be related to these human activities. However, the lower concentrations observed in fish from Santarém should be further investigated. When compared to other metals, fish have low concentrations of copper since their metabolism readily regulates this essential metal [23].

Iron concentrations of the 23 species analyzed ranged from 5 μg.g^{-1} w.w. for *M. schomburgki* to 286 μg.g^{-1} w.w. for *P. squamosissimus* (Table 2). No significant differences were observed between Fe concentrations of the carnivorous and non-carnivorous fish (Figure 1C) and also between fish collected at different sites (Figure 2C). Iron is a very important essential metal for diverse species and participates in cellular respiration as the transport of O_2 and CO_2 [24, 25]. The results may be associated with the geochemical composition of the region, where iron is abundant and has good mobility [26]. The iron concentrations in water from Tapajós near Santarém city were between the limits allowed by CONAMA one and two classes (for freshwater) (0.3 mg.L^{-1}), in almost all sampling points [19]. Therefore, there is a great availability of Fe concentrations in the water.

Studies that evaluated Zn, Cu and/or Fe in the muscle of fish from Northern Brazil basins verified that the concentrations could vary according to the water physicochemical properties (e.g., pH and temperature) and sampling time Table 2 [20, 27].

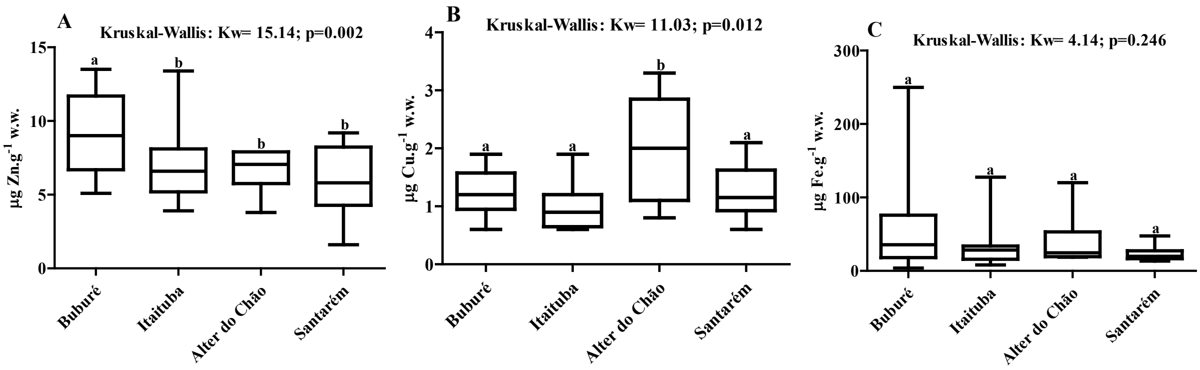

Figure 2. Zinc (A), copper (B) and iron (C) concentrations (μg.g^{-1} wet weight) in fish from four different sampling sites of Tapajós river. Box Plot represents: (-) Median ([]) 25% - 75% (I) Maximum and Minimum. Sampling sites that differ from each other (within each graph) at the significance level of 0.05 are presented with different letters.

Table 2. Zn, Cu and Fe concentrations (minimum-maximum) in muscle of fish from different sites of Northern Brazil.

Site	Zn	Cu	Fe	n	Reference
Tapajós river, PA, Brazil	**2.34-15.74**	**0.61-3.52**	**5-286**	**129**	**Present study**
Cassiporé river basin, AP, Brazil	0.04-1.14	0.01-0.08	-	246	Lima et al., 2015 [21]
Teles Pires basin, MT, Brazil	-	0.19-0.43	-	41	Matos et al., 2016 [27]
Belém, PA, Brazil	7.25-36.66	<0.52	<20-1,430	15	Cruz et al., 2015 [20]

In order to evaluate the human exposure to the metals determined in this study through the consumption of fish from Tapajós river, the values were compared to the maximum residue level (MRL) in food established by the Brazilian legislation Table 3 [28]. Comparing to MRL, it was observed that the concentrations of three metals in studied fish were below the maximum values permitted. Moreover, the estimated daily ingestion calculated also did not exceed the reference value of tolerable daily intake proposed by WHO (Table 4) [29]. Beyond the maximum intake, there is a recommended minimum daily intake for these three metals because they are essential. The minimum daily intake is 7 mg.day^{-1} for Zn (Anvisa, 2004), 0.9 mg.day^{-1} for Cu (Anvisa, 2004) and 14 mg.day^{-1} for Fe (FAO, 2001; Anvisa, 2004). Assuming that the riverside population consumed around 200 g of fish per day, the daily consumption of these metals by fish consumption reaches only 21% for Zn, 27% for Cu and 59% for Fe of the recommended minimum dose.

Table 3. Metal concentrations (minimum-maximum, in μg.g^{-1} wet weight) in fish from different sites of Tapajós river (present study) and maximum residue level (MRL) established by the Brazilian legislation (ANVISA, 2013), - there is no legislation about MRL of Fe.

	Zn	Cu	Fe
Tapajós river	2-14	1-3	4-250
MRL	50	30	-

Table 4. Estimated daily ingestion (EDI) calculated for fish from four different sites in Tapajós river assessed in the present study and reference value of provisional tolerable daily intake (PTDI) proposed by World Health Organization [29]. Values in μg.kg^{-1}.day^{-1}.

Site	Zn	Cu	Fe
Buburé	40	6	715
Itaituba	37	6	366
Alter do Chão	23	9	369
Santarém	26	6	103
PTDI value	**300**	**500**	**800**

3. Material and Methods

Fish were bought from local fisherman along Tapajós river basin in the upper/middle Tapajós (U/MTap) in October 2013 and the lower Tapajós (LTap) in May 2014. In the U/MTap, fish were collected in the Buburé and Itaituba cities (Figure 3). Both cities were surrounded by important gold mining sites mainly during 1970s and 1980s [4, 30]; and nowadays their economic activities are mainly mineral production, fishing and agriculture [31]. The municipality of Itaituba has an area of 62,042 km^2 and approximately 98,485 inhabitants [32]. Buburé, a region of Itaituba municipality, is where the port is located, and is approximately 100 km upstream from Itaituba. In the LTap section, fish were collected in the Santarém and Alter do Chão (Figure 3), located 800 km downstream from main garimpo areas [4]. Santarém city has an area of 23,000 km^2 and approximately 294,447 inhabitants [32]. Alter do Chão is an administrative district of the Santarém and is 40 km upstream from Santarém. The main economic activities of LTap region are agriculture, livestock, wildlife trade and manufacturing industries [33].

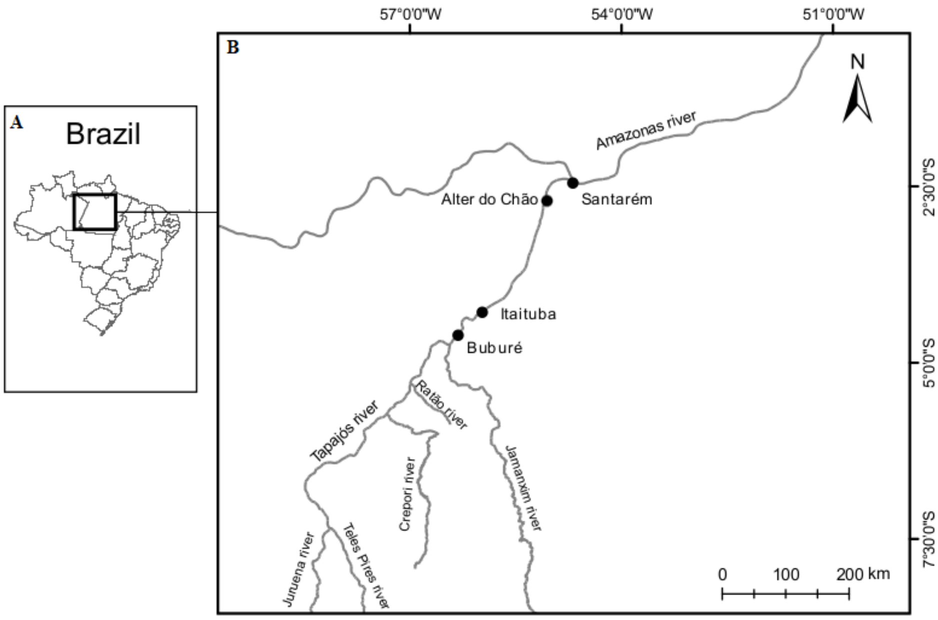

Figure 3. A) The map of Brazil highlights the geographical location of the studied region. B) Map of the study area, demonstrating the Tapajós River and the four fish sampling sites (Buburé, Itaituba, Alter do Chão and Santarém).

A total of 129 fish specimens (21% carnivores and 79% non-carnivores) belonging to 23 fish species (5 carnivores and 18 non-carnivores) were sampled and analyzed. Individuals were identified, and their feeding habits determined according to the literature [34]. Most of the species used in this study are consumed by the local population and are common in local fish markets. The white dorsolateral muscle of fish was removed, stored in polyethylene bags and frozen at -20 ºC. Aliquots of approximately 0.5 g were calcined (430 ºC) during 48 h and then solubilized with 3 mL of HNO_3:HCl solution (3:1 v:v; Tédia, Brazil) in a hot plate (85-90 ºC). Hydrogen peroxide (Vetec, Brazil) was added to the samples until complete transparency of the solutions, which were taken to dryness twice and wet with addition of pure hydrochloric acid (37%; Tédia, Brazil) and 0.1 M HCl (Tédia, Brazil) in the first and second times, respectively. Samples were filled to 15 mL with ultrapure water (18.2 MΩ cm, MilliQ system) [1]. All glassware was immersed in a solution of neutral detergent (5%; Extran, Merck) during 12 h, after in a solution of HNO_3 (5%; Tédia, Brazil) during 12 h and washed with deionized water. Element concentrations were determined by Flame Atomic Absorption Spectrometry (FAAS), using a Varian Spectrometer (AAS 240FS, Santa Clara, United States), equipped with deuterium background correction. Every batch of samples was processed with three analytical blanks in the same way as the environmental samples, and the average of them was used to subtract impurities from the samples. In order to assess the accuracy of method, TORT-2 (lobster hepatopancreas, N = 15) and DOM-3 (fish protein, N = 15) were analyzed. Our recovery results of metal determination (Table 5) in these two certified reference materials were satisfactory (85-115%), according to EPA criteria (EPA, 2000). All samples were analyzed in duplicate to assess the precision of the analytical method (coefficient of variation between duplicates below 15%).

Table 5. Certified and obtained values of elements concentration (μg.g^{-1} dry weight) in the certificate reference materials (TORT-2; lobster hepatopancreas) and (DORM-3; fish protein).

Elements	TORT-2 (n = 15)		DORM-3 (n=15)	
	Certified value	Obtained value	Certified value	Obtained value
Zn	180±6	165±5	51.3±3.1	45±8
Cu	106±10	109±5	15.5±0.63	16±2
Fe	105±13	126±25	347±20	320±11

The method detection limits (DL) were calculated using the equation 1:

$$DL = \frac{(3 \text{ x } Sb)}{Xb} \text{ x } \left(\frac{V}{M}\right) \qquad \text{Eq. 1}$$

where Sb is the standard deviation of 10 measurements of the analytical blank, Xb is the mean of the angular coefficient of the calibration curve, V is the final volume of the sample solution and M is the sample mass. The DL were 1.8, 0.1 and 3.0 μg.g^{-1} for Zn, Cu and Fe, respectively.

The estimated daily ingestion (EDI) for metals intake through fish consumption were calculated for each element (Zn, Cu and Fe) for each sampling site (Buburé, Itaituba, Santarém and Alter do Chão). This formula considered the concentration of metals (Zn, Cu and Fe) observed in fish of the present study (Conc.), the fish consumption for riverine population (200 g/inhabitant/day [4-6], and 70 kg of body weight for a Brazilian adult (Equation 2). This estimate was compared with the provisional tolerable daily intake (PTDI) suggested by the WHO [29].

$$EDI \left(\frac{\mu g.Kg^{-1}}{dia}\right) = \text{Conc.} \left(\frac{\mu g}{g \text{ w.w.}}\right) \text{x } 200 \left(\frac{g/\text{inhabitant/day}}{70 \text{ Kg}}\right) \qquad \text{Eq. 2}$$

Shapiro-Wilk's W test was used in order to test for normality of the data. Mann-Whitney was used to compare metal concentrations in carnivorous and non-carnivorous fish. Kruskal-Wallis and the post-hoc Dunn tests were used to compare metal concentration in fish from the four different sampling points. The adopted significance level was 5% for all tests

4. Conclusions

The bioaccumulation of zinc, copper and iron in fishes from Tapajós river is probably from the availability of these metals in the aquatic system from natural sources and also from their increase caused by anthropogenic activities in the basin. These fishes are suitable for human consumption with respect to these three metals. The riverine population does not reach the minimal daily intake of Zn, Cu and Fe by fish consumption, being necessary a complementary food that contains these elements.

Acknowledgments

The authors are thankful CNPq and FAPERJ for the financial support; Dario Pires de Carvalho for assistance with fieldwork; and the staff of Laboratory of Radioisotopes Eduardo Penna Franca (UFRJ) for their help with analyses.

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