Toxic and essential trace element concentrations in fish species in the Lower Amazon, Brazil

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Abstract

The Lower Amazon region (Western Pará, northern Brazil) is greatly affected by mining exploitations (particularly artisanal gold mines) and other industrial and intensive agricultural activities with potentially strong impacts on aquatic ecosystems. Although such impacts include contamination with various toxic elements, to date only the effects of Hg have been considered. In this study, toxic and trace element concentrations were determined in the flesh of 351 fish specimens, including detritivores (Acarí, *Pterygoplichthys pardalis*), omnivores (Piranha, *Pygocentrus nattereri;* Pirarucu, *Arapaima* sp.) and carnivores (Caparari, *Pseudoplatystoma fasciatum*; Tucunaré, *Cichla ocellaris*), during the dry and wet seasons in 2015 and 2016. The range of concentrations of toxic element residues were 2-238 µg/kg fresh weight for As, 1-77 µg/kg for Cd, 4-1922 µg/kg for Hg and 1-30 µg/kg for Pb. Only the maximum concentrations of Hg established in the Brazilian legislation for fish destined for human consumption (0.5 mg/kg) were exceeded (in 16% of carnivorous species). The large between-species and seasonal differences observed for all these toxic elements are probably related to the seasonal behaviour and ~~nutritional~~dietary habits of the different fish species. By contrast, essential trace element concentrations were low and not related to seasonal or ~~nutritional~~dietary factors, and the observed differences may be at least partly related to the metabolism of each species. The associations between Hg and the essential trace elements Se, Fe, Co and Mn deserve special attention, as these trace elements may play a role in Hg cycling and methylation and merit further evaluation with the aim of reducing Hg toxicity in aquatic environments.

Keywords: Hg, Selenium, Pirarucu, Tucunaré, mining activity, metal association

1. Introduction

~~The Amazon biome constitutes the most biologically diverse ecosystem on Earth and is an environmental icon with unique characteristics. The Amazon rainforest plays a major role in maintaining rainfall patterns throughout the continent and capturing carbon from the air and replacing it with clean oxygen, and its presence is essential to combat the impacts of climate change by slowing down global warming (Nepstad et al., 2008).~~

Since the last century, ~~the~~ high ~~serious~~ levels of mercury (Hg) contamination of aquatic environments in the Amazon basin, caused by artisanal gold mining activities (Lacerda and Pfeiffer, 1992; Pfeiffer and Lacerda, 1988; Veiga et al., 2002), have attracted the attention of the international research community. In addition to gold mines, other mining and oil extraction activities have caused environmental pollution, partly as a result of ~~important~~ spills into the rivers (Forte et al., 2007). The mining activities, together with ~~the~~ large-scale development of agriculture and livestock farming, have also indirectly aggravated pollution-related problems in the Amazon basin (Minervino et al., 2008). These activities trigger extensive deforestation associated with the construction of an extensive infrastructure network designed to facilitate access to various industrial operations.

The above-mentioned anthropogenic activities have a strong impact on local populations, which are highly dependent on the river for subsistence. The Amazon River is the basis of an artisanal fishing industry that provides livelihoods for around 40% of the households in the fishing communities (Almeida et al., 2004). In the Brazilian Amazon alone, the fishing sector generates employment for more than 160,000 people, including approximately 120,000 subsistence fishermen (Ferreira Filho and Fachinello, 2015). In addition, freshwater fish are the main source of protein for riparian communities. The annual average local consumption of fish is around 94 kilograms per person, i.e. almost six times the global average (Isaac and Almeida, 2011). Exposure to toxic elements varies widely in the fish species consumed in the Amazon region. The large carnivorous species at the top of the food chain accumulate significantly higher levels of ~~residues ~~Hg than other smaller herbivorous and detritivorous fish species (Lima et al., 2000). The Hg levels recommended by the WHO are exceeded in a large proportion of fish, particularly by carnivorous species (Barbosa et al., 2003), and the inhabitants (who consume these fish) have levels of Hg in their hair that indicate toxicity (Costa Junior et al., 2018; Santos et al., 2003). Within the Brazilian Amazon, Western Pará is one of the areas most affected by ~~the ~~mining activities. In the municipality of Itaituba, gold extraction is a common, often unregulated activity; in Porto Trombetas (Oriximiná), bauxite exploitation has been carried out since 1969; and in Juruti, a bauxite exploration project was initiated within the last 10 years. Numerous mining-related accidents have been reported in the region, such as the direct discharge of effluent comprising bauxite tailings rich in Fe, Al and Si oxides into ~~the ~~Batata ~~l~~Lake in Oriximiná between 1979 and 1989 (Callisto and Esteves, 1996, 1995; Lin and Caramaschi, 2005) and the historic Hg release ~~mining accident ~~in the Tapajos River~~river~~ basin associated with artisanal gold mining (Lacerda and Pfeiffer, 1992; Lima, 2016; Miranda et al., 2009; Santos et al., 2003). Agricultural activities, mainly corn and soybean cultivation, are important in the region and have led to intense deforestation (Aguiar et al., 2014; Toledo, 2012). These activities often lead to environmental contamination involving potentially toxic elements (other than and including Hg) as well as essential trace elements, which may have harmful effects on organisms if consumed in large quantities (Plessl et al., 2019; Ramos-Miras et al., 2019). Aquatic environments can be exposed to element contamination (Milačič et al., 2019) and monitoring metal concentrations in fish flesh is therefore important to ensure compliance with food safety regulations and consumer protection (Blanco et al., 2019; Fu et al., 2019; Jia et al., 2018; O'Mara et al., 2019). However, to date only the impact of Hg has been evaluated in the region (Berzas Nevado et al., 2010; Roulet et al., 1998).

The objective of the present study was to assess the degree of toxic and essential trace element contamination in the main fish species (including detritivorous, omnivorous and carnivorous species) consumed in the Lower Amazon region (Western Pará, northern Brazil). The seasonal effects related to ~~the~~ decreasing water volumes and changing habitats of the fish during the dry season were also considered.

2. Material and methods

2.1 Study site and ~~S~~sample collection

This research was conducted at Santarém, the largest city of the Lower Amazon region, with more than 200 thousand habitants, being the commercial center of the region from were several goods are distributed to the other cities through the rivers (Minervino and Brasileiro, 2019). Santarém does not have mining activity but is located downstream from mining activities (Figure 1).

A total of 351 specimens of different fish species were collected at the municipal market of Santarem (Mercadão 2000), the main fish supply centre in Western Pará during the end of the dry season (October to early December) and wet season (April to early June) in 2015 and 2016. The fish species selected were those considered to be the most important in the diet in the region and included detritivores (Acarí, *Pterygoplichthys pardalis,* n= 137, 81 dry 56 wet season), omnivores (Piranha, *Pygocentrus nattereri, n=29*, 20 dry 9 wet season; Piracurú, *Arapaima* sp., *n=21*, 8 dry 13 wet season) and carnivores (Caparari, *Pseudoplatystoma fasciatum, n=21*, 10 dry 11 wet season; Tucunaré, *Cichla ocellaris, n=143*, 63 dry 80 wet season). ~~.s~~Information about the fishing sites was recorded, to ensure that all specimens were from the region. The fish were packed in sealed airtight plastic bags and immediately transported to the laboratory at 4ºC.

2.2 Sample preparation

Once in the laboratory, each specimen was measured and weighed. Subsamples of approximately 1 g were accurately weighed and digested in a mixture of 5 ml of concentrated nitric acid (TMA, Hiperpure, PanReac, Spain) and 3 ml of 30% w/v hydrogen peroxide (PanReac, Spain) in a microwave-assisted digestion system (Ethos Plus; Milestone, Sorisole, Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 15 ml with ultrapure water according to previously described procedures and conditions (Minervino et al., 2018; Rey-Crespo et al., 2013).

2.3 Trace element analysis

The concentrations of the non-essential elements arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) and of the essential trace elements cobalt (Co), chromium (Cr), copper(Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) in the digested samples were determined by inductively coupled plasma mass spectrometry (ICP-MS; VG PQ Excel, Thermo Elemental, USA). A detailed description of the analytical conditions is provided elsewhere (Albuquerque et al., 2020; Luna et al., 2019; Rey-Crespo et al., 2013). Analytical quality control was applied throughout the study. Blank samples were processed at the same time as test samples, and the values obtained were subtracted from sample readings for calculation of the final values. The limits of detection (LOD) were calculated as three times the standard deviation of the reagent blanks and were based on the mean sample weight. In all cases, the LODs obtained were low enough to determine all essential and trace metals at the usual levels in the studied samples. The accuracy of determination was evaluated by comparison with the analytical recovery of certified reference materials (fish protein DORM- 3 National Research Council, Ottawa, Ontario, Canada), determined following the same procedure as for the shrimp samples. The good agreement between the measured and the certified values (Table 1) demonstrated the high accuracy of the method. As the CRM used was not certified for Co and Mo, the analytical recoveries of these elements were determined using samples spiked at concentrations yielding absorbance values 2–10 times higher than the usual levels in muscle. The mean recoveries were 91% and 94% respectively. The precision of the analytical method, calculated as the relative standard deviation (RSD) of 10 different extractions of the same sample, ranged between 5.4 and 9.6%.

2.4 Statistical and chemometric analysis

According to the results of the trace element determinations, an X_{351x13} matrix was constructed for the study, in which the rows corresponded to the 351 fish samples and the columns to the levels of the 13 elements measured by ICP-MS analysis (As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, and Zn). The data normality was verified by means of Kolmogorov–Smirnov test. As the data were not normally distributed, they were log-transformed before analysis. The results are thus presented as geometric means. Differences in toxic and trace element concentrations in the fish species in relation to season were studied using a general linear model in which "*species*" and "*season*" were included as main fixed factors and "*length*" as a covariate. When significant interactions between species and season were identified, individual/restricted comparisons (between seasons for each fish species and between fish species in each season) were performed by one-way ANOVA followed by post-hoc analysis (Tukey's test). The effect of fish length on trace element accumulation and the association between Hg and other toxic and trace element analysis was determined by Spearman rank correlation analysis. All of these statistical tests were performed with IBM SPSS for Windows v.21 (IBM Corporation, Armonk, NY, USA).

Principal component analysis (PCA), an unsupervised chemometric technique, was used to reveal latent structures in the data set (X_{mxn}, where m is the number of samples and n the number of variables) and to explore the relationship between samples and variables. PCA is a dimension reduction and display chemometric procedure in which the original data matrix is transformed as a product of two other matrices, the first of which (score matrix S_{mxPC}) includes information about the samples and the second of which (loadings matrix L_{PCxPC}) contains information about the variables. If the number of the principal components (PC) selected is lower than the original number of variables (n), then PCA provides substantial dimension reduction and simplification of the original X data matrix. PCA was consequently applied to study the latent data structure in a reduced dimension, but preserving the maximum amount of variance present in the data (Jolliffe et al., 1986). In order to prevent the potential effect of the different ranges of the original variables in PCA, an autoscaling procedure was performed (the average of the variable is subtracted from each data value and the result is divided by its standard deviation). After this preprocessing method, new variables of similar size (all with zero mean and unit variance) were obtained for PCA analysis (Roussel et al., 2014). Both the autoscaling data pretreatment and PCA were carried out with Statgraphics Centurion XVI v16.1.15 software package (Statgraphics Technologies, Inc., The Plains, Virginia, USA).

3. Results and discussion

~~A General Linear Model was used to examine the effect of *fish species* (Acarí, Caparari, Piranha, Pirarucú and Tucunaré) and *season* (dry and wet) as the main variables, with *fish length* included as a covariate, on the toxic and essential trace element concentrations in fish flesh.~~ The results of the GLM that examine the effects of fish species and season on metal concentration are presented in Table 2. Overall, *fish species* was a significant factor for most elements (except Fe and Ni), whereas *season* was only significant for Cd. However, there were significant interactions between *fish species* and *season* for the toxic elements As, Cd, Hg and Pb, indicating a different pattern of accumulation of toxic elements in the fish species considered in this study throughout the year. On the basis of these results, the essential trace element data were analysed for both seasons together, while for toxic elements individual paired-comparisons were carried out separately for each season (dry or wet). Finally, the GLM~~general linear model~~ also revealed a strong effect of fish length ($p<0.001$) on trace element accumulation for Hg and Se, as well as for Mn ($p<0.01$) and Fe and Co ($p<0.05$).

3.1 Toxic element concentrations

The results regarding toxic element concentrations in the different fish species considered in this study, in both the wet and dry seasons, are presented in Figure ~~1~~2. As previously stated (GML analysis) each fish species showed a particular seasonal pattern of toxic element accumulation in the flesh (which also differed within the omnivorous and carnivorous species). These patterns are possibly related (at least partly) to the particular seasonal behaviour and ~~nutritional~~dietary habits of the different species. In the Amazon basin, the main difference between the seasons, in addition to the volume of water (which may have a dilution effect), is that large areas of the rainforest are flooded in the wet season. Well-defined seasonal flooding causes the water level to rise by as much as 20 m (March–April, high-water season) above the low-water level in August–September (Bastos et al., 2007; Junk, 1989). The recorded river level of dry and wet seasons in 2015 were 132 and 597 cm respectively; this rise (4.65 m) is considered low corresponding to a weak rainy season (ANA, 2020). Seasonal inundation leads to important changes in the ~~nutritional~~dietary habits of fish, as well as changes in the ~~toxic element~~ bioavailability of toxic elements. For example, flooding ~~during the wet season~~ affects Hg methylation rates ~~,~~increasing the foraging area and the interaction between terrestrial and aquatic life; this in turn may lead to changes in Hg transfer throughout the food chain (Dorea et al., 2006).

Detailed analysis of the data obtained (Figure ~~1~~2) revealed some interesting results. First, the strong seasonal pattern of Hg accumulation observed for Pirarucú and Tucunaré, with significantly ($p<0.001$) higher (5 times in Pirarucú) residues in the flesh of fish caught during the wet season, and much higher than in any of the other fish species in both seasons, deserves special attention. This pattern may be associated with the behaviour of these two predator fish, which migrate to flooded areas of the rainforest during the wet season (Braga et al., 2016; Castello, 2008; Saint-Paul and Bayley, 1979), indicating that exposure to Hg in this part of the ecosystem is higher than in the main river channel/basin. According to Bastos et al. (2007), naturally occurring Hg in the soil is the principal source of Hg in the Madeira River (the largest tributary of the Amazon River) ecosystem, and human activities, such as deforestation for agricultural projects, damming for a hydroelectric power plant, and alluvial gold extraction, that are clearly associated with soil-Hg release. During the wet season, flooding facilitates transfer of Hg from the soil to the water. By contrast, As and Cd (only in Pirarucu) concentrations were higher in the specimens caught during the dry season, which following the same reasoning may indicate that Cd and As exposure is higher in the main river basin than in the flooded areas of the rainforest. Both elements could have an anthropogenic origin associated with the activities of bauxite exploration in the region in recent decades (they are found in the geological formation of the ore in its inert form; UNCTAD, 1995) but it is also possible that could have a natural origin since Andine rivers have dramatically high concentrations of As, both in solution or absorbed in iron oxides and hydroxides particles (Scarpelli, 2005). Secondly, no statistically significant seasonally-related differences were found for Acari and Caparari in any of the toxic element considered in our study. Acari is a benthic~~benthonic~~, detritivorous species that feeds on debris on the river bottom and sediments (Armbruste, 2004). Caparari is a carnivorous species which, unlike Pirarucu and Tucunaré, prefers deep waters in the beds of the main rivers and is rarely found in flooded waters in the forests (Coronel et al., 2004; Santos and Santos, 2005). Our findings also indicate that dilution of the toxic elements in the water during the wet season is not a main factor explaining the accumulation of toxic elements in fish in our study.

Finally, it is worth noting that Piranha shows the opposite seasonal pattern to Tucunaré and Pirarucu, with significantly higher concentrations of As and lower concentrations of Pb and Cd during the wet season. Unlike Tucunaré and Pirarucu, Piranha prefer lentic environments and can tolerate~~assimilate~~ variations in water quality. This enables Piranha to reside for long periods close to cities, leading to prolonged contact with effluent of the fluvial and socioeconomic activities of large cities located at the margins of the great Amazonian rivers (Freeman et al., 2007; Saint-Paul et al., 2000). Consequently, the seasonal pattern of toxic element accumulation detected in Piranha may be related to anthropogenic activities in urban areas, although a dilution effect (lower concentrations of residues during the wet season, except for As) is also possible. Similar results have been described by Bastos et al. (2007), who reported higher Hg residues in Piranha during the dry season (unlike in other piscivorous species, including Tucunaré).

In an attempt to better understand the observed pattern of toxic element accumulation in the fish and with the aim of studying all the toxic elements (As, Cd, Hg and Pb) simultaneously, PCA was applied to these four elements in order to evaluate the relationship between variables as well as to display the location of the samples in the four dimensional space according to the different species and seasons. After data autoscaling, the PCA selected two principal components (representing 61% of the total variance in the data). This selection enabled concurrent examination of the four elements by using two-dimensional loading and score plots. The loadings for the four toxic elements are represented in the reduced space of the first two principal components (Figure ~~.~~ 3~~2~~A). The loadings of the elements considered are located in the four different cardinal directions in the 2D-space of the principal components, with almost 90º angles between them. This indicates they are probably not correlated, and different patterns can therefore be expected for each. ~~Examination of the score plots also revealed interesting results.~~

The location of the fish samples in the score-space of the two principal components are presented separately according to the season (dry or wet) as a bubble diagram in which the colour of the bubbles is coded for each species, and the size of each bubble is proportional to the content of the element considered (Figure ~~. 2B~~3B). Higher levels of each metal coincide with the direction of the loading for the corresponding metal, although this differs slightly depending on the species considered. In the case of Hg, as expected, higher concentrations of Hg residues were detected in samples of Pirarucu and Tucunaré during the wet season and in Tucunaré and Piranha during the dry season. Predatory have high concentration of Hg ~~fish end up accumulating large amounts of Hg as a function of fish size~~due to bioaccumulation through the food chain (Barbosa et al., 2003). Thus, in the Amazonian aquatic ecosystem, in which a large number of predatory fish species co-exist, Hg is transferred through the aquatic food chain rather than in the form of waterborne Hg (Barbosa et al., 2003).

Cadmium and Pb are closely associated with Acari in both seasons (particularly Pb during the dry season), which may reflect the tendency of both toxic elements to be deposited on the river sediments (Ali et al., 2016; Demirak et al., 2006; Trefry et al., 1985). These results indicate that the concentrations of Cd and Pb in the sediments may constitute the main factor determining exposure in fish. Finally, the higher As residues in fish flesh found in our study were not obviously associated with any particular fish species. The accumulation of As in different components of the food chain in aquatic ecosystems depends upon the availability of As in both the water and the sediment: inorganic As is found as the predominant form ~~species~~ in sediments and waters, whereas organo-As compounds predominate in organisms (Francesconi et al., 1999). The fact that the higher concentrations of As residues were found both in detritivorous and carnivorous species (in the latter independently of seasonal behaviour) may suggest low exposure across the aquatic ecosystem.

Comparison between the toxic element residues measured in fish in the present study (range 2-238 µg/kg fresh weight for As, 1-77 µg/kg for Cd, 4-1922 µg/kg for Hg and 1-30 µg/kg for Pb) and the maximum concentrations established in the Brazilian legislation for fish destined for human consumption (As: 1 mg/kg; Cd: 1 mg/kg; Hg: 0.5 mg/kg; Pb: 1 mg/kg) revealed that these limits were only exceed for Hg in 16 % of the samples (particularly within the carnivorous species). This indicates that Hg contamination in fish is a public health concern in Amazonia. Indeed, numerous studies have been conducted in Amazonia in the last few decades to assess Hg accumulation in fish (Table 3). Most (if not all) of these studies show that a large proportion of carnivorous fish specimens have Hg concentrations exceeding the regulatory limits. Only a broad comparison can be made with previous studies because, as already indicated, Hg accumulation in fish follows a seasonal pattern, and precise information about the sampling collection period is not always provided, and some of the studies report Hg concentration in dry weight. Overall, the Hg concentrations determined in the present study are within the same order of magnitude as reported in previous studies in the region, and are higher in the carnivorous than in detritivorous species. By contrast, data on other toxic elements in fish in the Amazon region are scarce (Table 4). Overall, in the present study, the concentrations of toxic element residues were within the lower ranges reported in previous studies and much lower (up to orders of magnitude) than in Acari and Piranha (the only two species in common in both studies) in the Piracicaba river in Sao Paulo, an area well known for high pollution levels (Meche et al., 2010). Although only a broad comparison can be made with data from other regions/countries as the fish species evaluated in our study are almost exclusively found in Amazonia, the concentrations of As, Cd and Pb are within the same order of magnitude as reported in other studies elsewhere, representing a background environmental level of exposure (for review see Ali and Khan, 2018)

3.2 Essential element concentrations

The essential trace element concentrations in the different fish species considered in this study are shown in Table 5. Except for Fe and Ni, significant differences in trace element concentrations in flesh were found in the fish species analysed in our study, although no clear pattern was observed in relation to feeding habits (*e.g.* ~~in example,~~ carnivorous versus detritivorous species).

In comparison with the maximum tolerable levels (MTL) established by the Brazilian government and other international bodies such as FAO (note that the EU has no~~t~~ MTL for any essential trace element in fish), none of the fish specimens analysed in the present study exceeded the regulatory concentrations for any of the elements except Se (Brazilian MLR: 0.3 mg/kg); however, it should be noted that no other bodies have established MTL for Se and we consider that this limit is unrealistically low, as most studies of freshwater fish in Brazil (Table 5) and elsewhere (Peterson et al., 2009; Plessl et al., 2019) have reported Se concentrations higher than the MTL.

As with the other toxic elements (except Hg), data on essential trace elements in fish in the Amazon region is scarce (reviewed by Albuquerque et al., 2019). As far as we are aware, the most complete study is that conducted in the River Piracicaba in Sao Paulo, which included 16 fish species (Meche et al., 2010). The afore_mentioned study reported large (up two orders of magnitude) differences between fish species (Acari and Piranha showing the highest residues), which they ~~the authors~~ believed could not be explained simply by feeding behaviour. By contrast, inter~~between~~-species differences in trace element concentrations in muscle tissue ~~muscles~~ - known to occur in mammals (Mertz, 2012) - and even within different muscle tissue ~~muscles~~ in the same animal (Gálvez et al., 2019) may be partly related to the homeostatic regulatory mechanisms and trace element metabolism in each species. Differences between fish species may be greater when fish are exposed to high environmental levels (both related to the geographical origin and the anthropogenic sources of pollution), which may overload homeostatic mechanisms in the fish. Comparison of the present findings with those of other studies carried out in the same fish species in the Amazon region (Table 4) indicates that the trace element concentrations measured were within the low range ~~of metal deposition~~ in all cases. Moreover, these results possibly indicate a low level of natural/background environmental exposure not related to anthropogenic or urban sources of pollution. At this level of exposure, trace element metabolism is well regulated by homeostatic mechanisms, and differences between fish species are related to physiological factors. This is consistent with the lack of seasonal variation in the trace element concentration in the fish flesh observed in the present study.

3.3 Effect of fish length on trace element accumulation

As already stated, fish length was strongly associated ($p<0.001$) with tissue concentrations of Hg and Se (Table 2), and a lower effect was also observed for Mn ($p<0.01$) and for Fe and Co ($p<0.05$). Figure 4 shows the association of Hg concentration and fish length in the five analyzed species. In aquatic ecosystems, Hg generally accumulates over time in fish flesh, particularly in organisms at the top of the food chain (Renzoni et al., 1998). Selenium is known to play an essential role in protecting living organisms against the toxic effects of Hg by the in vivo formation of mercuric selenide (HgSe), a stable and biologically inert complex, and also being an important component of selenoenzymes (Yang et al., 2008). However, as far we are aware, there is no available information about possible interactions between Hg and the essential trace elements Mn, Fe and Co. It is possible that the effect of these essential elements on fish may be related to physiological variations during the lifespan of the organism.

In order to determine the possible effect of Hg accumulation and/or fish length (indicative of animal development) on the essential trace element concentrations, the correlation between these variables was determined (Table 6). As Hg accumulation followed a different pattern in the fish species analysed in our study, it was done separately for each. Interestingly, Hg residues were significantly correlated with length in Acari, Caparari and Piranha, but not in Pirarucu or Tucunaré. However, separate analysis of data by season revealed a significant association between Hg residues in fish flesh and length for both species, although only during the wet season. Our results are consistent with those of Dorea et al. (2006), who evaluated Hg bioaccumulation in different Amazonian fish species and observed different bioaccumulation patterns related to ~~the~~ feeding behaviour and hydrological cycles. These researchers concluded that changes in feeding behaviour dominate in importance in Hg bioaccumulation over water characteristics~~description factors~~. By contrast, when considering the essential trace elements (Co, Fe, Mn and Se), only variable (affecting 1 or 2 fish species) and weak correlations with length were observed (Table 6), suggesting that changes in physiological factors during development are not the main reason for the variation in the concentrations of these trace elements in individuals of different lengths.

When evaluating the associations between the concentrations of Hg and these essential trace elements in fish flesh, significant positive correlations with Se (for Pirarucu and Tucunaré only in the wet season) and negative correlations with Fe were observed for all species considered (Table 6; Figure 5~~4~~ and 6~~5~~). As already mentioned, the relationship between Hg and Se in fish is well described in the literature. The high binding affinity of Se and Hg leads to a protective potential of Se, decreasing Hg bioavailability and, subsequently, its toxicity to organisms. ~~the latter exerting a protective role against the toxic effects of Hg~~The Se:Hg molar ratio in fish has been proposed to assess Hg toxicological hazards: Se:Hg molar ratios approach or exceed 1 would potentially protect them and their consumers against Hg toxicity (Peterson et al., 2009). In our study most specimens (96.2%) have Se:Hg molar ratios <1—being only <1 in a low number of omnivorous (3 Pirarucu and 4 Piranha samples) and carnivorous specimens (8 Tucunaré)—suggesting a low toxicological potential for consumers. Some recent studies have evaluated the relationship between Hg and Se in different species of fish from the Amazon River, showing inconsistent results. Whereas only significant correlations between Hg and Se were observed in carnivorous species (Lino et al., 2018a), another study reported significant positive associations between both elements in carnivorous and omnivorous species (Lima et al., 2005). A study in the Tapajós River reported only a positive association between Hg and Se in herbivorous and omnivorous but not in piscivorous/carnivorous fish species (Sampaio da Silva et al., 2006). In the latter study the association between Hg and Se was evaluated separately in the wet and dry season and, as in the present study, the trend was variable and related to species. This finding led the study authors to conclude that the Se cycle in the Amazon is far from being understood and that further knowledge is necessary in order to obtain a more complete picture of the presence of this element in tropical aquatic ecosystems as well as the potential risks and benefits that it represents to the health of fish resources. By contrast, as far we know the possible association between Hg and Fe in fish has not previously been described. Early studies in rats suggest that administration of milk with a high Fe content decreased the absorption of Hg in the gut (Kostial et al., 1980); however, the mechanisms involved in this interaction have not been elucidated. Recent studies indicate the role of Fe in mercury methylation by iron-reducing bacteria in aquatic ecosystems

(Li and Cai, 2013; Paranjape and Hall, 2017). While the reduction in Fe(III) can stimulate MeHg ~~FeHg~~ formation, higher concentrations of Fe(III) can suppress mercury methylation by complexing Hg and making it unavailable for methylation (Si et al., 2015). Finally, significant associations between Hg and Co and Mn were also observed in some of the fish species analysed in the present study (Table 6). Although less well studied, the influence of these two redox-sensitive and microbially important elements (Mn and Co) on Hg cycling and methylation has been suggested (Ekstrom and Morel, 2008; Faganeli et al., 2012), as has their potential to reduce Hg toxicity in aquatic environments.

4. Conclusions

Accumulation of toxic elements in fish in Western Pará showed large differences between species and throughout the year, possibly related to the particular seasonal behaviour and ~~nutritional~~dietary habits of each species. Of the toxic elements, only Hg seems to be of concern for the local population. By contrast, the concentrations of trace elements were low and did not show any seasonal or diet-related patterns ~~seasonally or nutritionally-related patterns~~, with observed differences between species possibly at least partly related to the fish metabolism. The associations between Hg and the essential trace elements Se (well described in the literature) and also Fe, Co and Mn deserve special attention. As far we are aware, this is the first time that such associations have been found in fish exposed to high environmental levels of Hg and they could play a role in Hg cycling and methylation. Further evaluation should be conducted with the aim of reducing Hg toxicity in aquatic environments.

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Author contributions

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Declarations of interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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Table 1. Analytical quality program expressed as mean±standard deviation used to determine trace elements

		DORM-3	
	Detection limit (µg/L)	**Level determined (mg/Kg)**	**Certified level * (mg/Kg)**
As	0.4	6.62 ± 0.38	6.88 ± 0.30
Cd	0.2	0.291 ± 0.062	0.290 ± 0.020
Hg	0.1	0.348 ± 0.021	0.382 ± 0.060
Pb	0.1	0.367± 0.046	0.395 ± 0.050
Co	0.1	0.200 ± 0.014	--
Cr	0.4	1.74 ± 0.61	1.89 ± 0.17
Cu	2.8	14.9 ± 1.2	15.5 ± 0.63
Fe	7	352 ± 43	347 ± 20
Mn	1.0	3.36 ± 0.33	(4.6)
Mo	0.9	0.661 ± 0.010	--
Ni	0.3	1.27 ± 0.25	1.28 ± 0.24
Se	0.8	3.51 ± 0.32	(3.3)
Zn	5	48.1 ± 2.2	51.3 ± 3.1

*Values in parenthesis are informative only

Table 2. Summary of the general linear model used to evaluate the influence of fish species and season as main factors and length as covariate on toxic and essential trace element accumulation in fish flesh. Statistically significant effects at P <0.05 (*), P<0.01 (**) and P<0.001 (***).-- not significant.

	species		season		species*season		length	
	F	P	F	P	F	P	F	P
As	$F_{5,365}=9.750$	***	$F_{1,365}=2.543$	--	$F_{4,365}=3.132$	**	$F_{1,365}=3.431$	--
Cd	$F_{5,365}=4.657$	***	$F_{1,365}=5.033$	*	$F_{4,365}=3.633$	**	$F_{1,365}=3.291$	--
Hg	$F_{5,365}=12.906$	***	$F_{1,365}=3.714$	--	$F_{4,365}=5.259$	***	$F_{1,365}=10.914$	***
Pb	$F_{5,365}=5.964$	***	$F_{1,365}=0.641$	--	$F_{4,365}=2.561$	*	$F_{1,365}=0.358$	--
Co	$F_{5,365}=8.677$	***	$F_{1,365}=2.320$	--	$F_{4,365}=1.672$	--	$F_{1,365}=5.020$	*
Cr	$F_{5,365}=5.589$	***	$F_{1,365}=0.093$	--	$F_{4,365}=0.920$	--	$F_{1,365}=2.253$	--
Cu	$F_{5,365}=5.709$	***	$F_{1,365}=1.290$	--	$F_{4,365}=0.716$	--	$F_{1,365}=1.792$	--
Fe	$F_{5,365}=1.567$	--	$F_{1,365}=3.355$	--	$F_{4,365}=1.810$	--	$F_{1,365}=5.224$	*
Mn	$F_{5,365}=6.627$	***	$F_{1,365}=1.290$	--	$F_{4,365}=0.662$	--	$F_{1,365}=6.913$	**
Mo	$F_{5,365}=34.133$	***	$F_{1,365}=0.517$	--	$F_{4,365}=1.408$	--	$F_{1,365}=2.508$	--
Ni	$F_{5,365}=2.175$	--	$F_{1,365}=1.044$	--	$F_{4,365}=1.561$	--	$F_{1,365}=1.056$	--
Se	$F_{5,365}=20.179$	***	$F_{1,365}=0.245$	--	$F_{4,365}=0.258$	--	$F_{1,365}=10.857$	***
Zn	$F_{5,365}=4.476$	***	$F_{1,365}=1.045$	--	$F_{4,365}=1.851$	--	$F_{1,365}=0.805$	--

Table 3. Mercury concentrations in fish (expressed in mg/kg fresh weight, unless indicated) from the Amazon River. Only studies including at least three of the fish species considered in our study are shown.

Local	**Fish species**					**Reference**
	Mean±SD and (range)					
	Pirarucu	**Tucunaré**	**Piranha**	**Caparari**	**Acari**	
Western Pará						
Tapajós River	0.17[a]	0.40±0.19	0.27±0.12	0.36[a]	0.03±0.01	(Lebel et al., 1997)
Tapajós River		0.357±0.092	0.342±0.188	0.435±0.166	0.069±21.05	(Sampaio da Silva et al., 2006)
Tapajós River		0.753±0.31	0.413±0.243		0.08[a]	(Passos et al., 2008)
Tapajós River		0.268±0.121	0.138±0.064	0.381±0.058		(Castilhos et al., 1998)
Tapajós River		0.267±0.049	0.219±0.204	0.385		(Brabo et al., 1999)
Tapajós River	0.406[a]	0.549±46.7	0.437±35.25	0.545±36.5		(Uryu et al., 2001)
Tapajós River		0.420±0.190	0.100±0.038	0.460±0.06		(Bidone et al., 1997)
Tapajós River		0.306±0.261	0.280±0.087	0.199±0.073		(Lima et al., 2000)
Amazon Lower	0.147±0.102					(Martinelli and Mc Grath, 1999)
Other areas of Amazonia						
Madeira River	0.343	0.437	0.781	0.785	0.198	(Bastos et al., 2007)
	(0.231-0.730)	(0.012-1.316)	(0.017-2.168)	(0.046-2.890)	(0.006-0.644)	
Madeira River		0.409	0.832	0.197	0.080	(Oliveira et al., 2010)
		(0.001-1.488)	(0.555-1.097)	(0.101-0.518)	(0.061-0.100)	
Madeira River		0.45±0.32	0.275±0.1	0.41±0.52	0.045±0.02	(Hacon et al., 2014)
Upper Solimões					0.029±0.01	(Silva et al., 2019)
Madeira river basin		0.533±0.14	0.423±0.3 (0.14-0.91)	0.825		(Reuther, 1994)
Madeira river basin		0.103±0.001[b]	0.265±0.002[b]	0.192±0.002[b]		(Queiroz et al., 2018)
Upper Madeira River			0.634±0.02 (1.206-1.233)	0.859 (0.327-2.304)	0.016 (0.014-0.018)	(Maurice-Bourgoin et al., 2000)

[a] only one specimen analysed, [b] dry matter

Table 4. Toxic and trace element concentrations in fish (expressed in mg/kg fresh weight, except * in dry matter) from the Amazon region

Species	Origin	As	Cd	Pb	Cr	Cu	Fe	Mn	Mo	Ni	Se	Zn	Reference
Acari													
	Cassiporé River		0.009	0.065	0.025	0.052						0.193	(De Lima et al., 2015)
	Piracicaba River	0.93*	0.92*	1.41*	1.52*	1.72*		3.24*	1.48*	1.27*	1.61*	9.25*	(Meche et al., 2010)
	Corral Wire Stream					7.11*	49.22*					27.33*	(Rosso et al., 2015)
Caparari													
	Santarém					1.3	17				0.41	7	(Lino et al., 2018ab)
	Cachoeira do Piriá										5.08		(Lima et al., 2005)
	Cassiporé River		0.034	0.175	0.006	0.061						0.099	(De Lima et al., 2015)
	Paraopeba River		0.13	2.12	0.48							19.76	(Arantes et al., 2016)
	São Francisco River	0.096	0.112	0.578								10.53	(Gobbi et al., 2011)
Piranha													
	Itaituba					1.2	40				0.13	8	(Lino et al., 2018ab)
	Gelado River		1.84		0.85								Barros et al 2010
	Cassiporé River		0.015	0.191	0.100	0.05						0.254	(De Lima et al., 2015)
	Piracicaba River	0.73*	0.72*	0.66*	1.11*	4.18*		1.67*	0.82*	1.33*	1.22*	16.3*	(Meche et al., 2010)
Tucunare													
	Tapajós River										0.32		(Faial et al., 2015)
	Tapajós river basin					1	119.6				1.0		(Lino et al., 2018ab)
	Gelado River		1.67		1.45								(Barros et al., 2010)
	Xingu River			0.17*	0.39*	0.21*				0.06*			(Ribeiro et al., 2017)

Table 5. Essential trace element concentrations (expressed in mg/kg fresh weight) in fish from Western Pará. GM: geometric mean. MRL: maximum recommended levels. Different superscript letters within each column indicate statistically significant differences between species.

	Co			Cr			Cu		
	GM	median	range	GM	median	range	GM	median	range
Acarí	0.003^{b}	0.002	0.001-0.044	0.018^{bc}	0.016	0.005-0.076	0.121^{b}	0.108	0.000-1.566
Caparari	0.001^{a}	0.002	0.001-0.004	0.017^{abc}	0.013	0.010-0.071	0.084^{a}	0.078	0.056-0.176
Piranha	0.003^{bc}	0.004	0.001-0.008	0.012^{ab}	0.010	0.007-0.053	0.096^{ab}	0.087	0.058-0.194
Pirarucu	0.005^{c}	0.005	0.001-0.014	0.012^{a}	0.011	0.006-0.043	0.119^{ab}	0.112	0.058-0.313
Tucunaré	0.002^{ab}	0.003	0.001-0.040	0.013^{ab}	0.011	0.006-0.091	0.113^{ab}	0.103	0.057-0.634
MRL Brazil				*0.1*			*30*		
FAO				*1*			*30*		

	Fe			Mn			Mo		
	GM	median	range	GM	median	range	GM	median	range
Acarí	3.05	2.69	0.57-26.67	0.169^{b}	0.118	0.037-1.356	0.002^{a}	0.002	0.000-0.010
Caparari	3.05	1.89	0.62-12.09	0.079^{a}	0.081	0.032-0.175	0.002^{a}	0.002	0.001-0.003
piranha	3.22	4.75	0.64-11.29	0.098^{ab}	0.079	0.026-0.806	0.007^{b}	0.016	0.001-0.022
pirarucu	3.05	2.57	1.23-15.43	0.111^{ab}	0.101	0.061-0.481	0.008^{b}	0.016	0.001-0.019
Tucunaré	3.15	2.69	1.09-18.57	0.123^{ab}	0.120	0.021-1.075	0.008^{b}	0.018	0.000-0.023
MRL Brazil									-
FAO									

	Ni			Se			Zn		
	GM	median	range	GM	median	range	GM	median	range
Acarí	0.008	0.008	0.002-0.054	0.326^{cd}	0.306	0.057-1.594	2.98^{ab}	3.02	1.31-10.26
Caparari	0.008	0.008	0.005-0.016	0.235^{b}	0.241	0.155-0.295	2.73^{ab}	2.75	1.83-4.53
piranha	0.007	0.007	0.004-0.011	0.159^{a}	0.195	0.051-0.349	3.34^{b}	3.13	1.87-9.53
pirarucu	0.007	0.007	0.002-0.023	0.244^{bc}	0.237	0.171-0.371	3.41^{b}	2.89	1.53-16.72
Tucunaré	0.008	0.007	0.004-0.040	0.347^{d}	0.346	0.142-1.008	2.79^{a}	2.72	1.74-7.30
MRL Brazil	*5*			*0.3*			*50*		
FAO							*50*		

Table 6. Summary of the correlation analysis (Spearman rank correlation coefficient) showing the association between the length of the fish specimen and trace element concentrations in the flesh, as well as between mercury and trace elements in the flesh

	Acarí	Cacararí	Piranha	Pirarucú[1]	Tucunaré[1]
Length vs					
Hg	0.334**	0.764**	0.423*	0.281 (0.790***)	-0.046 (0.463*)
Se	0.242**	0.196	0.178	0.277 (0.555**)	0.531**
Fe	-0.121	-0.728**	-0.013	-0.023	-0.207*
Mn	-0.164	-0.228	0.212	-0.049	-0.302**
Co	-0.257**	-0.532*	0.547**	-0.041	-0.226**
Mercury vs					
Se	0.449**	0.478*	0.825**	0.427 (0.595**)	0.163 (0.381*)
Fe	-0.490**	-0.591**	-0.676**	-0.719**	-0.368**
Mn	0.038	-0.182	0.530**	-0.039	-0.243**
Co	-0.257**	-0.355	0.252	-0.456*	-0.170*

[1] Values shown in parenthesis were only calculated for specimens collected during the wet season

Figure legends

Figure 1. Study site. (A). Map of Brazil with the Amazon biome marked in light green; (B). Region impacted by mining activity: 1 Santarém city; 2 Porto Trombetas district (bauxite exploitation); 3 Itaituba (artisanal gold mining); 4 Jurutí city (bauxite exploitation). Light blue arrows indicate the rivers flux direction; (C). Santarém urban area and the location of the main fish market.

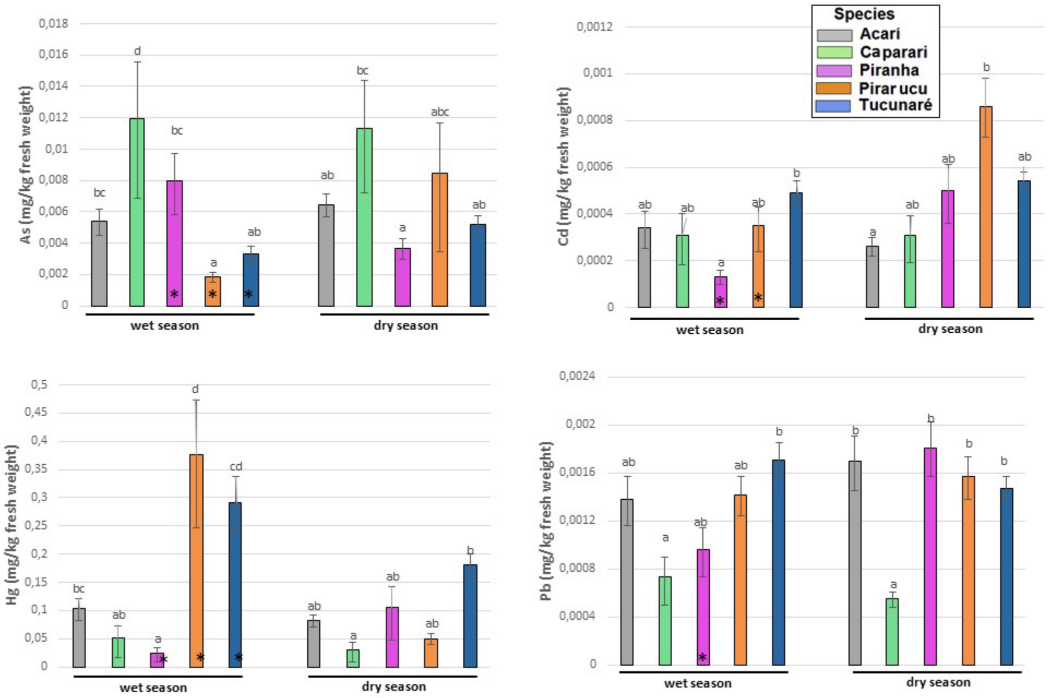

Figure ~~1~~2. Toxic element concentrations (expressed as geometric mean and geometric intervals in fish from Western Pará. Different letters indicate statistically significant differences between species for each season, * indicates statistically significant differences between seasons for each fish species.

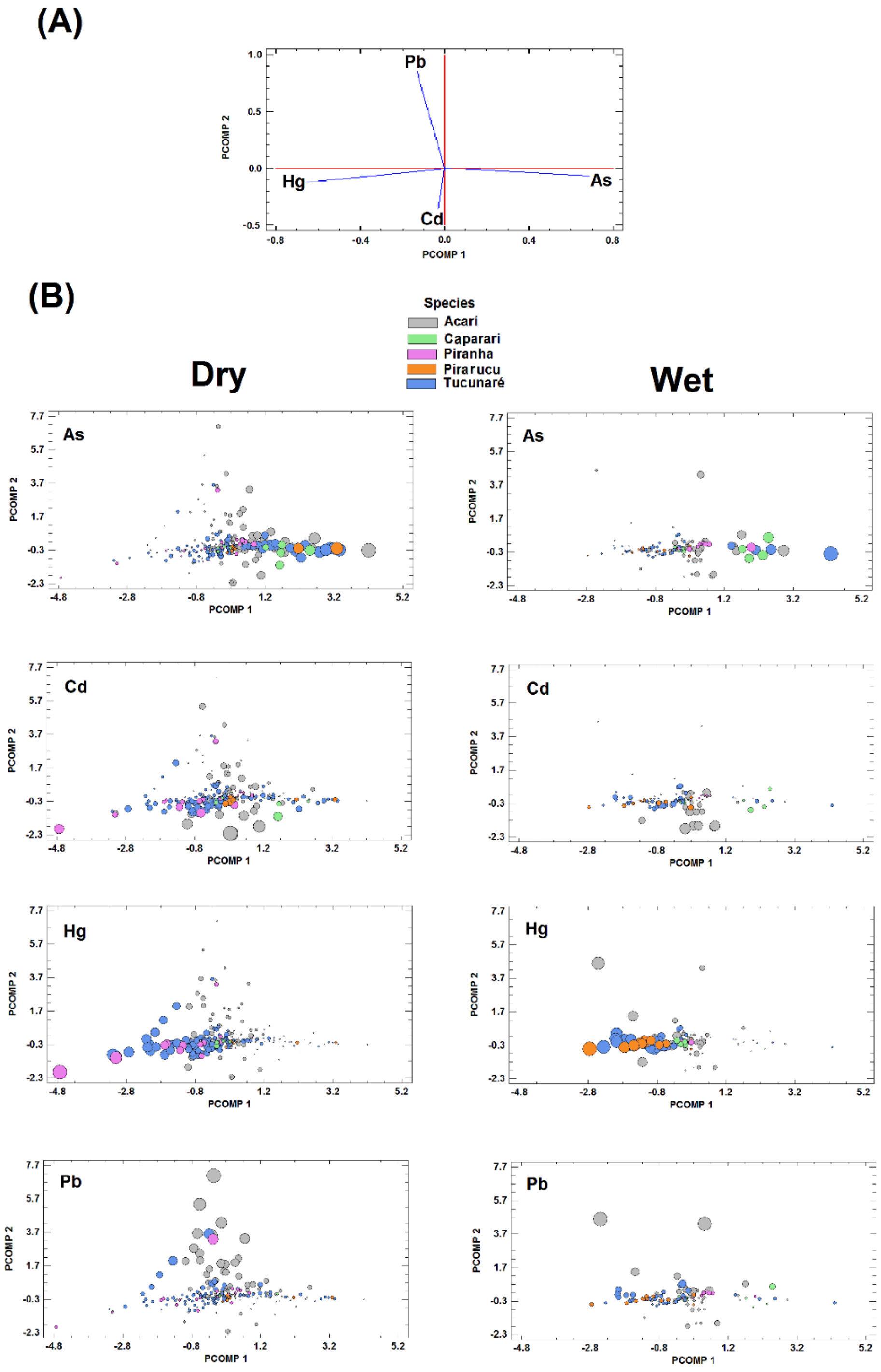

Figure ~~2~~3. (A). Loadings-plot for the toxic elements in the space of the two first principal components. (B) Bubble diagrams for the score-plots of the fish samples according to the season (the size of the bubble is proportional to the concentration of the indicated element in fish flesh).

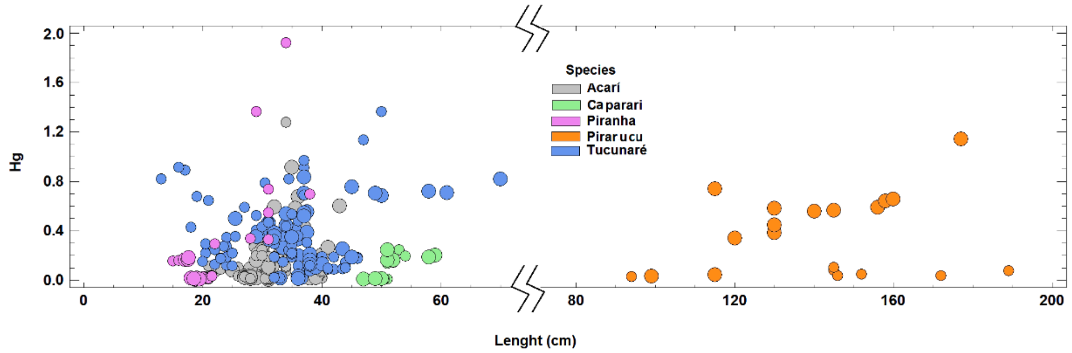

Figure ~~3~~4. Hg concentration *vs*. fish length according to the species and season (small bubbles: dry season: large bubbles: wet season).

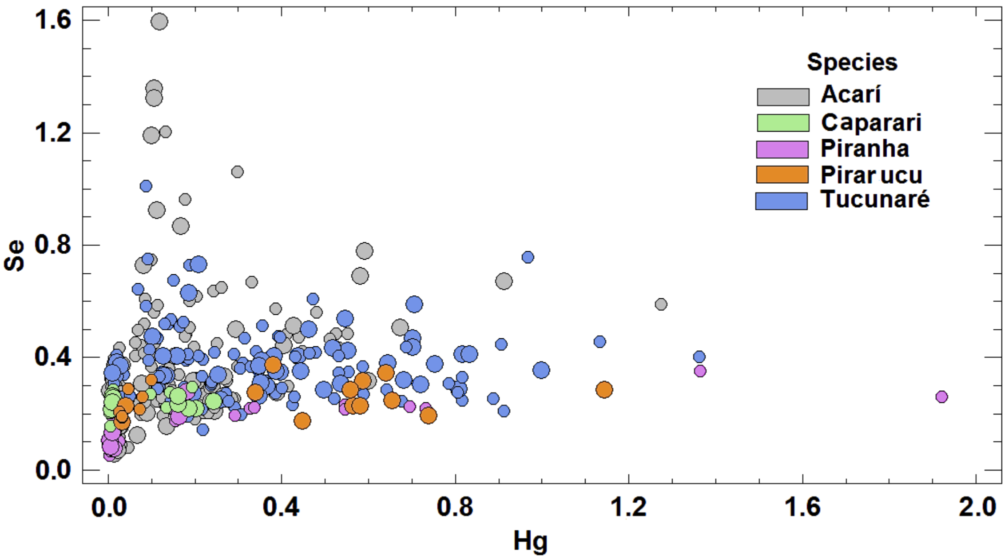

Figure 45. Hg *vs*. Se concentrations in fish flesh according to species and season (small bubbles: dry season: large bubbles: wet season).

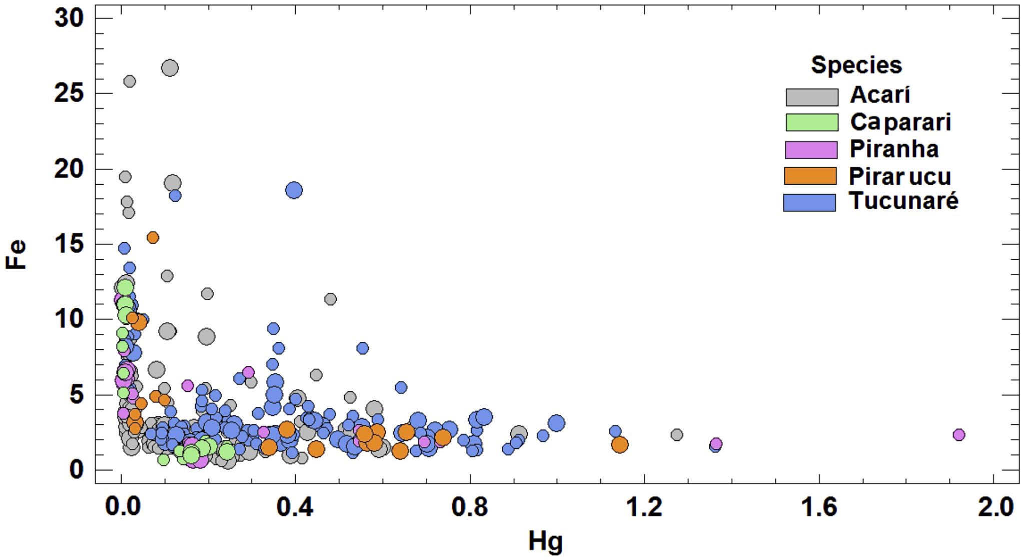

Figure ~~5~~6. Hg *vs*. Fe concentrations in fish flesh according to species and season (small bubbles: dry season: large bubbles: wet season).