~~Biomonitoring toxic and essential trace elements in the Lower Amazon region using fish tissues~~

Feasibility of using fish tissues to biomonitor toxic and essential trace elements in the Lower Amazon

Fabio Edir Amaral Albuquerque[a,f], Carlos Herrero-Latorre[b], Marta Miranda[c], Raimundo Alves Barrêto Júnior[d], Francisco Leonardo Costa Oliveira[e], Maria Cláudia Araripe Sucupira[e], Enrico Lippi Ortolani[e], Antonio Humberto Hamad Minervino[a*], Marta López-Alonso[f]

[a]Laboratory of Animal Health, LARSANA, Federal University of Western Pará, UFOPA. Rua Vera Paz, s/n, Salé, CEP 68040-255, Santarém, PA, Brazil

[b]Research Institute on Chemical and Biological Analyses (IIAQBUS), Department of Analytical Chemistry, Nutrition and Bromatology. Faculty of Sciences, University of Santiago de Compostela, 27002 Lugo, Spain

[c]Department of Anatomy, Animal Production and Clinical Veterinary Sciences, Veterinary Faculty, University of Santiago de Compostela, 27002 Lugo Spain.

[d]Department of Animal Science, Federal Rural University of the Semiarid Region (UFERSA). Av. Francisco Mota, s/nº - Bairro Pres. Costa e Silva, CEP 59625-900, Mossoró, RN, Brazil.

[e]Department of Clinical Science, College of Veterinary Medicine and Animal Science, University of Sao Paulo (FMVZ/USP). Av. Prof. Orlando Marques de Paiva, 87, Cidade Universitária, CEP 05508-270, São Paulo, SP, Brazil.

[f]Department of Animal Pathology, Veterinary Faculty, University of Santiago de Compostela, 27002 Lugo Spain.

Abstract

Brazilian soils can have high concentrations of toxic elements, mainly mercury (Hg) and arsenic (As), metals also associated with anthropogenic activities (e.g. intensive agriculture, mining, deforestation and hydroelectric plants). This can lead to large amounts of these elements reaching and/or being mobilized in the aquatic ecosystem, which constitutes a serious threat to the environment and to the health of local populations. Thus, we evaluate the feasibility of analyzing the tissues of freshwater fish species for monitoring toxic and trace element accumulation within the aquatic ecosystem in the Lower Amazon, Brazil. Two fish species were considered: *Cichla temensis* (Tucunaré), a carnivorous species, and *Pterygoplichthys pardalis* (Acari), a detritivorous species. Samples of liver and muscle from both species were evaluated in relation to their potential use for biomonitoring purposes. The study findings clearly demonstrate the value these fish species and tissues, particularly liver, for biomonitoring toxic and trace element concentrations in the aquatic environment across the study region. While Tucunaré liver proved the best option for biomonitoring elements that accumulate through the food chain (e.g. Hg), Acari liver better reflected elements that typically accumulate in the sediments (e.g. As). Moreover, the trace element profiles, determined using chemometric (multivariate) techniques, differed greatly in specimens from waters in the Andean mountain range (sampling sites located in the main course of the Amazon River) with high sediment concentrations, and in specimens from the Guyana and Brazilian shields (Porto Trombetas on the Trombetas River and Itaituba on the Tapajós River). The findings also indicate that deposition of elements in freshwater fish in this area is mainly associated with the geological origin of the soils and that large amounts of toxic elements can reach the aquatic ecosystem due to anthropogenic activities, thereby posing a serious danger to the environment and the health of the riverside communities.

Key words: Biomonitoring; Amazon river; trace and toxic elements; fish liver and muscle.

1. Introduction

Aquatic ecosystems can be polluted by a wide range of contaminant substances, such as heavy metals and pesticides, that may be deposited in the sediments and/or accumulated through the trophic chain, (Lescord et al., 2020; Varol and Sünbül, 2017; Zupo et al., 2019). The Amazon region is an environmental icon with unique characteristics. Amazonian forests host a quarter of terrestrial species in the world, and they account for about 15% of global terrestrial photosynthesis and act as the engine of global atmospheric circulation (Malhi et al., 2008), which is essential to combat the impacts of climate change (Nepstad et al., 2008). The Amazon basin produces about 20% of the world's freshwater discharge, and the aquatic environment is vital for the local population using it for food supply (fish), transportation, and economical activities (Latrubesse et al., 2017).

Since the last century, Amazonian freshwater ecosystems have been facing a serious problem of mercury (Hg) contamination associated with artisanal gold mining. The devastating consequences of the mining activities have been widely monitored in several studies in the past few years, with particular focus on human exposure through consumption of fish (Castilhos et al., 2015; Crespo-Lopez et al., 2021; Passos et al., 2008). In addition, other mining activities and oil extraction have led to important spills of toxic chemicals into the rivers in the area (Soares et al., 2017). Mining activities, together with the large-scale development of agriculture and intensive livestock farming, have indirectly aggravated problems associated with environmental pollution in the Amazon. Such activities have triggered extensive deforestation and are associated with the construction of extensive infrastructure networks designed to facilitate access to various industrial operations. However, the impact of these activities on other toxic elements, including interactions with trace elements that are essential for living organisms, has not been widely evaluated.

Within the Brazilian Amazon, the state of Pará is particularly seriously affected by mining exploitations. In the municipality of Itaituba, gold extraction is common (and often unregulated and clandestine); in Porto Trombetas (municipality of Oriximiná), bauxite mining has been carried out since 1969; and in Juruti, a bauxite exploitation project has also been initiated within the last 10 years. Numerous mining-related accidents have been reported in the region, including the direct discharge of effluent comprising bauxite tailings rich in iron (Fe), aluminium (Al) and silica (Si) oxides into the Batata Lake~~lake~~ in Oriximiná between 1979 and 1989 (Lin and Caramaschi, 2005) and the historic mercury contamination in the Tapajos river basin associated with artisanal gold mining (Lino et al., 2019). The Pará region is also well known for agricultural production (mainly corn and soybean), which has led to intense deforestation (Fearnside, 1990). All of these activities are very likely to have a significant impact on environmental pollution, the consequences of which have not been evaluated in depth.

According to the results of a previous study assessing toxic element contamination in the main fish species consumed in Santarém (the main city in western Pará), Hg pollution is of serious concern in the area (Albuquerque et al., 2020b) and m~~. M~~aximum admissible Hg concentrations in fish destined for human consumption (0.5 mg/kg fresh weight) are frequently exceeded in carnivorous species. According to the patterns observed in this study, it seems that mercury is transferred through the aquatic food chain rather than in the form of waterborne mercury, although other essential trace elements, such as selenium (Se), Fe, manganese (Mn) and cobalt (Co) in addition to exert an essential role in the organism (Watanabe et al., 1997) may play an important role in Hg methylation in the environment. Although other toxic elements, i.e. arsenic (As), cadmium (Cd), and lead (Pb), were found at relatively low concentrations in the fish flesh, some interesting patterns were observed that deserve further investigation. Liver plays an essential role in trace element metabolism and is the main organ in which metals accumulate. Analysis of liver tissue may therefore provide more useful information than muscle in relation to assessing toxic element exposure and bioaccumulation in the aquatic ecosystem.

The objective of this study was to evaluate and compare the feasibility of using liver and muscle tissue from two different freshwater fish species as analytical samples for monitoring toxic and trace element accumulation in the aquatic ecosystem in the western part of the state of Pará. For this goal, the concentrations of thirteen toxic and trace elements were determined in liver and muscle tissue extracted from a carnivorous fish species, *Cichla temensis* (Tucunaré) and a detritivorous fish species, *Pterygoplichthys pardalis* (Acari), captured at various sampling points across the study area. Chemical data

were analyzed by using unsupervised chemometric techniques to elucidate the relationships between samples, essential and toxic elements, species and sampling points.

2. Material and methods

2.1. Fish species and sampling procedure

Samples were obtained at 5 different points in the western part of the state of Pará (**Figure 1**). Three points were selected within the main course of the Amazon River, from the source to the mouth of the river: Faro, the most western point of the state of Pará, which is free of mining activities (sampling point 1, SP1); Jurutí, where a bauxite exploitation project was initiated within the last 10 years (sampling point 2, SP2); and Santarém, the most important urban area in the region (sampling point 3, SP3). Another two sampling sites were also established in rivers from the Guyana and Brazilian shields: the first one at Itaituba, on the Tapajós River (sampling point 4, SP4), where the largest small-scale gold mining sites called "garimpos" are located; and at Porto Trombetas on the Trombetas River (sampling point 5, SP5), a region/area where a bauxite exploitation has been carried out since 1969. A total of 258 specimens of two different fish species largely distributed in the Amazon River and fished for human consumption, -with distinct feeding patterns were obtained from these sampling sites. Thus, 125 of the samples corresponded to *Pterygoplichthys pardalis* (Acari or Amazon Sailfin Catfish), a detritivorous, nocturne, benthonic species that mainly feeds on detritus and algae scraped or rasped from the bottom substrates of the river, participating in the pre-mineralization phase of organic matter present in the sediments. Acari lives in groups on sites with decomposed organic matter and do not perform systematic migrations, remaining in shoals in the original environments. When necessary, they make small lateral movements during the ebb or flow moving between the lakes and the seasonally flooded areas. The Acari is extremely tolerant to inhospitable environments (*e.g.* low O_2) (Armbruster, 2004; Soares et al., 2006). -The remaining 133 samples were of *Cichla temensis* (Tucunaré), a sedentary, voracious predatory carnivorous fish that lives both in the middle courses of the slow flowing rivers and in flooded areas during the rainy season. In the rainy season, the fish is usually found lurking near the shore where it feeds on smaller species. They have diurnal habits, can use different places of food, and occasionally they also eat invertebrates such as crustaceans and insects. In general, Tucunaré are sedentary, although they can perform lateral movements between the main river channel and the flood plains for foraging (Kullander and Ferreira, 2006). Most of Tucunaré was found moving only one km in a capture-recapture study, but a few larger specimens moved up to 21 km (Hoeinghaus et al., 2003). In each sampling site, specimens of Acari and Tucunaré were obtained directly from professional fishermen and were measured and weighted, and packed in airtight plastic bags and immediately transported to the laboratory at 4ºC. Since nematode infection can change mineral status, fish was ~~era~~ examined and only parasite-free specimens were used (Rosa Leite et al., 2020). Summarized data of the specimens included in our study are presented in **Table 1**; no statistical significant differences in length and weight were observed between specimens collected in different areas.

2.2. Sample preparation

Once in the laboratory, each specimen was measured and weighed, and tissue samples were prepared immediately to prevent possible sample deterioration and/or potential contamination. Samples of muscle and liver tissue were extracted and dried in an oven at 85ºC for 24 hours until constant weight. Subsamples of approximately 1 g, were weighed accurately 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 (Albuquerque et al., 2020a; Rey-Crespo et al., 2013).

2.3. Toxic and trace element analysis

The concentrations of the non-essential elements As, Cd, Hg, Pb and of the essential trace elements Co, chromium (Cr), copper (Cu), Fe, Mn, molybdenum (Mo), nickel (Ni), Se and zinc (Zn) in the digested samples were determined by inductively coupled plasma mass spectrometry (ICP-MS; VG PQ Excel,

Thermo Elemental, USA). The analytical conditions have been described in detail in previously published studies (Albuquerque et al., 2020b; Luna et al., 2019). Samples were analyzed in triplicate and an analytical quality control programme was applied throughout the study. Blank solutions were processed and measured alongside samples, and blank values obtained were subtracted from sample readings before calculation of the final results. Limits of detection (LOD) were calculated as three times the standard deviation of the reagent blanks. In all cases, the LODs were sufficient to enable determination of the essential and trace metals at the levels found in the samples of both fish species. The accuracy of the determinations was evaluated by comparison with a certified reference material (CRM) of fish protein DORM-3 (National Research Council, Ottawa, Ontario, Canada), which was analyzed following exactly the same procedure as for fish samples. The results obtained (summarized in **Table 2~~1~~**) showed satisfactory agreement between measured and certified values, thus demonstrating that the accuracy of the method was acceptable. As there is no CRM available for Co and Mo, the analytical recoveries of these elements were determined by using spiked samples at concentrations producing absorbance values up to 10 times the usual levels in muscle. The mean recoveries were 89% and 92%, respectively. The precision of the analytical determinations, calculated as the relative standard deviation (RSD) of 10 distinct digestions of the same sample, ranged between 4.3 and 7.6%.

2.4. Data matrices and chemometric procedures

Two different data matrices were obtained from the chemical data: *Liver.set* and *Muscle.set*. The first matrix corresponds to the analytical data for liver tissue, and the second incorporates the data on the mineral composition of muscle. Each matrix (X_{258x13}) is composed of the concentrations of the 13 mineral elements (columns) in the 258 samples (rows) from both fish species (Acari and Tucunaré). The data normality was checked by means of the Kolmogorov–Smirnov test. As the data were not normally distributed, they were log-transformed before analysis. Differences in toxic and trace elements concentrations in the fish species from the different sampling points were studied using a general linear model in which "species" and "sampling point" were the main fixed factors. The statistical analysis was performed using IBM SPSS for Windows v.24 (IBM Corporation, Armonk, NY, USA).

Chemometric analysis of the data was carried out ~~with the main objective of for~~to test~~ing~~ the potential use of mineral concentrations in liver and/or muscle of the two fish species as markers of bioaccumulation. Therefore, ~~the *Liver.set* and *Muscle.set* ~~data sets were processed by chemometric ~~(multivariate)~~ techniques in order to evaluate the capacity of the different fish species (Acari *vs*. Tucunaré) and the diverse target tissues (liver *vs*. muscle) for this purpose. In addition, the capacity of the selected biomarkers to differentiate between sampling sites affected by different levels of environmental pollution (associated with diverse anthropogenic activities) was also evaluated. ~~The chemometric study consisted of the use of ~~Two unsupervised ~~multivariate ~~display chemometric techniques: Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were applied to explore the relationships between samples and variables and to reveal the latent structure of the chemical information contained in both X_{258x13} data matrices. ~~As 13 metals were determined for both data sets (*Liver.set* and *Muscle.set*), each fish sample was therefore characterized by a point in a 13-dimensional space of the variables. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) were used to examine these data sets. PCA is a useful technique in exploratory data analysis involving numerous samples and variables (Massart et al., 2003). This display technique enables better visualization of the data set in a reduced dimension of the principal components (PC) with minimum loss of total information. PCA decomposes the original data matrix X_{nxm} into a product of another two matrices. The first of these, the score matrix S_{nxm}, contains information about the samples, while the second, the loadings matrix L_{nxn}, includes information about the variables. If the number of principal components selected is lower than the number of original variables (m), PCA enables considerable reduction and simplification of the original data matrix X. Thus, in the case at hand,~~ PCA was first carried out ~~used ~~to visualize the 13-dimensional data matrices in a reduced dimension, thereby preserving the maximum ~~information contained in the ~~data variance (Massart et al., 2003) After that, HCA ~~(Massart and Kaufman, 1983) is another multivariate display chemometric technique ~~(usually applied in combination with PCA) was performed ~~used ~~to establish clusters of ~~group ~~similar samples (or variables) on the basis of the Euclidean distance between them as a ~~into a number of clusters based on the observed~~

values of the variables. In the present case, clusters were obtained according to the Ward agglomerative procedure on the basis of the squared Euclidean distance between samples as the similarity measure. The final result produced by HCA is a graphical tree diagram representing the clusters, i.e. a called dendrogram, which is a two-dimensional plot of the sample similarities in the 13-dimensional space of the variables. Before PCA and HCA, pretreatment consisting of an autoscaling procedure was applied to both *Liver.set* and *Muscle.set* matrices to prevent the diverse ranges of the different elements analyzed affecting the outcome. In this common data pretreatment procedure, each value of a variable is subtracted from the mean value of the variable and divided by the standard deviation. This produces new variables with zero mean and unit variance in all cases, thus preventing the undesired influence of the different size of original variables. The multivariate techniques (PCA and HCA) All chemometric procedures were carried out using Stat graphics Centurion XVI *v.* 16.1.15 (Statistical Graphics Corp., Rockville, MD, USA).

3. Results

3.1. Toxic and trace element concentrations in liver and muscle

The data on toxic and trace element concentrations in liver and muscle of the fish specimens were analyzed using a General Linear Model in order to evaluate the influence of the fish species (Acari *vs.* Tucunaré) and sampling site (SP1 to SP5). The results are presented in **Table 32**. In general, for the liver, strong ($p<0.001$) statistically significant differences between fish species and geographical regions were detected for most elements (except Mn and Mo). By contrast, for the muscle, significant differences were only observed for the toxic elements (As and Hg) and Se. However, as the interaction between both factors (fish species and geographical region) proved significant, accumulation of toxic and trace elements does not follow the same geographical pattern in both fish species, and it should therefore be studied separately.

Detailed data on the toxic and essential trace element concentrations in the liver and muscle of specimens of Acari and Tucunaré from the different geographical areas are presented in **Figures 2** and **3,** respectively. For the toxic elements, the highest geographical variations corresponded to Hg and As, although for both elements geographical variations depended on the fish species considered. Mercury concentrations were particularly high in the liver of Tucunaré collected in the mining areas of Itaituba (SP4) and Porto Trombetas (SP5),) — ii.e. up to 26.7 mg/kg dry weight (0.5 mg Hg/kg fresh weight is the maximum residue level in the Brazilian and other international legislations), with mean values 5 times higher in the other areas (see box-whisker-plots of Figure 2, note the different scales on the y-axis for liver and muscle samples). The mean Hg concentrations were generally one order of magnitude lower in Acari than in Tucunaré, but they were significantly higher in the specimens from Porto Trombetas (SP5) and Faro (SP1) than in specimens from the other sampling sites. The Hg concentrations were lower (ca. 50 %) in muscle than in liver of both fish species, but with the same pattern of accumulation (Pearson correlation coefficient for Hg in liver and muscle 0.793 and 0.830 ($p<0.001$) for Acari and Tucunaré, respectively). By contrast, the highest As concentrations were detected in both liver and muscle of Acari (approximately 5 times higher than in Tucunaré), although the same geographical pattern was detected in both species: the As concentrations were highest in the fish samples from the main course of the Amazon River (Faro [SP1], Juruti [SP2] and Santarém [SP3]). The concentrations of As in liver and muscle were closely correlated in both fish species (Pearson correlation coefficient 0.696 and 0.772, $p<0.001$), as observed for Hg. The Cd concentrations were higher in liver than in muscle, as expected due to the bioaccumulation capacity of this element; the concentrations were similar in both fish species, with similar geographical variation. Finally, Pb concentrations were generally low in both fish species, and except for the liver of Acari (Pb concentrations were higher in specimens caught in Faro [SP1]), no significant geographical variations were found. None samples exceeded the maximum residue levels for As, Cd and Pb in the Brazilian (1, 1 and 2 mg/kg fresh weight) and other international legislations (1-2, 0.5-1, 0.5 mg/kg fresh weight respectively; for review see Albuquerque et al., 2020a)

The distributions of essential trace element concentrations are shown in box-whisker plots on the basis of fish sample, tissue analyzed and sampling point (**Figure 3)**. Essential trace element concentrations in the fish species studied were generally within the adequate physiological ranges (Albuquerque et al., 2020b).

As in the case of toxic elements, the concentrations were higher and more variable in the liver than in muscle. For these elements the main geographical variations (statistically significant for all the elements, except Mn) were found in the liver of Acari, and in most cases the concentrations of essential trace elements were highest in fish from Faro (SP1) (as for the toxic elements). For the other categories of samples, significant differences were observed only for Fe in the Tucunaré liver (as with Hg, the highest concentrations corresponded to the mining areas of Itaituba [SP4] and Porto Trombetas [SP5]) and Se in the muscle of Acari (concentrations were highest in specimens from Faro [SP1]).

3.2. Chemometric analysis

Although the individual analysis (for each element) of toxic and trace element deposition in liver and muscle of Acari and Tucunaré revealed significant differences between geographical areas, this did not enable identification of a clear pattern of exposure to metals in the studied area. For all elements studied, an overlap between the specific metal levels in liver and muscle of both species was observed. This finding indicated that, despite some differences being detected, neither of the metals analyzed in the two species seems to be able to reflect the metal exposure in this geographical region. Therefore, a multivariate approach was applied for joint consideration of all variables.

3.2.1. Fish species

Application of PCA to the *Liver.set* matrix, examination of score and loadings plot afforded interesting results. As it can be seen in **Figure 4a**, in which the scores of the fish samples are presented in the space defined by the first three principal components (accounting for 63% of the total data variance), two natural groups of the samples were detected according to the fish species (Acari and Tucunaré). The samples of each species are thus located in different zones of the 13-dimensional space, implying a different mineral profile for each species when all the variables are evaluated together, although none by themselves were capable of producing this separation. The sample grouping revealed by PCA was verified by using other display chemometric techniques with a different mathematical basis. In this case, HCA was used to reveal the groups of samples in the 13-dimensional space according to the squared Euclidean distance between them as a similarity measurement and using the Ward method as agglomerative procedure for identification of clusters. The result obtained in the form of dendrogram is presented in **Figure 5**. Two main groups of samples (coded as clusters A and cluster B) were identified: cluster A, composed of Acari samples, and cluster B composed of Tucunaré samples. Additionally, cluster C, including samples from the two fish species, illustrates the minimum overlap between the two main groups in the multidimensional space. Moreover, the level of similarity within Tucunaré samples is higher than the similarity between Acari samples. The intra-species variability for Tucunaré is therefore lower than that observed for Acari. The concordant conclusions obtained by two diverse chemometric techniques confirm the very different total metal profiles in liver samples from the two fish species.

In order to study the relationships between variables, the same display chemometric techniques were applied to the X_{238x13} *Liver.set*. Examination of the loadings obtained in the PCA showed that the variables contributing most to the first principal component were Cd, Co, Fe, Hg and Se, while As, Cr, Mo, Ni and Pb were the dominant variables in the second PC. This finding can be interpreted with the help of the PCA biplot (presented in **Figure 4b**), produced by a 3D-graph plotting function (Biplot) in which samples and variables are plotted in the same space defined by the first three PCs. The graph produced is very useful for evaluating the associations between the samples (fish species) and variables (mineral elements). As it can be seen in this figure, the variables contributing most to PC1 are mainly related to Tucunaré, while those with the highest loadings in PC2 correspond to the Acari samples. Following an approach similar to the previously applied to the samples, this result was verified by HCA. The result for the variable association produced by this chemometric technique clearly revealed two groups of variables: the first one is composed of Fe, Cd, Se, Co, Hg and Mo (which mainly coincides with the most important variables in PC1) and it is related to Tucunaré species, and the second cluster is formed by the variables Cr, Ni, Pb, Mn, As, and Zn (most with high loadings in PC2) and is related to Acari. Thus, the variable association was confirmed by a second different display multivariate chemometric technique. A similar multidimensional approach was used to examine the *Muscle.set* matrix. Samples of both fish species also form separate groups in the

multidimensional space of the variables. However, a higher degree of overlap in the muscle data was demonstrated, as can be seen in the score plot of muscle samples in **Figure 6a**. This greater overlap was also confirmed by HCA (**Figure 7**). This means that muscle mineral patterns for the two species of fish are not as different as observed for the liver. On the other hand, when the relationships between variables and samples were examined in the corresponding biplot, very different patterns were observed relative to those observed for liver. The only evident association was for Hg levels in muscle of Tucunaré (**Figure 6b**). For the other variables associated with Acari, two subsets were identified, the first composed of As, Cr and Ni, and the second determined by the remaining variables. This result was also confirmed by HCA.

3.2.2. Geographical distribution/sampling sites

In order to explore the associations between fish samples and the sampling sites, PCA was applied to the *Liver.set* and *Muscle.set* matrices separately for each fish species on the basis of the sampling locations. The results obtained are presented in the form of score plots in **Figure 8**. Differences between differentiated polluted areas were observed. Liver samples from Acari formed three separate groups: the first and most numerous group is comprised of samples from the main course of the Amazon basin (including Faro [SP1], Jurutí [SP2] and Santarém [SP3]), the second group is composed of Itaituba (SP4) samples, while the last one is formed by Porto Trombetas (SP5) samples, which are very different from the other two groups. The same pattern can be observed for muscle samples from Acari. In this case, both target tissues are sensitive to different pollution areas. However, examination of the Tucunaré data ~~-~~only identified two groups: the first is composed of all the samples from the main course of the Amazon River (SP1, SP2 and SP3), but the second group includes samples from both Itaituba (SP4) and Porto Trombetas (SP5). Very similar results were obtained for Tucunaré muscle samples.

4. Discussion

The study findings demonstrate the potential feasibility of using fish tissues for biomonitoring toxic and trace elements in the western Pará aquatic ecosystem. However, as different information was obtained in relation to the toxic and trace elements in each type of sample and species (liver or muscle, Acari or Tucunaré) in the different geographical areas, the selection of the most appropriate type of sample will depend on the particular objectives of the biomonitoring study.

The interest in Hg biomonitoring studies is obvious considering that contamination with this element is one of the main concerns in Lower Amazon, regarding both the environmental risk and the health risks to the inhabitants of the area, whose diet is largely fish-based. From this point of view, muscle of carnivorous fish species seems to be the best choice of sample. The tissue concentrations of Hg have been studied in a broad selection of fish species in the Amazonian region in the last few decades (Azevedo et al., 2020; Bastos et al., 2015; Lima et al., 2015; Lino et al., 2019; Martín-Doimeadios et al., 2014; Silva et al., 2019) and there is a general agreement that Hg accumulation occurs mainly in the form of methyl-Hg and is highest in carnivorous fish species. In previous studies, Hg concentrations were often found to be above the maximum levels allowed in the Brazilian as well as in other international legislation (Rabitto et al., 2011). These high levels are toxic for humans, and they must be closely monitored. In Amazonian fish, Hg is transferred through the aquatic food chain rather than in the form of waterborne Hg (Barbosa et al., 2003), and therefore fish species at the top of the food chain can provide the best information about Hg pollution. It is generally assumed that gold-mining activities constitute the main source of Hg contamination in Amazonia (Hg has been historically used, since the times of the Spanish colonizers in the XVI century, in the amalgamation process used in the extraction of gold from ore). Since the 1980s, a new gold rush has been occurring in South America, particularly in Brazil, where almost 90% of gold production is associated with often unregulated and small-scale gold mining sites called "*garimpos*" (Lacerda and Salomons, 1998). In Brazil alone, artisanal-scale gold mining accounts for 30 tonnes of gold per year, 26% of which is produced in the Tapajós River Basin, with the largest area of *garimpos* located at ~~is in~~ Itaituba (Lobo et al., 2016)~~, the central area of the Tapajós River basin, where it is estimated that approximately 500 tons of gold has been recovered since 1980~~. In addition to this clearly anthropogenic source of Hg, high concentrations of Hg have been found in the mineral horizons of soils from different sub-basins in the Amazon, which are

responsible for the high Hg concentrations detected in fish in areas not affected by the gold-mining exploitations (Barbosa et al., 2003). Soil erosion, intensified by human activities such as forest clearing, agriculture and other mining activities, has been shown to be an important natural source of Hg in local aquatic systems (Berzas Nevado et al., 2010). The findings of the present study indicate that the main sources of Hg exposure in the aquatic ecosystems under study are the Hg used in the amalgamation process involved in gold mining in the municipality of Itaituba in the Tapajos River and also the earthworks and perforation of soils in the Porto Trombetas bauxite exploitations (See Figure 2).

The results of the PCA and HCA showed that the metal profiles of Acari and Tucunaré are different (see Figure 4a), and therefore the species provide different information about the metal status of the sampled areas. Tucunaré is the most appropriate choice for biomonitoring Hg, as it is more sensitive to this metal (in fact Hg is one of the metals with the highest score in PC1: see section 3.2.1, Figure 4). In addition, the pattern of Hg accumulation is similar in the liver and muscle of Tucunaré. Both tissues could therefore be useful for monitoring purposes; however, as Hg accumulation was much higher in the liver than in the muscle, liver is preferred, particularly for poorly contaminated or unpolluted areas, or when the limits of detection of the analytical technique used for Hg quantification is high (i.e. fails to detect when there is limited concentration of metals in the tissues) using methods such as cold vapor atomic fluorescence spectrometry or direct mercury analysers, reported in studies from South America (Pestana et al., 2019; Ribeiro et al., 2017). Therefore, Tucunaré liver is considered potentially useful for biomonitoring Hg exposure in the aquatic ecosystem in the Amazonia. In addition, the chemometric analysis also showed that other trace elements are also closely associated with Hg accumulation in the liver of Tucunuré, e.g. Se, Fe and Co (See Figure 4b). Selenium is a well-known antagonist of Hg as it sequesters this element and reduces its environmental bioavailability, thereby protecting living organisms from its toxic effects (Sørmo et al., 2011). Thus, only determination of the amount of Hg present in the environment or in food sources may provide an inadequate reflection of the potential health risks generated by this element if the protective effects of Se are not also considered (Ralston et al., 2008). In fact, the Se:Hg molar ratio in tissues has been proposed to estimate the protective role of Se against Hg toxicity: Se:Hg molar ratios >1 being considered protective (Ralston et al., 2008). In our study (data not shown) the Se:Hg molar ratio was well above 1 in most samples, the only exception being 12of 133 muscle specimens of Tucunaré; these ratios being statistically higher ($p<0.001$) in the liver (mean value: 53.93) compared to the muscle (20.33) and in Acarí (61.43) compared to Tucunaré (11.68). ~~Further research on Hg-Se interactions will help to clarify the consequences of Hg exposure and identify populations that may be protected or at greater risk from the toxic effects of Hg.~~ Moreover, it has recently been suggested that Fe and Co may play an important role in mercury methylation (Albuquerque et al., 2020b). If these results are confirmed, liver samples from carnivorous species could provide additional information to better understand the metabolic methylation of Hg and consequently its toxicity. Moreover, these elements ~~-~~may potentially play an important role in bioremediation strategies (Paranjape and Hall, 2017).

Interestingly, Cd also appears to be associated with Hg, Fe, Co and Se in Tucunaré liver (Figure 4b). ~~This is not surprising, because Cd (like Hg) is a toxic element that is bioaccumulated and biomagnified at the top of the food chain.~~ In fish, as observed in other animal species, including mammals, Cd is accumulated by being bound to hepatic metallothionein in the liver, kidney and gills for very long periods. However, Cd concentrations in the muscle are very low and are generally not related to Cd exposure, although some samples from Amazonian fish may have Cd concentration higher then maximum allowed limit ~~-~~(Alcala-Orozco et al., 2020). Indeed, the study findings confirmed very low Cd concentrations in muscle (2 orders of magnitude lower than in the liver) and also a stable pattern not related to the different geographical areas, thus ruling out this type of sample as a useful biomarker of Cd exposure. With the only exception of the specimens collected in Itaituba (SP4), Cd concentrations were similar in the liver of Acari and Tucunaré, with similar geographical variations. This finding suggests that sediments are the main source of Cd exposure in the habitats of both species (the main course of the rivers for Acari and in flooded areas for Tucunaré), particularly in Acari given its position at the bottom of the food chain.

On the other hand, if the objective of biomonitoring is to detect other toxic (mainly As) and essential trace elements, then the liver of Acari is the optimum type of sample. This species is extraordinarily sensitive to

As (in fact As is one of the metals with high score in PC1: see section 3.2.1, Figure 4). Acari is a detritivorous species and therefore feeds on debris (sediments) on the river bottom, where most toxic and trace elements typically accumulate (Palacios-Torres et al., 2020). Although As contamination receives less attention than Hg contamination, it is also a major concern in the Amazon River. A recent study evaluating soil and tailing samples from gold mining areas at Amazon demonstrated that concentrations of As were exceeding by far the acceptable value from Brazilian regulatory agency (258 times higher), with high concentration in all exploration areas, especially in tailings, inorganic reactive fractions of As being a serious risk for the local population (Souza Neto et al., 2020). Waters and rivers in the Andes mountains have extremely high concentrations of As, both in solution or absorbed in iron oxides and hydroxides particles. Arsenic (originated from the common occurrences of arsenic-bearing sulphites along the Andean mountain range) appears in these environmental waters at concentrations close to the maximum value accepted for potable water of 10 µg L^{-1} (Scarpelli, 2005). Moreover, Andean waters contain high loads of sediments containing very high concentrations of As, up to four orders of magnitude higher than in the water itself (Scarpelli, 2005). Arsenic concentrations were very high in the liver of Acari in this study (one order of magnitude higher than in Tucunaré and than reported in other international studies studies (Liu et al., 2012; Squadrone et al., 2013) and they accurately reflect the high levels of As residues found in sediments and in water throughout the Amazon basin (Seyler and Boaventura, 2001). In these selected samples, As concentrations were significantly higher in the Andean tributaries and in their area of influence (sampling points located in the main Amazon course SP1, SP2 and SP3) than in the non-Andean areas (Itaituba [SP4] in the Tapajós River and Porto Trombetas [SP5] in the Trombetas River).

Finally, considering all of the information on the concentrations of the thirteen toxic and trace elements in the samples, assessed as potential biomarkers of pollution in relation to their geographical origin, Acari samples (both liver and muscle) best reflect the differences in the three sampling areas, as can be seen in section 3.2.2 (Figure 8). Samples from this type of fish were capable of distinguishing between the specimens from the main course of the Amazon River (from the source to the mouth of the river, Faro [SP1], Juruti [SP2] and Santarém [SP3], representing the Andean waters) and specimens from the tributary rivers, i.e. Tapajos (Itaituba [SP4]) and Trombetas (Porto Trombetas [SP5]). These results are consistent with the type of waters contributing to the Amazon River. On the one hand, Andean tributary rivers (including Solimoes and Madeira) carry high loads of sediments, which give the water a distinctive brown colour. The large sediment loads are due to the high erosion rates at the high elevations of the Andes range (up to 4000 m) with steep slopes and a thin cover of vegetation (Scarpelli, 2005). It is estimated that the 97% of the total suspended sediments in the mouth of the Amazon River are derived from the Andean tributaries. On the other hand, the Amazon tributaries from the Guyana shield (e.g. the Trombetas River) and from the Brazilian shield (e.g. the Tapajós River) carry small loads of sediments, as both are characterised by surface elevations rarely surpassing a few hundred metres, with intense rainfall and thick vegetation and a low rate of erosion (Scarpelli, 2005). Information on toxic and trace element concentrations (with the exception of Hg) in the Amazon basin is scarce (see Seyler and Boaventura, 2001); however, it seems that the patterns observed in fish in the present study are related to the natural origin of the soils and to the rate of erosion. In a recent study from Atrato River, an ecosystem also impacted by impacted by gold mining, but at the Colombian Pacific, pollution by Cr, Ni, Cu, As, Cd and Pb was found and linked to gold mining (Palacios-Torres et al., 2020).

In general, in the Amazon basin ~~is relatively free of industrial and other human inputs of toxic elements~~the type of anthropogenic activities (mainly mining) makes that~~, and~~ the concentrations in the aquatic environment are probably ~~therefore~~ closely related to the background concentrations in the soil. The soils in the Brazilian Amazon are geochemically very diverse, reflecting the distinct territorial areas and the different source materials and factors involved in soil formation (Nascimento et al., 2018). This explains why, for some elements, the highest residues were found in SP1-Faro, a region free of mining and other relevant anthropogenic sources of trace elements, but that on the contrary, is most strongly influenced by the Andean waters. Finally, the study findings also indicated that Acari tissues (both liver and muscle) are capable of providing more information about toxic and trace element exposure than Tucunaré tissues. The patterns of accumulation in Acari samples ~~is~~ are different in specimens collected in the Amazon River (SP1, SP2 and SP3), in Itaituba (SP4) and in Porto Trombetas (SP5) However, for Tucunaré samples these last

two areas appeared together and overlapped in the chemometric analysis showed in Figure 8). This finding probably is related to the detritivorous nature of Acari, which feeds on the debris on the bottom of the river where most trace elements typically concentrate. However, it is also possible that this primitive fish species has more rudimentary homeostatic mechanisms that lead to higher accumulation in the liver when the fish are feeding in polluted environments, but this hypothesis need further evidence.

5. Conclusions

The findings of the present study clearly demonstrated the feasibility of using fish species to biomonitor toxic and trace element concentrations in the aquatic environment of the western part of the state of Pará (Brazil). Liver tissue from Tucunaré and Acari proved more useful for this purpose than muscle tissue. Although Tucunaré (a carnivorous species) liver is the best candidate for biomonitoring elements that accumulate through the food chain (*e.g.* Hg), Acari (a detritivorous species) liver better reflects elements that typically accumulate in the sediments (e.g. As). Moreover, the findings indicate that element deposition in freshwater fish in this area seem to be more closely related to the geological origin of the soils than to the anthropogenic activities. The trace element profiles show a clear difference between specimens feeding in waters originating in the Andean mountain range, with a high sediment concentrations (sampling sites located in the main course of the Amazon River), than fish from the Brazilian and the Guyana shields (the Tapajós and Trombetas rivers respectively). The abnormally high concentrations of several toxic elements in some Brazilian soils (mainly Hg and As) favour large amounts of these elements reaching aquatic ecosystem due to anthropogenic activities (including intensive agriculture, mining and deforestation and energy production in hydroelectric plants), thus posing a serious risk to the environment and to the health of the riverside communities. New studies, including analysis of toxic and trace element concentrations in water, suspended particulate matter and sediments, as well as the physical and chemical properties of the water would help to better understand the dynamic of these elements in the aquatic ecosystem.

Acknowledgements

This research was supported by the Coordination of Superior Level Staff Improvement (CAPES), Visiting Research Program (PVE) grant #88881.062153/2014-01. We also are grateful to CAPES for Ph.D. sandwich fellowships grated (to FEAA and FLCO). The authors thank RIAIDT-USC, Spain, for the use of analytical facilities. AHHM is grateful to the National Council for Scientific and Technological Development (CNPq) for a research productivity fellowship. FEAA is grateful to Amazon Foundation to Support Studies and Research (FAPESPA) for his Doctoral fellowship and FLCO is grateful to The São Paulo Research Foundation (FAPESP). The authors are grateful to Prof. Domingos Diniz (*in memorian*) for support with funding acquisition.

Author contributions

Fabio Albuquerque Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing - original draft, Writing - review & editing; **Carlos Herrero-Latorre:** Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing - review & editing; **Marta Miranda:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing - review & editing;; **Antonio Minervino:** Conceptualization, Data curation, Funding acquisition, Investigation, Project administration, Supervision, Writing - review & editing; **Raimundo Barrêto JR:** Conceptualization, Funding acquisition, Methodology, Supervision, Writing - review & editing; **Francisco Oliveira:** Data curation, Formal analysis, Investigation, Methodology, Software, Visualization; **Maria Sucupira:** Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing - review & editing; **Enrico Ortolani:** Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing - review & editing; **Marta López-Alonso:** Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing - original draft, Writing - review & editing

Declarations of interest

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

References

Albuquerque, F.E.A., Minervino, A.H.H., Miranda, M., Herrero-Latorre, C., Barrêto Júnior, R.A., Oliveira, F.L.C., Dias, S.R., Ortolani, E.L., López-Alonso, M., 2020a. Toxic and essential trace element concentrations in the freshwater shrimp Macrobrachium amazonicum in the Lower Amazon, Brazil. J. Food Compos. Anal. 86, 103361. https://doi.org/10.1016/j.jfca.2019.103361

Albuquerque, F.E.A., Minervino, A.H.H., Miranda, M., Herrero-Latorre, C., Barrêto Júnior, R.A., Oliveira, F.L.C., Sucupira, M.C.A., Ortolani, E.L., López-Alonso, M., 2020b. Toxic and essential trace element concentrations in fish species in the Lower Amazon, Brazil. Sci. Total Environ. 732, 138983. https://doi.org/10.1016/j.scitotenv.2020.138983

Alcala-Orozco, M., Caballero-Gallardo, K., Olivero-Verbel, J., 2020. Biomonitoring of Mercury, Cadmium and Selenium in Fish and the Population of Puerto Nariño, at the Southern Corner of the Colombian Amazon. Arch. Environ. Contam. Toxicol. 79, 354–370. https://doi.org/10.1007/s00244-020-00761-8

Armbruster, J.W., 2004. Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and the Ancistrinae. Zool. J. Linn. Soc. 141, 1–80. https://doi.org/10.1111/j.1096-3642.2004.00109.x

Azevedo, L.S., Pestana, I.A., da Costa Nery, A.F., Bastos, W.R., Souza, C.M.M., 2020. Mercury concentration in six fish guilds from a floodplain lake in western Amazonia: Interaction between seasonality and feeding habits. Ecol. Indic. 111, 106056. https://doi.org/10.1016/j.ecolind.2019.106056

Barbosa, A.C., De Souza, J., Dórea, J.G., Jardim, W.F., Fadini, P.S., 2003. Mercury biomagnification in a tropical black water, Rio Negro, Brazil. Arch. Environ. Contam. Toxicol. 45, 235–246. https://doi.org/10.1007/s00244-003-0207-1

Bastos, W.R., Dórea, J.G., Bernardi, J.V.E., Lauthartte, L.C., Mussy, M.H., Lacerda, L.D., Malm, O., 2015. Mercury in fish of the Madeira river (temporal and spatial assessment), Brazilian Amazon. Environ. Res. 140, 191–197. https://doi.org/10.1016/j.envres.2015.03.029

Berzas Nevado, J.J., Rodríguez Martín-Doimeadios, R.C., Guzmán Bernardo, F.J., Jiménez Moreno, M., Herculano, A.M., do Nascimento, J.L.M., Crespo-López, M.E., 2010. Mercury in the Tapajós River basin, Brazilian Amazon: A review. Environ. Int. 36, 593–608. https://doi.org/10.1016/j.envint.2010.03.011

Castilhos, Z., Rodrigues-Filho, S., Cesar, R., Rodrigues, A.P., Villas-Bôas, R., de Jesus, I., Lima, M., Faial, K., Miranda, A., Brabo, E., Beinhoff, C., Santos, E., 2015. Human exposure and risk assessment associated with mercury contamination in artisanal gold mining areas in the Brazilian Amazon. Environ. Sci. Pollut. Res. 22, 11255–11264. https://doi.org/10.1007/s11356-015-4340-y

Crespo-Lopez, M.E., Augusto-Oliveira, M., Lopes-Araújo, A., Santos-Sacramento, L., Yuki Takeda, P., Macchi, B. de M., do Nascimento, J.L.M., Maia, C.S.F., Lima, R.R., Arrifano, G.P., 2021. Mercury: What can we learn from the Amazon? Environ. Int. https://doi.org/10.1016/j.envint.2020.106223

Fearnside, P.M., 1990. The Rate and Extent of Deforestation in Brazilian Amazonia. Environ. Conserv. 17, 213–226. https://doi.org/10.1017/S0376892900032355

Hoeinghaus, D.J., Layman, C.A., Arrington, D.A., Winemiller, K.O., 2003. Movement of Cichla species (Cichlidae) in a Venezuelan floodplain river. Neotrop. Ichthyol. 1, 121–126. https://doi.org/10.1590/s1679-62252003000200006

Kullander, S.O., Ferreira, E.J.G., 2006. A review of the South American cichlid genus Cichla, with descriptions of nine new species (Teleostei: Cichlidae). Ichthyol. Explor. Freshwaters 17, 289–398.

Lacerda, L.D., Salomons, W., 1998. Mercury from Gold and Silver Mining: A Chemical Time Bomb?, Mercury from Gold and Silver Mining: A Chemical Time Bomb? https://doi.org/10.1007/978-3-642-58793-1

Latrubesse, E.M., Arima, E.Y., Dunne, T., Park, E., Baker, V.R., D'Horta, F.M., Wight, C., Wittmann, F., Zuanon, J., Baker, P.A., Ribas, C.C., Norgaard, R.B., Filizola, N., Ansar, A., Flyvbjerg, B., Stevaux, J.C., 2017. Damming the rivers of the Amazon basin. Nature. https://doi.org/10.1038/nature22333

Lescord, G.L., Johnston, T.A., Heerschap, M.J., Keller, W. (Bill), Southee, F.M., O'Connor, C.M., Dyer, R.D., Branfireun, B.A., Gunn, J.M., 2020. Arsenic, chromium, and other elements of concern in fish from remote boreal lakes and rivers: Drivers of variation and implications for subsistence consumption. Environ. Pollut. 259, 113878. https://doi.org/10.1016/j.envpol.2019.113878

Lima, D.P., Santos, C., Silva, R. de S., Yoshioka, E.T.O., Bezerra, R.M., 2015. Contaminação por metais pesados em peixes e água da Bacia do rio Cassiporé, Estado do Amapá, Brasil. Acta Amaz. 45, 405–414. https://doi.org/10.1590/1809-4392201403995

Lin, D.S.C., Caramaschi, É.P., 2005. Responses of the fish community to the flood pulse and siltation in a floodplain lake of the Trombetas River, Brazil. Hydrobiologia 545, 75–91. https://doi.org/10.1007/s10750-005-2186-x

Lino, A.S., Kasper, D., Guida, Y.S., Thomaz, J.R., Malm, O., 2019. Total and methyl mercury distribution in water, sediment, plankton and fish along the Tapajós River basin in the Brazilian Amazon. Chemosphere 235, 690–700. https://doi.org/10.1016/j.chemosphere.2019.06.212

Liu, F., Ni, H.G., Chen, F., Luo, Z.X., Shen, H., Liu, L., Wu, P., 2012. Metal accumulation in the tissues of grass carps (Ctenopharyngodon idellus) from fresh water around a copper mine in Southeast China. Environ. Monit. Assess. 184, 4289–4299. https://doi.org/10.1007/s10661-011-2264-7

Lobo, F., Costa, M., Novo, E., Telmer, K., 2016. Distribution of Artisanal and Small-Scale Gold Mining in the Tapajós River Basin (Brazilian Amazon) over the Past 40 Years and Relationship with Water Siltation. Remote Sens. 8, 579. https://doi.org/10.3390/rs8070579

Luna, D., Miranda, M., Minervino, A.H.H., Piñeiro, V., Herrero-Latorre, C., López-Alonso, M., 2019. Validation of a simple sample preparation method for multielement analysis of bovine serum. PLoS One 14, e0211859. https://doi.org/10.1371/journal.pone.0211859

Malhi, Y., Roberts, J.T., Betts, R.A., Killeen, T.J., Li, W., Nobre, C.A., 2008. Climate change, deforestation, and the fate of the Amazon. Science 319, 169–172. https://doi.org/10.1126/science.1146961

Martín-Doimeadios, R.C.R., Berzas Nevado, J.J., Guzmán Bernardo, F.J., Jiménez Moreno, M., Arrifano, G.P.F., Herculano, A.M., Nascimento, J.L.M., Crespo-López, M.E., 2014. Comparative study of mercury speciation in commercial fishes of the Brazilian Amazon. Environ. Sci. Pollut. Res. 21, 7466–7479. https://doi.org/10.1007/s11356-014-2680-7

Massart, D.L., Vandeginste, B.G.M., Deming, S.N., Michotte, Y., Kaufman, L., V., 2003. Chemometrics: A textbook, 1 st. ed. Amsterdan.

Nascimento, C.W.A., Lima, L.H.V., da Silva, F.L., Biondi, C.M., Campos, M.C.C., 2018. Natural concentrations and reference values of heavy metals in sedimentary soils in the Brazilian Amazon. Environ. Monit. Assess. 190. https://doi.org/10.1007/s10661-018-6989-4

Nepstad, D.C., Stickler, C.M., Soares-Filho, B., Merry, F., 2008. Interactions among Amazon land use, forests and climate: Prospects for a near-term forest tipping point, in: Philosophical Transactions of the Royal Society B: Biological Sciences. Royal Society, pp. 1737–1746. https://doi.org/10.1098/rstb.2007.0036

603 Palacios-Torres, Y., de la Rosa, J.D., Olivero-Verbel, J., 2020. Trace elements in sediments and
604 fish from Atrato River: an ecosystem with legal rights impacted by gold mining at the
605 Colombian Pacific. Environ. Pollut. 256, 113290.
606 https://doi.org/10.1016/j.envpol.2019.113290

607 Paranjape, A.R., Hall, B.D., 2017. Recent advances in the study of mercury methylation in aquatic
608 systems. Facets 2, 85–119. https://doi.org/10.1139/facets-2016-0027

609 Passos, C.J.S., Da Silva, D.S., Lemire, M., Fillion, M., Guimarães, J.R.D., Lucotte, M., Mergler,
610 D., 2008. Daily mercury intake in fish-eating populations in the Brazilian Amazon. J. Expo.
611 Sci. Environ. Epidemiol. 18, 76–87. https://doi.org/10.1038/sj.jes.7500599

612 Pestana, I.A., Almeida, M.G., Bastos, W.R., Souza, C.M.M., 2019. Total Hg and methylmercury
613 dynamics in a river-floodplain system in the Western Amazon: Influence of seasonality,
614 organic matter and physical and chemical parameters. Sci. Total Environ. 656, 388–399.
615 https://doi.org/10.1016/j.scitotenv.2018.11.388

616 Rabitto, I. da S., Bastos, W.R., Almeida, R., Anjos, A., de Holanda, Í.B.B., Galvão, R.C.F., Neto,
617 F.F., de Menezes, M.L., dos Santos, C.A.M., de Oliveira Ribeiro, C.A., 2011. Mercury and
618 DDT exposure risk to fish-eating human populations in Amazon. Environ. Int. 37, 56–65.
619 https://doi.org/10.1016/j.envint.2010.07.001

620 Ralston, N.V.C., Ralston, C.R., Blackwell, J.L., Raymond, L.J., 2008. Dietary and tissue selenium
621 in relation to methylmercury toxicity. Neurotoxicology 29, 802–811.
622 https://doi.org/10.1016/j.neuro.2008.07.007

623 Rey-Crespo, F., Miranda, M., López-Alonso, M., 2013. Essential trace and toxic element
624 concentrations in organic and conventional milk in NW Spain. Food Chem. Toxicol. 55,
625 513–518. https://doi.org/10.1016/j.fct.2013.01.040

626 Ribeiro, D.R.G., Faccin, H., Molin, T.R.D., de Carvalho, L.M., Amado, L.L., 2017. Metal and
627 metalloid distribution in different environmental compartments of the middle Xingu River
628 in the Amazon, Brazil. Sci. Total Environ. 605–606, 66–74.
629 https://doi.org/10.1016/j.scitotenv.2017.06.143

630 Rosa Leite, L.A., dos Reis Pedreira Filho, W., Kozlowiski de Azevedo, R., Doro Abdallah, V.,
631 2020. Patterns of distribution and accumulation of trace metals in Hysterothylacium sp.
632 (Nematoda), Phyllodistomum sp. (Digenea) and in its fish host Hoplias malabaricus, from
633 two neotropical rivers in southeastern Brazil. Environ. Pollut. 116052.
634 https://doi.org/10.1016/j.envpol.2020.116052

635 Scarpelli, W., 2005. Arsenic in the rivers of the Amazon Basin. Terræ 2, 20–27.

636 Seyler, P.T., Boaventura, G.R., 2001. Trace Elements in the Mainstem Amazon River, in:
637 McClain, M.E., Victoria, R., Richey, J.E. (Eds.), The Biogeochemistry of the Amazon
638 Basin. Oxford University Press, London, pp. 307–327.

639 Silva, S.F. da, Oliveira, D.C., Pereira, J.P.G., Castro, S.P., Costa, B.N.S., Lima, M. de O., 2019.
640 Seasonal variation of mercury in commercial fishes of the Amazon Triple Frontier, Western
641 Amazon Basin. Ecol. Indic. 106, 105549. https://doi.org/10.1016/j.ecolind.2019.105549

642 Soares, B.E., Cabral, G.L., Estrella, F., Caramaschi, É.P., 2017. Two-decade remaining effects of
643 bauxite tailings on the fish taxonomic structure of a clear-water floodplain lake in central
644 Amazon (Batata lake, Pará state, Brazil). Oecologia Aust. 21, 311–322.
645 https://doi.org/10.4257/oeco.2017.2103.08

646 Soares, M.G.M., Menezes, N.A., Junk, W.J., 2006. Adaptations of fish species to oxygen
647 depletion in a central Amazonian floodplain lake. Hydrobiologia 568, 353–367.
648 https://doi.org/10.1007/s10750-006-0207-z

649 Sørmo, E.G., Ciesielski, T.M., Øverjordet, I.B., Lierhagen, S., Eggen, G.S., Berg, T., Jenssen,
650 B.M., 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. Environ.

Sci. Technol. 45, 6561–6566. https://doi.org/10.1021/es200478b

Souza Neto, H.F. de, Pereira, W.V. da S., Dias, Y.N., Souza, E.S. de, Teixeira, R.A., Lima, M.W. de, Ramos, S.J., Amarante, C.B. do, Fernandes, A.R., 2020. Environmental and human health risks of arsenic in gold mining areas in the eastern Amazon. Environ. Pollut. 265, 114969. https://doi.org/10.1016/j.envpol.2020.114969

Squadrone, S., Prearo, M., Brizio, P., Gavinelli, S., Pellegrino, M., Scanzio, T., Guarise, S., Benedetto, A., Abete, M.C., 2013. Heavy metals distribution in muscle, liver, kidney and gill of European catfish (Silurus glanis) from Italian Rivers. Chemosphere 90, 358–365. https://doi.org/10.1016/j.chemosphere.2012.07.028

Varol, M., Sünbül, M.R., 2017. Organochlorine pesticide, antibiotic and heavy metal residues in mussel, crayfish and fish species from a reservoir on the Euphrates River, Turkey. Environ. Pollut. 230, 311–319. https://doi.org/10.1016/j.envpol.2017.06.066

Watanabe, T., Kiron, V., Satoh, S., 1997. Trace minerals in fish nutrition, in: Aquaculture. Elsevier, pp. 185–207. https://doi.org/10.1016/S0044-8486(96)01503-7

Zupo, V., Graber, G., Kamel, S., Plichta, V., Granitzer, S., Gundacker, C., Wittmann, K.J., 2019. Mercury accumulation in freshwater and marine fish from the wild and from aquaculture ponds. Environ. Pollut. 255, 112975. https://doi.org/10.1016/j.envpol.2019.112975

Table 1. Summary of the samples collected

region	Acarí			Tucunaré		
	N	Weight(g)	Length (cm)	N	Weight(g)	Length (cm)
Faro	19	382±44	32,9±1.2	14	496±87	32,6±2.1
Juruti	20	355±25	32,3±0.7	21	556±49	34,3±1,1
Santarém	41	354±12	32,1±0.5	54	540±44	32,6±0,9
Itaituba	21	344±26	31.6±0.7	20	541±98	33,4±2,0
Porto Trombetas	24	335±21	32,8±0.5	24	555±50	36,6±1,0

Table 21. Results of the analytical quality control programme applied in the ICP-MS determination of toxic and trace elements in this study: limits of detection and analysis of certified reference material. Results are expressed as mean± standard deviation.

		CRM DORM-3[a]	
	Detection limit (µg L^{-1})	**Analyzed levels (mg Kg^{-1})**	**Certified levels[b] (mg Kg^{-1})**
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	7.0	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	5.0	48.1 ± 2.2	51.3 ± 3.1

[a] Fish protein DORM- 3, National Research Council, Ottawa, Ontario. [b] In parenthesis only informative values. CRM: certified reference material.

Table 23. Summary of the general linear model used to evaluate the influence of the sampling point (Faro [SP1], Jurutí [SP2], Santarém [SP3], Itaituba [SP4], Porto Trombetas [SP5]) and fish species (Acari and Tucunaré) on toxic and essential trace element accumulation in liver and muscle in this study. Statistically significant effects at *P <0.05* (*), *P<0.01* (**) and *P<0.001* (***), -- not significant.

	Liver			**Muscle**		
	Sampling Point (SP)	**Fish Species (FS)**	**SP*FS**	**Sampling Point (SP)**	**Fish Species (FS)**	**SP*FS**
As	***	***	**	***	***	**
Cd	***	***	**	--	--	--
Hg	***	***	***	***	***	***
Pb	***	**	**	--	--	--
Co	--	***	--	--	--	--
Cr	***	***	***	*	*	--
Cu	***	***	***	--	--	--
Fe	--	***	***	--	--	--
Mn	--	*	--	--	--	--
Mo	--	--	--	--	--	--
Ni	***	***	***	--	*	--
Se	*	***	*	***	--	--
Zn	***	--	*	*	*	--

Figure captions

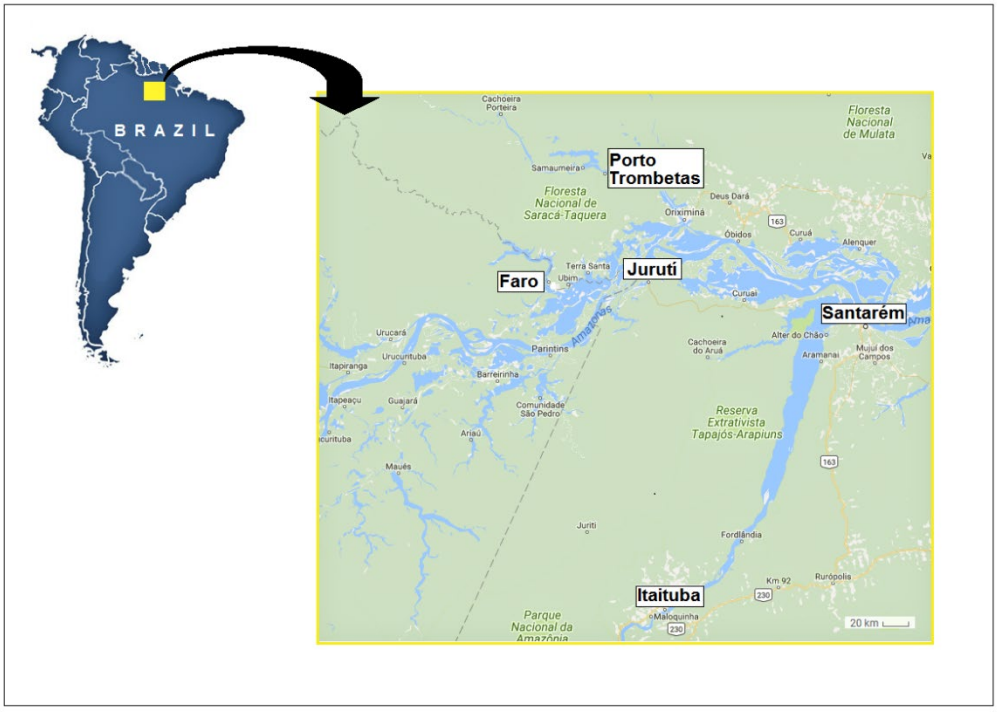


Figure 1. Identification and location of sampling sites in the western part of the state of Pará. 1-Faro, 2-Jurutí, 3-Santarém, 4-Itaituba, 5-Porto Trombetas. The symbols in the map indication gold-mining are not precise geographical location since there are several small scale artisanal mining activity in the region.

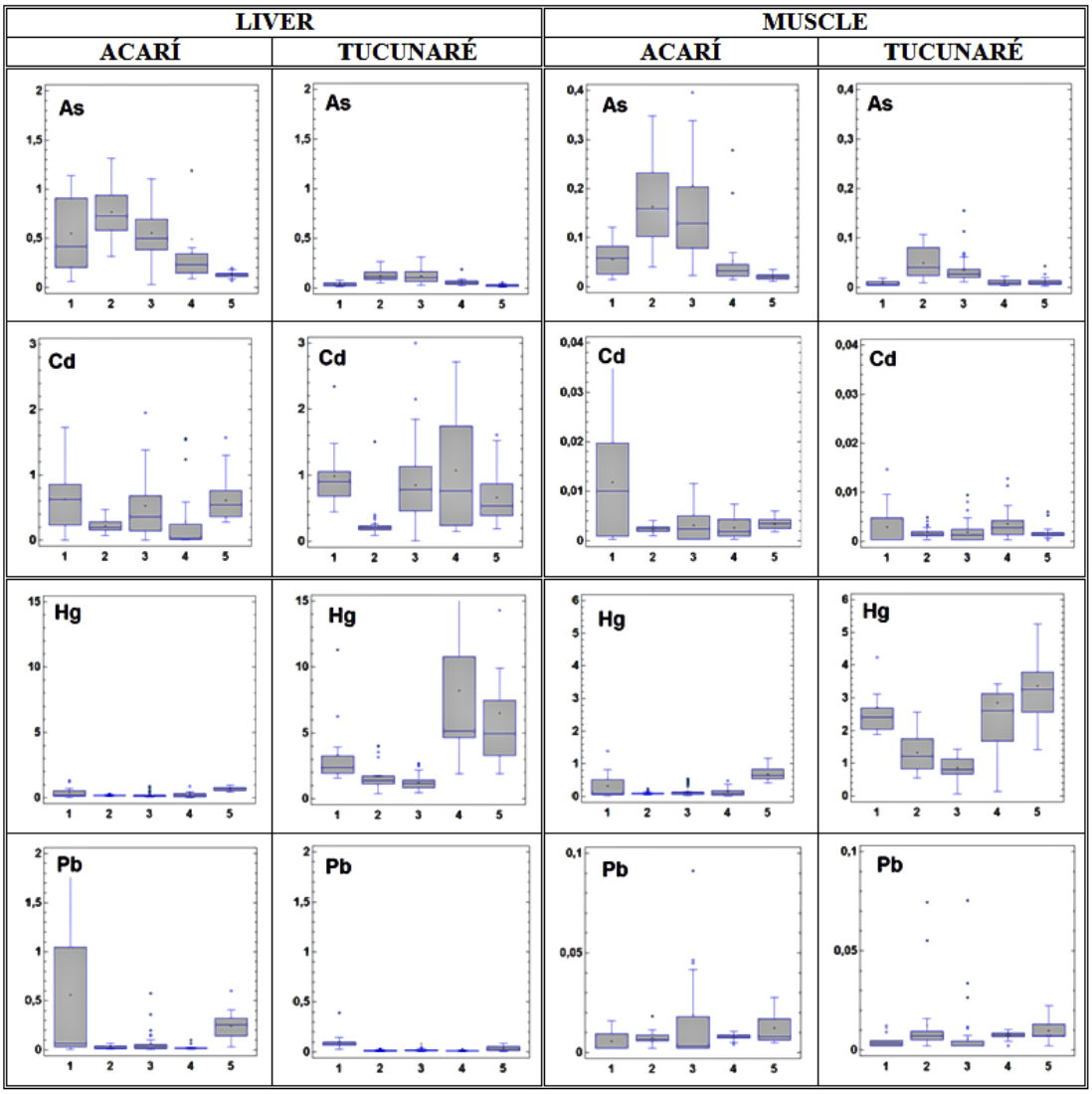
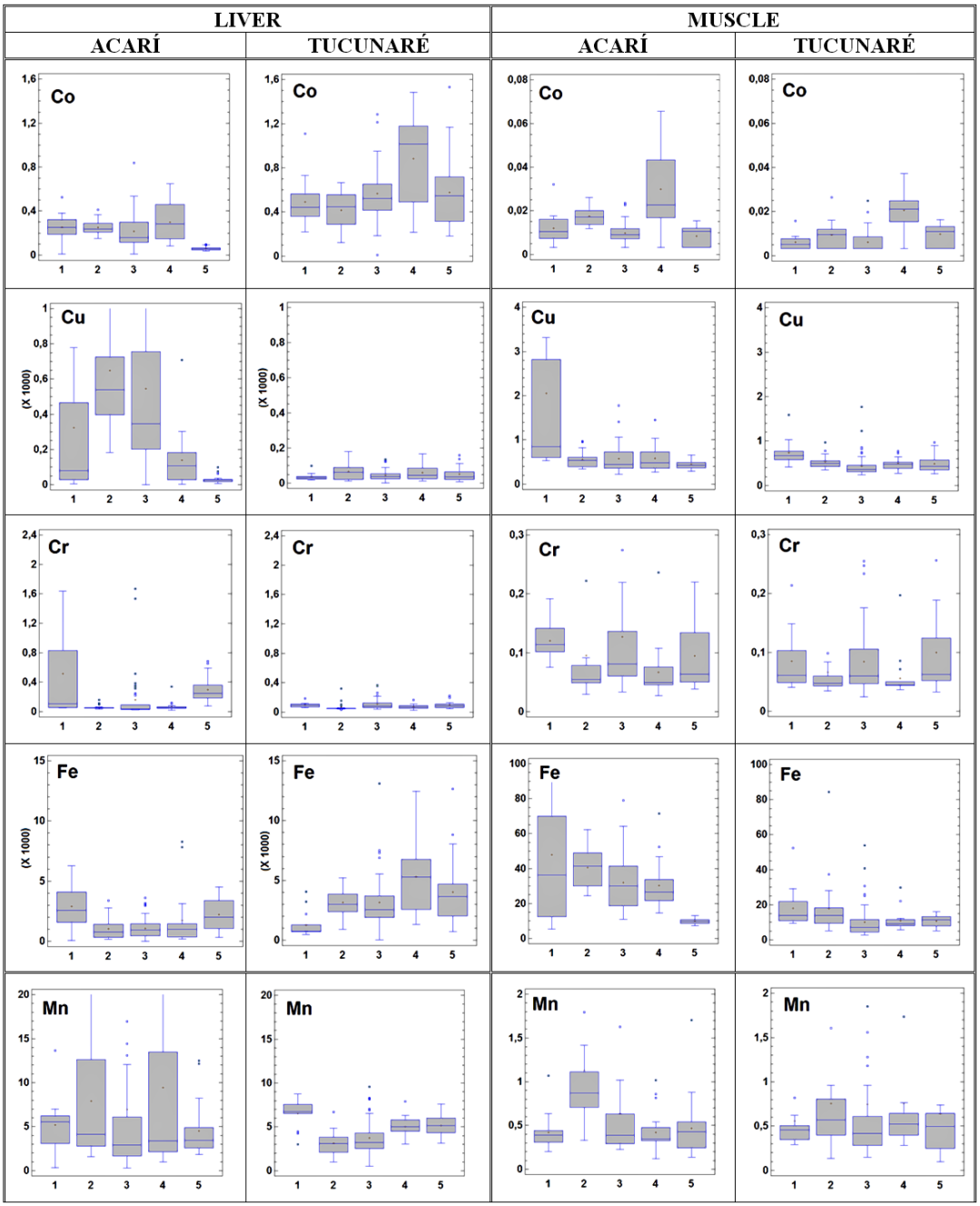


Figure 2. Box-whisker plots of the toxic element concentrations (in mg kg^{-1} dry weight) in the liver and muscle of Acari and Tucunaré for the five sampling sites considered in this study. Sampling site codes: 1: Faro; 2: Jurutí; 3: Santarém; 4: Itaituba; and 5: Porto Trombetas.


LIVER
MUSCLE
ACARÍ
TUCUNARÉ
ACARÍ
TUCUNARÉ
Co
Cu
Cr
Fe
Mn
(X 1000)

701

702

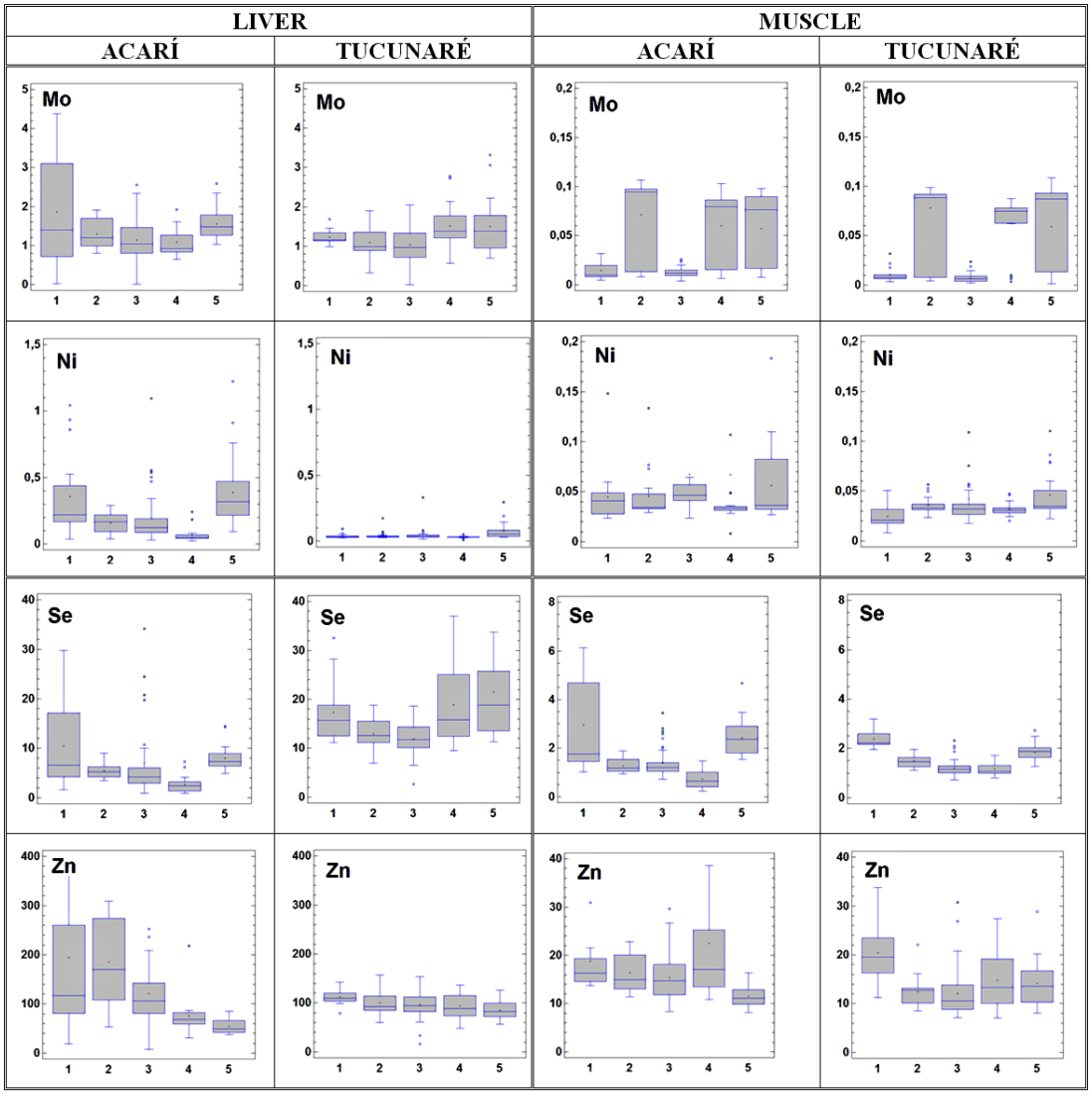


Figure 3. Box-whisker plots of the essential trace element concentrations (in mg kg^{-1} dry weight) in the liver and muscle of Acari and Tucunaré for the five sampling sites considered in this study. Sampling sites codes: 1: Faro; 2: Jurutí; 3: Santarém; 4: Itaituba; and 5: Porto Trombetas. A: plots for Co, Cu, Cr, Fe, Mn. B: plots for Mo, Ni, Se, and Zn.

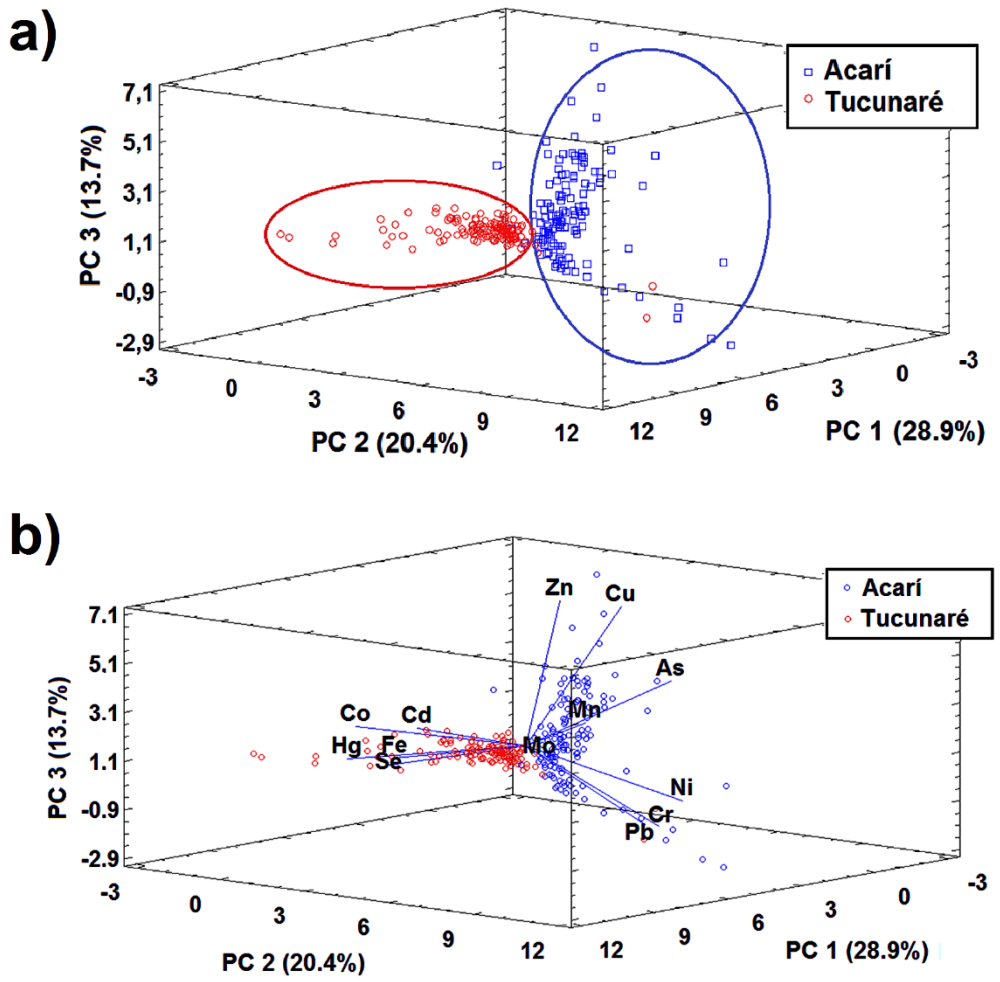


Figure 4. a) PCA-Score plot for the Acari and Tucunaré fish samples of the *Liver.set* in the space of first three principal components (63.0% of total data variance). b) PCA-Biplot for the Acari and Tucunaré fish samples of the *Liver.set* in the space of first three principal components.

717

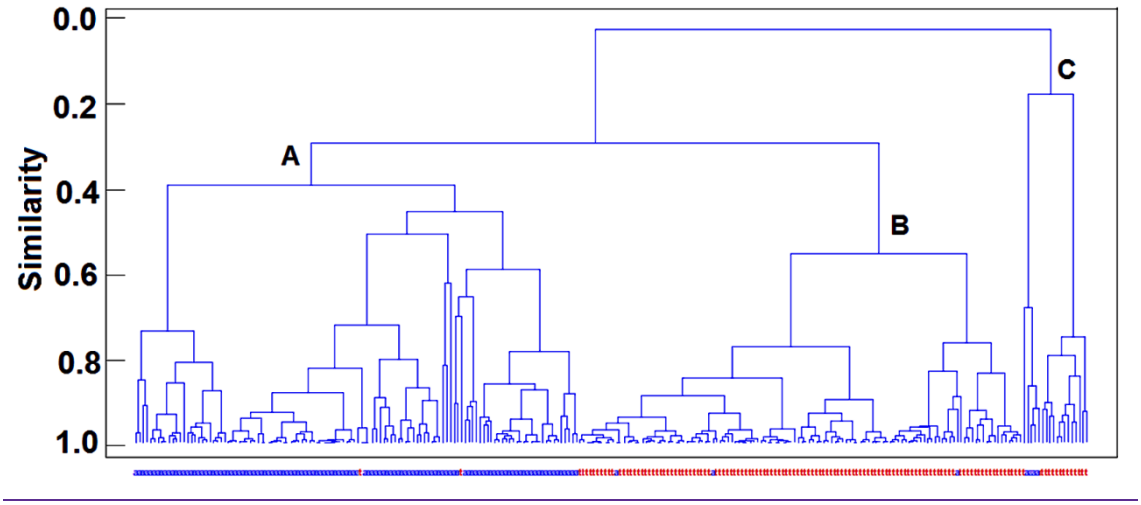


Figure 5. HCA-Dendrogram for the Acari and Tucunaré fish samples of the *Liver.set*. (Codes a: Acari; t: Tucunaré).

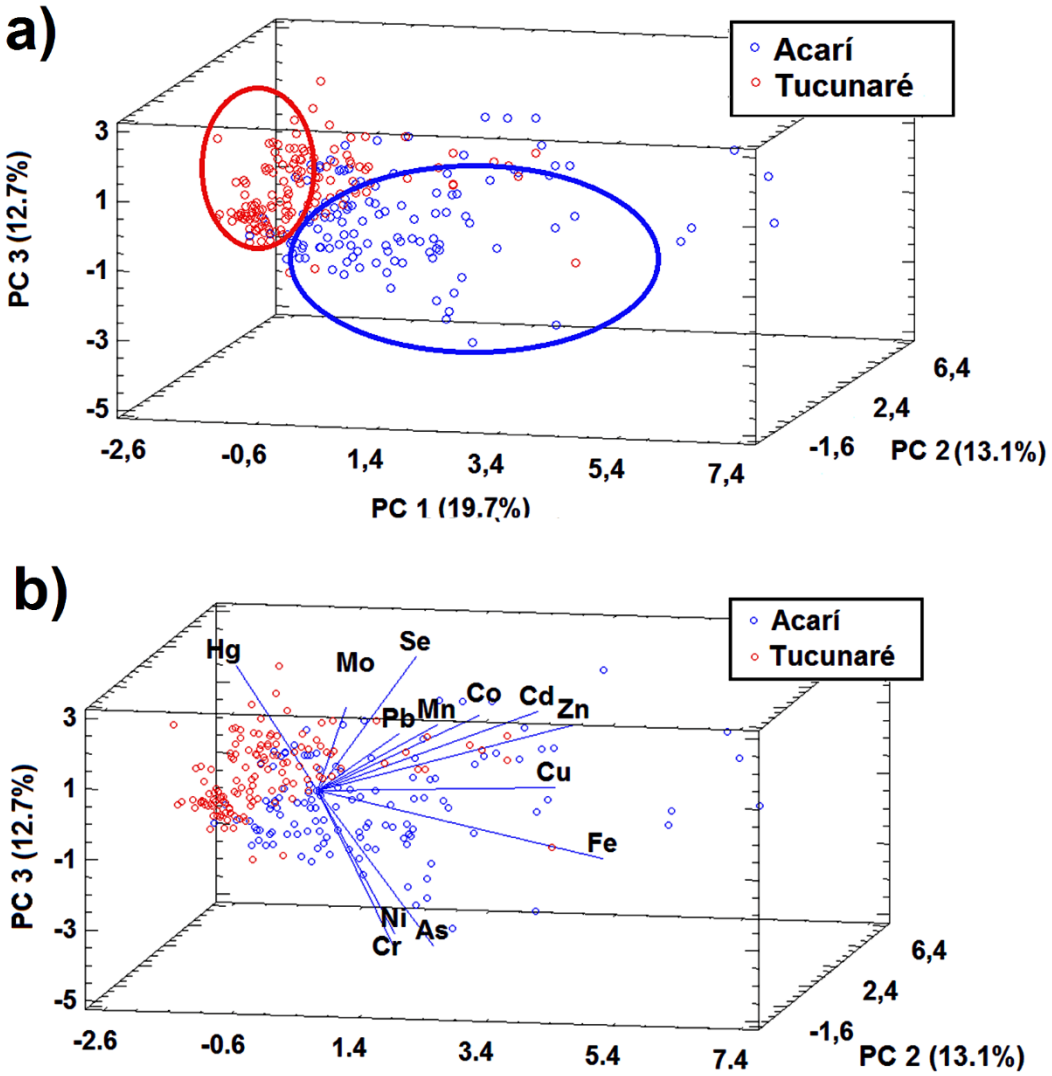


Figure 6. a) PCA-Score plot of the Acari and Tucunaré fish samples of the *Muscle.set* in the space of first three principal components (45.5% of total data variance). b) PCA-Biplot of the Acari and Tucunaré fish samples of the *Muscle.set* in the space of first three principal components.

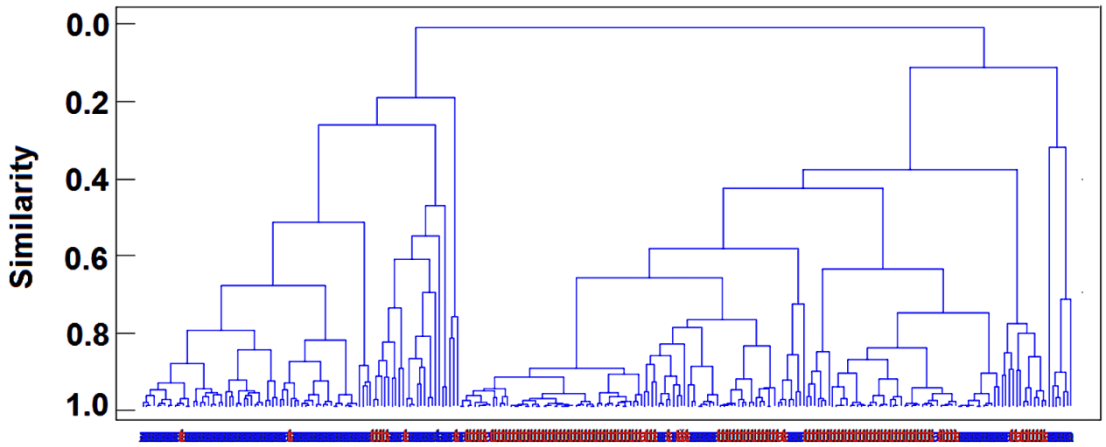


Figure 7. HCA-Dendrogram of the Acari and Tucunaré fish samples of the *Muscle.set*. (Codes a: Acari; t: Tucunaré).

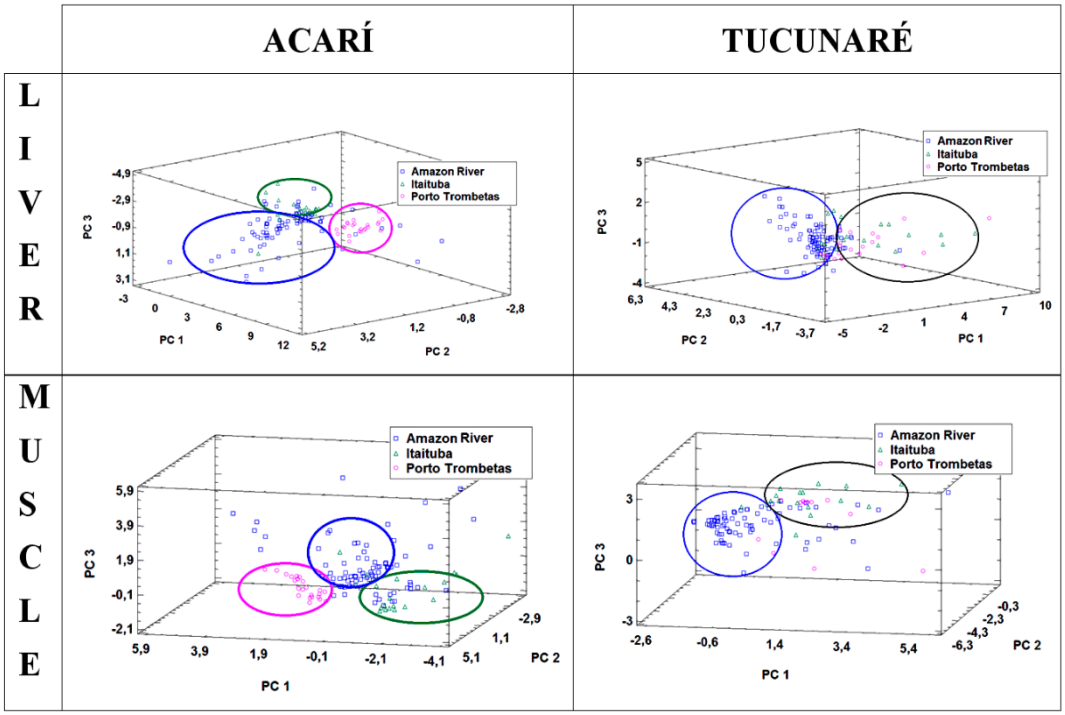


Figure 8. PCA-Score plots for fish samples and target tissues according to the sampling site (Amazon river (1-Faro, 2-Jurutí, 3-Santarém) Itaituba and Porto Trombetas).