



HEAVY METAL DISTRIBUTION IN THE SEDIMENT AND HOPLOSTERNUM LITTORALE FROM MANAUS INDUSTRIAL DISTRICT

G. P. SANTANA

Universidade Federal do Amazonas, Departamento de Química
E-mail: gsantana@ufam.edu.br

RESUMO: *We collected sediment samples in Igarapé do Quarenta (IQ40) to extraction of heavy metal into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual according to Tessier et al. (1979). To the sequential extraction procedure in a 50 mL tube added 1 g of sediment sample. To analyze from heavy metal present into muscle and liver from *Hoplosternum littorale*, we collected twenty species and analyzed heavy metals in *Hoplosternum littorale* and sediment sequential fractions by Flame Absorption Atomic Spectrometry (FAAS). As a tool alternative statistical analysis, we suggest the use of beanplot in the results obtained by sequential fraction. The results show high heavy concentrations in the exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter fractions. The beanplot shows being an important tool to understand the behavior of the heavy metal in sequential fractions present in contaminated sediment. High Zn and Cu concentration found exchangeable fraction explain the high concentration in muscle and live from *Hoplosternum littorale*.*

KEYWORD: Sequential extraction; sediments; heavy metal; FAAS.

1. INTRODUCTION

Studying the ecological risks in polluted rivers is a complicated task, such as heavy metal pollution is widespread by aquatic ecosystem (Smolders et al., 2003). However, the concentration and bioavailability of heavy metal bound to sediments depend on many factors including, redox potential, pH, salinity, dissolved metals species and sediment composition (Duquesne et al., 2006). Accordingly, the sediment forms a trap for heavy metals, and thus, concentrations of heavy metal in the sediment has several orders of magnitude greater than in the overlying water. Metals found in the sediment directly threaten detrital and deposit-feeding benthic organisms, remaining as a long-term source of contamination higher up the food chain (Pulatsü and Topçu, 2015).

The heavy metal encompasses several solid compounds with carbonate, sulfate or sulfur; sink to the bottom accumulated in the sediment. Conceptually, toxicity of an element is strongly dependent on its chemical form in sediment. Additionally, metal concentration in the aqueous phase associated with a sediment depends on the formation of well-defined or poorly soluble compounds; interaction of the dissolved species with solid sediment; and

adsorption or precipitation of the particulate phases (Ure et al., 1995).

Tessier et al. (1979) developed a sequential extraction procedure for the partitioning of heavy metals into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter, and residual. This operational speciation is the principal technique for assessing the availability and predicting disorders or toxicity of heavy metal (Ure, 1991). Despite of the certified reference material (BCR) recommendation for using of sequential metal extraction (Sahuquillo et al., 1999), the literature had recommended the procedure proposed by Tessier et al. (Wojtkowska et al., 2016). The reason for the use of Tessier et al. procedure based on the urgently assessment of heavy metals contamination in sediments, which requires an interpretation about the bioavailability, mobility and toxicity and provide basic information for use and supervision.

The interpretation of the heavy metal level depend on assemble of information of contaminated site. Obviously high heavy concentrations have associated the industrial activities and/or in urbanized regions. In Manaus city, such as the high heavy metal level had reported to the IQ40 in the Manaus Industrial District. The IQ40 stream in the Educandos hydrographic basin (03°07'16.6 "S and 59°59'08.0" W) encompasses an aquatic system formed by 12 small streams. It is possible to find 1.30-4.00 m wide and 0.30-1.6 m deep streams along its 38 km course. IQ40 harbors 250,000 inhabitants as well as more than 400 industrial enterprises showing a high technological index. The electric-electronic sector predominates and is responsible for 55% of its whole production. The Urban and industrial district is responsible by heavy sewage discharges into IQ40.

The high heavy metal level had detected in some ecosystem compartments in IQ40 according the following findings. (Santana, 2015) reported that Zn concentration above established by CETESB (reference value 60 mg kg⁻¹) in exchangeable and residual fraction from Ultisol soil. Valle et al. (2005) reported high Hg concentration (average 108 ng g⁻¹) in Ultisol (fraction < 2000 µm). Ferreira et al. (2012) reported that heavy metal distribution in contaminated water varies according to seasonal period (dry or rainy). Pio et al. (2014) reported the macrophyte *Lemna aequinoctialis* ability in removing heavy metal from water contaminated IQ40. (Santana, 2015) shows the pH influence on bioavailability of heavy metal in contaminated sediments, as well as the Fe, Cu and Zn with sulfides, carbonates and organic fraction association.

Despite IQ40 contamination, it is still possible to find some preserved forest patches within the area and only the fish species *Hoplosternum littorale* survive in this environment. The *Hoplosternum littorale* physiology explains as this fish resist the heavy metal high level in sediment from IQ40 (Affonso et al., 2004). However, Meche et al. (2010) related high levels of Ni and Cr in *Hoplosternum littorale* absorbed of the contaminated Piracicaba River, mainly tannery industry wastewater.

The aim of this study was to propose or use of Beanplot as a tool for analysis of results of heavy metal concentrations obtained from sequential extractions in sediment samples. In addition, establishing a relationship between heavy metal concentration in sediment and *Hoplosternum littorale* that are in a contaminated area as IG40 from the Industrial District of Manaus region.

2. MATERIALS AND METHODS

2.1. Sediment sampling and sequential extraction

We collected about 300 g of sediment (0-10 cm) sediment samples in nine sites (about 27 sediment samples) to polyethylene scoops used exclusively for sampling and storage. The sampling site occurred according to Santana, (2015), shown in the Figure 1. To applied sequential extraction according to Tessier et al. (1979), the samples for a week used an oven at 55 °C and sieved through at 53 mm. To the sequential extraction procedure in a 50 mL tube added 1 g of sediment sample. After exposition to the several reagents under agitation (Table 1), we separated by twice wash with 8 mL of deionized water the exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter fractions from the supernatant by centrifugation at 10,000 rpm for 30 min.. To obtain residual fraction we digested 12 mL of 5:1 mL HF-HClO₄ acid mixture and evaporation to near dryness in the residue organic matter fraction.

2.2. Fish samplings and chemical analyses

To analysis from heavy metal present into muscle and liver from *Hoplosternum littorale* we collected twenty species (Figure 1), being thawed at room temperature and the length and weight recorded. The analysis of fishes samples prepare consisted:

- Dissection with stainless steel scalpels and Teflon forceps on a laminar flow bench;
- Separation of a part of the muscle (skinless dorsal fin) and liver;
- Lyophilization for one week;
- Homogenization of dry tissue;
- Acid digestion of about 0.2 g dried liver and 0.4 g dried muscle by 12 h at room temperature and for 4 h at 100 °C in Teflon beakers.

Table 1 – Operating conditions for Tessier et al. sequential extraction procedures

Fraction	Agitation time (hour)	Added volume (mL)	Reagents
Exchangeable	1	8	1 mol MgCl ₂ pH 7.0
Bound to carbonates	5	8	1 mol NaOAc adjusted to pH 5.0 with HOAc
Bound to Fe-Mnoxides	6	20	0.3 mol Na ₂ S ₂ O ₄ + 0.175 mol Na-citrate + 0.025 mol H-citrate
Bound to organic matter	3	8	3 ml o 0.02 mol HNO ₃ 30% and 5 mL of H ₂ O ₂ adjusted pH 2.0 with HNO ₃

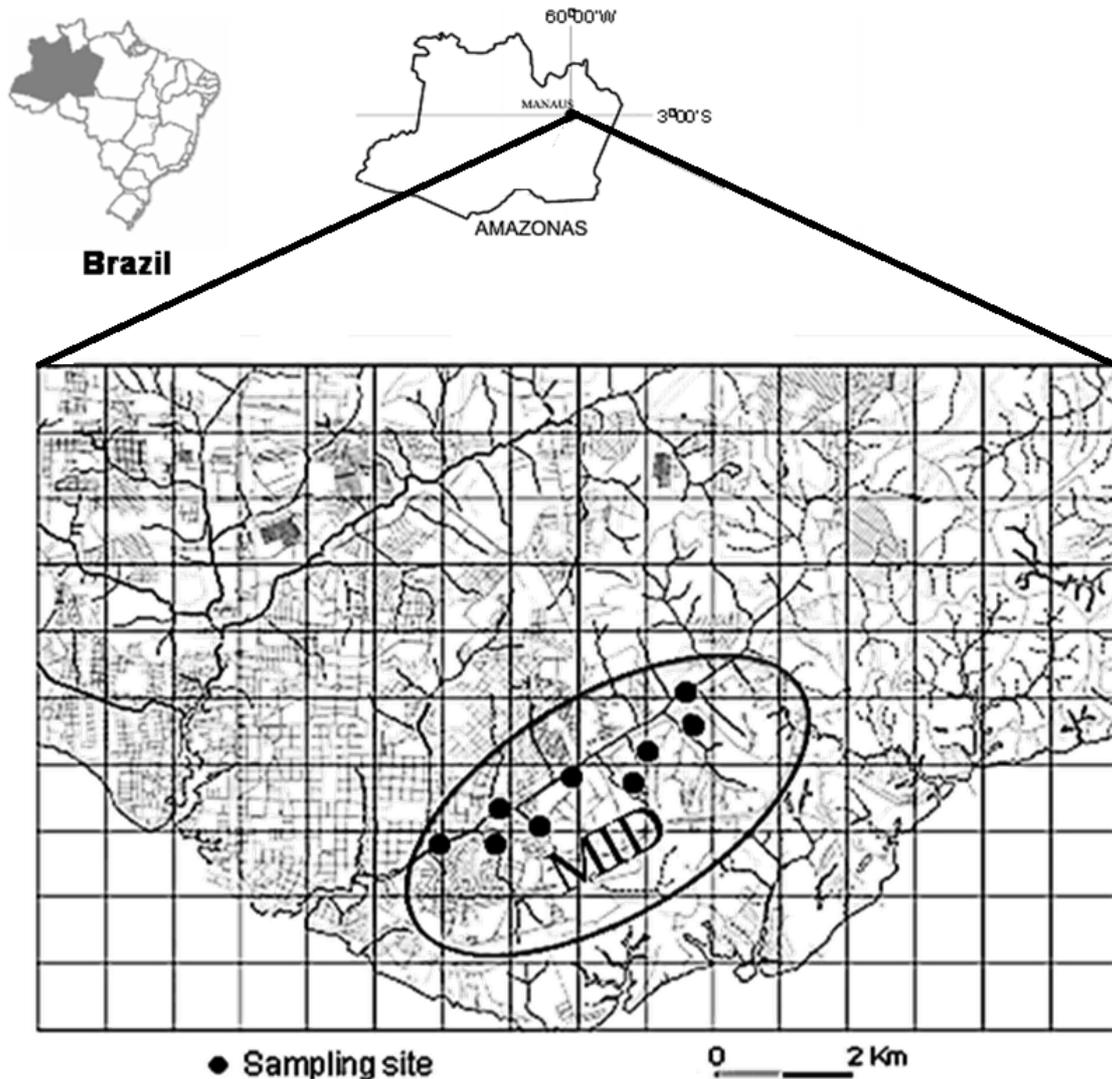


Figure 1- Map showing the sampling sites along the Igarapé do Quarenta stream (Santana, 2015).

2.3. Heavy metal chemical analysis by FAAS

To find the Fe, Zn, Cu, Cr and Pb concentrations present in the extracted and digested solutions used FAAS (Perkin Elmer, model ASS 3300), air/acetylene flame, standard calibration and standard reagent certificates (SpecSol) with concentrations of $1.000 \pm 0.003 \text{ mg g}^{-1}$. For all elements, the readings used calibration curves presenting $r^2 \geq 0.99$. Linear correlation and residual standard deviation (RSD) $< 9\%$ for $n=10$ was the criteria for calculating figure of merit to heavy metal analyses. Our quality control consisted of analytical blanks, certified standard solutions of all elements ready, and analyzed using the

same way and reagents. We calculated the limits of detection (LOD) as under, $LOD (3 \times s/m)$, where s is the standard deviation of 10 measurements of the blank and m is the slope of the calibration graph obtained for each case, the LODs ($\mu\text{g g}^{-1}$); Cr (0.04), Zn (0.005), Cu (0.01), Cr (0.07) and Pb (1.1). The quantification limit found by $(10 \times s/s)$ were Fe (0.90), Zn (0.01), Cu (0.02), Cr (0.07) and Pb (1.1).

The laboratory analytical performance was evaluated through the Certified Reference Material BCR-701 analysis to check accuracy and the recovery percentages obtained for the metals analyzed were in all cases between 80 and 105% (Sahuquillo et al., 1999).

3. RESULTS AND DISCUSSION

The Table 2 shows the mean concentrations with associated standard deviations. Each individual heavy metal displays a wide variation in concentration, as reflected by the large standard deviation values. Highest spread in the heavy metal concentration occur commonly in chemical composition of sediments. As the contaminant distribution in an environmental phase such as sediment has the control of a continuous exchange with other environmental phase such as water and biota (Shea, 1988), these findings affected the mean concentration via the extremely high or low values of the heavy metal concentration. Additionally, Tessier e Campbell (1987) claim that the great variety of solid phases of trace metals have relation with scavenging action, concentration, amorphous character and other associations. It suggests that spatial variation within each sampling sediment site varies because of the metal contamination within Manaus Industrial District region.

A reasoned solution to available these findings is the replacing by the mean and standard deviation by median. Reimann et al. (2005) suggest the boxplot use for identification of extreme values. As a result, the boxplot displays show the heavy metal composition variation in sediment as well as outliers attributed the contamination source in sediments. Boxplot encompasses dependent on the empirical cumulative distribution plots, normal or lognormal. The box length is the interquartile range. Outliers are values between 1.5 and 3 box lengths from the upper or lower edge of the box. Far outliers are values more than 3 box lengths from the edge of the box. To compare univariate data often uses Boxplot and its variants. Boxplots have the disadvantage that they are not easy to explain to non-mathematicians, and that some information is not visible. A beanplot is an alternative variant of the boxplot for visual comparison of univariate data between groups (Potter et al., 2010). As small lines in a one-dimensional scatter plot, the beanplot show the individual observations. It is easy to compare different groups of data in a beanplot and to see if a group has enough observations to make the group interesting from a statistical point of view. In a beanplot observes anomalies, such as bimodal distributions and duplicate measurements. For groups with two subgroups (e.g., male and female), there is a special asymmetric beanplot (Kampstra, 2008).

The Figure 2 shows beanplot for heavy metal sequential extractions a form of a violin plot. This plot combines the standard box plot with a density trace to exploit the information contained in both types of diagrams. The beanplot density alters according to the sequential fraction in two known distributions: bimodal and normal. The bimodal distribution occurs only in the chromium concentrations, mainly bound to Fe-Mn oxides and residual fraction.

The other metals and fraction the data obey the normal distribution with variation between class I, IIA and IIIB (Hintze and Nelson, 1998). Despite of the observed outlier's beanplots show the normal distribution as representing the data. Additionally, the violin plots show outside points and upper adjacent values in all beanplots.

Table 2 – Mean heavy metal concentration values (mg kg^{-1}) from IQ40 sediment analyzed by geochemical fractions

Metal	Mean	DP	Min	Max
	Exchangeable			
Cr	6.38	2.48	ND	10.53
Cu	14.89	14.47	1.08	67.8
Ni	191.34	388.20	ND	1468
Pb	26.34	31.32	6.83	158
Zn	538.88	829.83	19.7	4061
Bound to carbonates				
Cr	5.08	6.00	ND	17.7
Cu	26.19	24.57	ND	110.7
Ni	21.35	34.25	ND	126
Pb	25.63	24.27	ND	128
Zn	119.66	186.83	6.26	771
Bound to iron and manganese oxides				
Cr	95.45	96.33	7.61	401
Cu	52.27	56.34	ND	241
Ni	31.73	48.41	ND	179
Pb	30.85	43.86	ND	162
Zn	163.98	206.51	5.88	665
Bound to organic matter				
Cr	214.48	278.75	ND	1155
Cu	231.41	278.58	27.4	1155
Ni	48.09	39.06	4.15	130
Pb	53.88	42.41	0.98	123
Zn	84.48	108.01	28.7	405
Residual				
Cr	12.87	2.66	8.19	21.1
Cu	2.38	0.50	1.39	3.75
Ni	0.72	0.42	ND	1.56
Pb	2.76	1.59	1.34	9.2
Zn	1.78	1.51	0.34	7.13

Nd = non detected, mean ($n=27$) \pm standard deviation (DP)

The exchangeable and bound to carbonate fractions have not a density trace of a normal distribution corresponding a form of polygon shape. These beanplot suggest that high

variability of the heavy metal concentrations in two sequential fractions. Another hypothesis is a high heavy metal mobility in two sequential fractions because of the physical and chemical environmental conditions. The exchangeable fraction depends on the particle surfaces (e.g. clays, humic acids, metal oxyhydroxides) to adsorb the heavy metal. The exchangeable beanplot suggest low levels of adsorb layer of sediment samples. On the other hand, as in the Manaus region has low carbonate concentration, the acid-extractable fraction, operationally defined as bound to carbonates, has high and varied (Souza and Santana, 2014).

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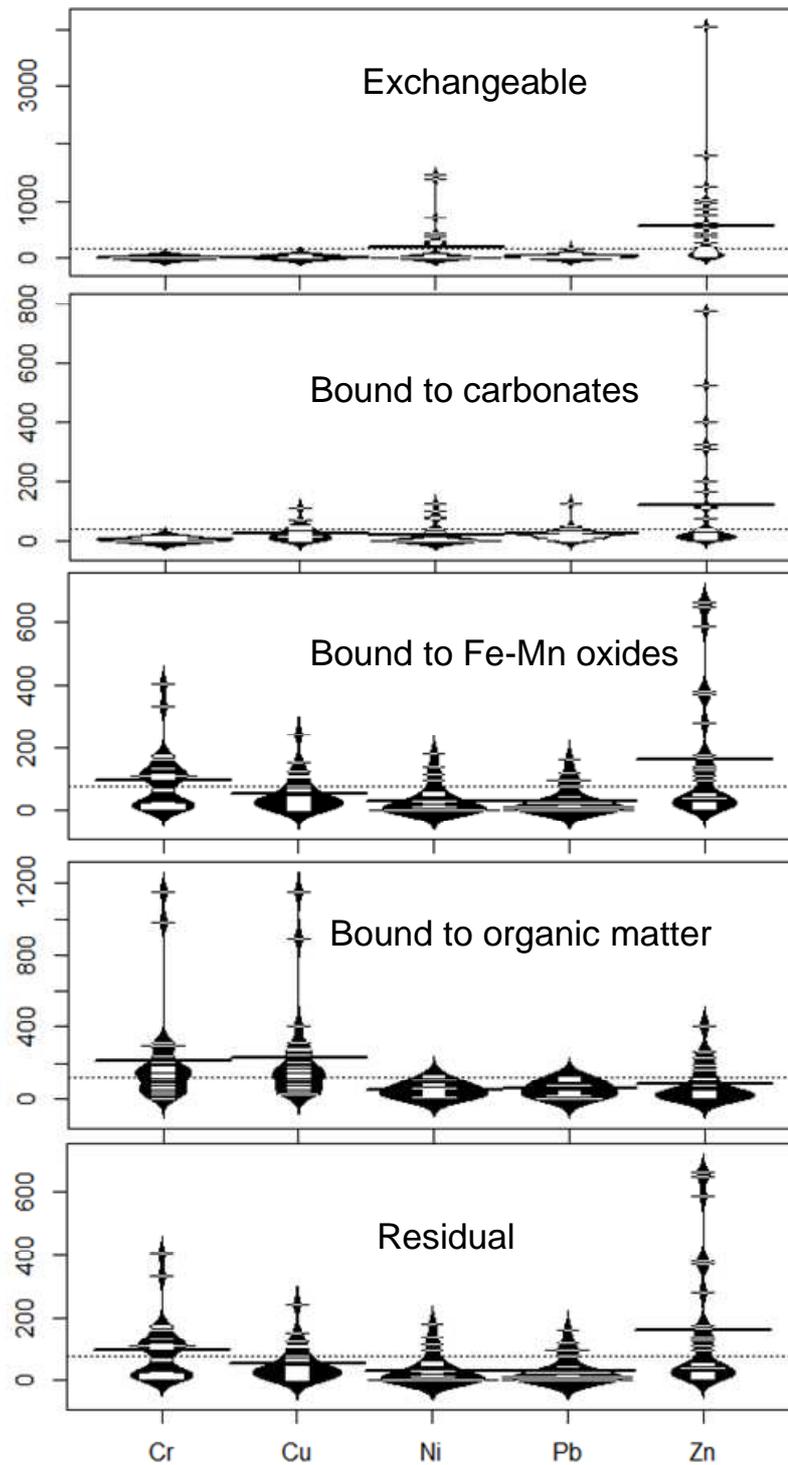


Figure 2 – Beanplot of the heavy metal sequential extraction.

The Figure 3 shows the partitioning patterns for Zn, Cr, Cu, Pb, Ni and Co sediment samples. The lower residual fraction suggests the entering of high heavy metal in the IQ40 water caused by discharging of domestic and industrial effluents. The sum of exchangeable

fraction point out the high mobility of heavy metal. The Ni partitioning indicates its affinity towards the sediment organic matter fraction.

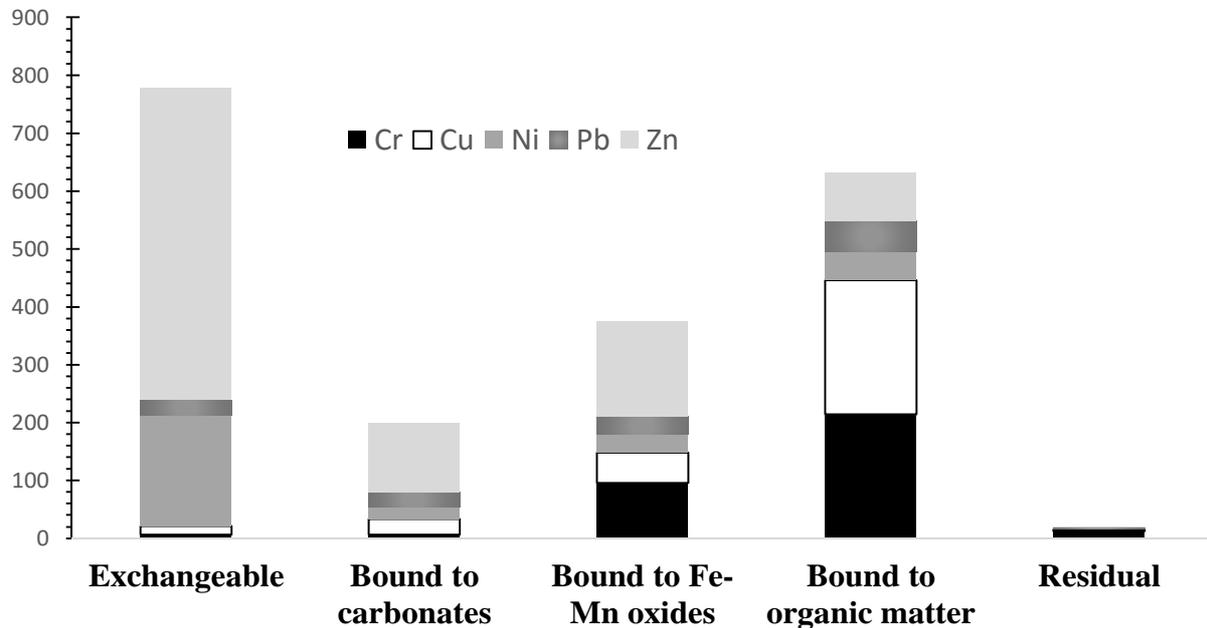


Figure 3 – Sequential extractors of the heavy metal present in sediment samples from Ig40.

The sequential extraction procedure indicates Cr dominant presence in the residual fraction and exchangeable. The sediment-distributed Zn shows it to have a large amount bound to exchangeable. The Pb prominent associated to exchangeable fraction and bound to iron/manganese oxide suggest a higher bioavailability of this metal throughout the IQ40 water. Bound to organic matter and bound to carbonate have high concentration associated to Cu. The metal association with organic matter especially Cu, Pb and Fe with humic substances occurs naturally with organic matter (Sekhar et al., 2003).

Fish study

In general, heavy metal contents present in *Hoplosternum littorale* are above those established by the Brazilian Ministry of Health (MS - MINISTÉRIO DA SAÚDE, 1965). As a result, consuming this fish can contaminate the IQ40 inhabitants with heavy metal (Table 3). Findings show higher Zn, Cu and Cr concentration in liver than muscle. Both liver and muscle have Pb, Ni and Cd concentration close. These results corroborate those found before another author, in which shows that fish generally concentrate metallic ions in their body organism, directly or indirectly through ingested food (Alinnor, 2005). Several fish species accumulates heavy metal mainly in metabolic organs such as liver and others. These organs store metals to remove it by producing metallothioneins (Karadede and Unlü, 2000).

Additionally, Affonso et al. (2004) affirm that *Hoplosternum littorale* is a fish species the most tolerant the high level of the pollution. *Hoplosternum littorale* alters themethemoglobin concentration nor forms sulfhemoglobin with high levels of polluted such as sulfide in the water. It show an alternative metabolic route involving the pyruvate accumulation of products such as lactate.

Table 3 – Heavy metal contents (mg kg^{-1}) absorbed by *Hoplosternum littorale* tissues.

Metal	Muscle	Liver
Zn	58.5±0.1	130.0±0.4
Cu	13.6±0.4	114.3±0.3
Cr	2.6±0.1	51.7±0.2
Pb	17.5±0.7	18.5±0.7
Ni	8.6±0.2	8.6±0.6

Mean of twenty measurements \pm standard deviation

4. CONCLUSION

The results show characterized by high heavy concentrations in the exchangeable, bound to carbonates, bound to iron and manganese oxides, bound to organic matter fractions. Additionally, the standard deviation point out high dispersion of heavy metal concentrations in the region of Manaus Industrial District. The beanplot shows being an important tool to understand the behavior of the heavy metal in sequential fractions present in contaminated sediment. High Zn and Cu concentration found exchangeable fraction explain the high concentration in muscle and live from *Hoplosternum littorale*.

5. Acknowledgements

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DISTRIBUIÇÃO DE METAIS PESADOS NO SEDIMENTO E HOPLOSTERNUM LITTORALE DE DISTRITO INDUSTRIAL DE MANAUS

RESUMO: Foram coletadas amostras de sedimentos no Igarapé do Quarenta (IQ40) para extração de metais pesados em cinco frações: trocável, carbonácea, oxídica, matéria orgânica e residual de acordo com Tessier et al. (1979). Para o processo de extração sequencial num tubo de 50 mL adicionou-se 1 g de amostra de sedimento. Para a análise de metais pesados presente no músculo e no fígado a partir *Hoplosternum littorale* foram coletadas vinte espécies e analisados metais pesados em Tamoatá e frações sequenciais de sedimentos por Espectrometria de Absorção Atômica com Chama (FAAS). Como uma análise estatística ferramenta alternativa, sugerimos o uso de Beanplot para analisar os resultados obtidos das frações sequenciais. Os resultados mostram altas concentrações pesados no trocável, ligados a carbonatos, ligados a óxidos de ferro e manganês, ligados a frações da matéria orgânica. O Beanplot mostrou ser uma ferramenta importante para compreender o comportamento dos metais pesados em frações sequenciais presentes em sedimentos contaminados. As altas concentrações de Zn e Cu na fração trocável explica a alta concentração destes elementos no músculo e fígado do *Hoplosternum littorale*.

PALAVRAS-CHAVE: Extração sequencial; Sedimentos; Metal pesado; FAAS.