

## Deposition flow of Mercury and Selenium in Hair of riverine in habitants of the Amazon, Brazil

Kleber R. F. Faial<sup>1</sup>, Rosivaldo A. Mendes<sup>2</sup>, Adaelson C. Medeiros<sup>3</sup>, Kelson C. F. Faial<sup>4</sup>,  
Simonny C. S. Deus<sup>5</sup>, Antônio Miranda<sup>6</sup>, Mônia Silva<sup>7</sup>, Iracina Jesus<sup>8</sup>, Elisabeth Santos<sup>9</sup>,  
Jandecy Cabral Leite<sup>10</sup>, Ricardo J. A. Deus<sup>11</sup>

<sup>1,2,3,4,6,7,8,9,11</sup> Toxicology Laboratory, Institute Evandro Chagas – Health Ministry in Brazil, Environment Section, 67030-000 Pará, Brazil

<sup>5,11</sup> Environment and Conservation Research Laboratory, Institute of Biological Sciences Federal University of Pará, 66075-110, Brazil

<sup>10</sup> Galileo Institute of Technology and Education of the Amazon (ITEGAM). Av. Joaquim Nabuco N° 1950. CEP: 69005-080. Manaus - AM. Brazil.

Email: [dedeus@ufpa.br](mailto:dedeus@ufpa.br), [kleberfaial@iec.pa.gov.br](mailto:kleberfaial@iec.pa.gov.br)

### ABSTRACT

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This paper provides an estimation of Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels in hair samples of riverine inhabitants belonging to Itaituba municipality - Barreiras community (area impacted by gold mining underground) and Juruti municipality - Tabatinga community (area without mining impact) (masculine and feminine genus), Pará state (Amazon, Brazil), as well as Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels in 12 fish species (carnivores and non-carnivorous), which stand out as the main species consumed by riverside inhabitants, to evaluate a relationship between fish consumption frequency; and Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se concentration, and also to evaluate possible protection mechanisms (or non-protection) to Hg exposure by Se. Results showed that the levels of Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se found in the present study, which were an average of 15.21 µg g<sup>-1</sup> of Hg, 10.48 µg g<sup>-1</sup> of CH<sub>3</sub>Hg<sup>+</sup> and 3.64 µg g<sup>-1</sup> of Se for male inhabitants and an average of 13.89 µg g<sup>-1</sup> of Hg, 10.68 µg g<sup>-1</sup> of CH<sub>3</sub>Hg<sup>+</sup> and 3.60 µg g<sup>-1</sup> of Se for female inhabitants belonging to the Barreira community, were higher than the Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels found in hair samples from inhabitants of Tabatinga community and other gold-producing areas, indicating risk for river side populations along the Tapajós river and for Barreira community. From the information of protective effect (or not) of selenium in hair, given the variation in Se concentration and Hg exposition between the two populations, the molar ratio Hg/Se and Hg millimole revealed a plausible correlation averaged for hair samples of riverine inhabitants belonging to Barreiras (R<sup>2</sup> = 0.86, p<0.05) and Tabatinga (R<sup>2</sup> = 0.57, p<0.05) community. Similarly for the fish, the molar ratio Hg/Se and Hg millimole revealed a plausible correlation averaged for fish samples carnivorous and non-carnivorous (R<sup>2</sup> = 0.97, p<0.05).

**Keywords:** Mercury and Selenium; Hair; Fish; Risk assessment; Barreiras and Tabatinga community

### I. INTRODUCTION

Mercury is a toxic heavy metal and its neurotoxic effect is well known to be caused by Hg exposure in humans [1]. Its biomagnification in aquatic food webs has become a global problem [2-3] as a result of (1) its ability to be transported worldwide via the atmosphere and then be deposited far from its source [4], (2) its microbial methylation to monomethyl mercury (CH<sub>3</sub>Hg<sup>+</sup>) in aquatic sediment [5], and (3) the subsequent bioaccumulation and biomagnification of that CH<sub>3</sub>Hg<sup>+</sup> in aquatic food webs [6]. This combination has resulted in elevated mercury concentrations in fish even in remote areas far from major sources of the contaminant and in regions with low mercury levels in

surface waters and aquatic sediment [7]. These elevated mercury levels in fish adversely affect the health of both wildlife [9] and humans (1-3).

The U.S. Environmental Protection Agency has established a limit for MeHg of 0.1 micrograms of Hg/Kg of body weight per day (0.1µg/Kg/day) [10]. That amount is 4.7 times less than the amount of 0.47µg/Kg/day recommended by the World Health Organization [11]. However, in a sectional study to assess exposure to mercury among 330 Mundurukú Indians in the community known as Sai Cinza in the State of Pará, Amazon, [12] found large levels of fish consumption that were associated with mercury levels in hair tissue, 14.45 µg g<sup>-1</sup> for children ages 7

to 12 years,  $15.70 \mu\text{g g}^{-1}$  for women ages 14 to 44 years and  $14.1 \mu\text{g g}^{-1}$  study involving 251 indigenous women and children who were selected from along the Madeira River and the Kayapó Reserve found an average of mercury in hair of  $8.30 \mu\text{g g}^{-1}$  among women, with 25 of those women presenting levels above  $10.0 \mu\text{g g}^{-1}$ . The authors concluded that population exposure to mercury was related to high fish consumption their main source of protein in the diet.

While it is recognized that fish eating is associated with higher levels of mercury exposure [13-14], fish is also an important source of several beneficial nutrients, including long chain polyunsaturated fatty acids (particularly docosahexaenoic acid and arachidonic acid), choline, iodine and vitamin D, which are all critically important for health and contribute to lower rates of cardiovascular diseases [15]. Besides that, fish also contain high levels of selenium that, among many functions, plays an antioxidant role and may confer some protection against the most toxic form of mercury, methylmercury [16-18].

Several studies suggest that ingestion of selenium (Se) by aquatic organisms up to a certain toxic threshold may counteract the toxic effects of mercury (Hg) [19-22]. In the industrialized region of Sudbury, Canada, [23] showed that total mercury content (THg) was inversely related to Se content in muscle tissue of yellow perch (*Perca flavescens*) and pike (*Sander Vitreus*). The results obtained by the same research group led to the conclusion that Se plays an important role in limiting the assimilation of Hg at the low end of aquatic food chains [24]. Possible mechanisms of fish protection (or non-protection) to Hg exposure by Se have been reviewed by [19], while recent laboratory and field works have been reviewed by [20]. Interaction between Se and Hg in aquatic organisms is real but the true antagonism between these two elements has not yet been clearly shown [25]. More recently [26] proposed that Se concentrations in lake ecosystems could be considered as an indicator of the susceptibility of aquatic organisms to Hg toxicity.

$\mu\text{g g}^{-1}$  for other individuals. Para [14] Barbosa *et al.* (1998), in a

In this regard, the aim of this study was to investigate the levels of Hg,  $\text{CH}_3\text{Hg}^+$ , and Se in hair samples of riverine inhabitants belonging to Itaituba municipality - Barreiras community (area impacted by gold mining underground) and Juruti municipality - Tabatinga community (area without mining impact), Pará state (Amazon, Brazil) as well as analyze the levels Hg,  $\text{CH}_3\text{Hg}^+$ , and Se in samples fish species (carnivorous and non-carnivorous) consumed in the said municipalities, seeking to correlate the possible human exposure with territorial occupation and/or fish consumption in areas with anthropic and non-anthropoc action.

## II. MATERIALS AND METHODS

### II.1 STUDY AREA

This study is limited in Itaituba municipality - Barreiras community (area impacted by gold mining underground) and Juruti municipality - Tabatinga community (area without mining impact), Pará state (Amazon, Brazil) (Figure 1). The Itaituba municipality - Barreiras community is located on the banks of the Tapajós river, with geographical coordinates at S  $04^{\circ}05'45.9''$  and W  $55^{\circ}41'17.9''$ , southwestern State of Pará, Brazil. The economic structure in the municipality is set up around mineral production, agriculture and fishing, although placer gold mining is the principal base [27-29]. Access to the Barreiras community from Itaituba is mainly by river along the Tapajós River and by land via a municipal road with an extension of 70 km. The Juruti municipality - Tabatinga community is situated on the right bank of the Amazon river, in a  $8.342 \text{ km}^2$  area in the state of Para, Brazil. The municipal seat has the following geographical coordinates: S  $02^{\circ}09'09''$  and W  $56^{\circ}05'42''$ .

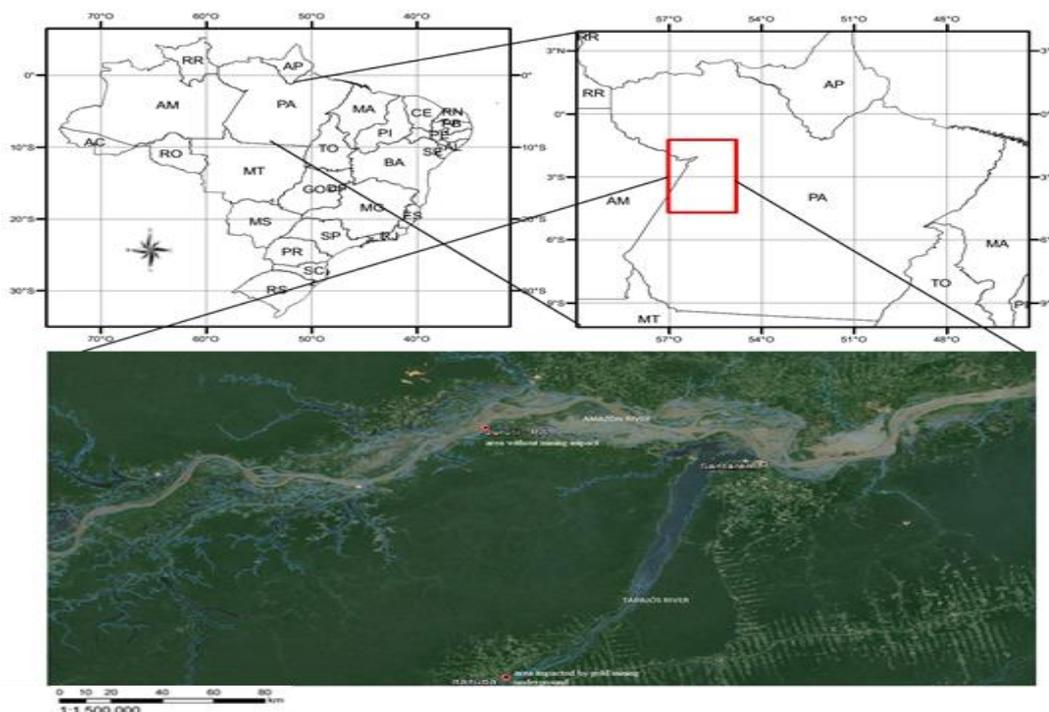


Figure 1: Location of sampling sites (Itaituba municipality - Barreiras community (area impacted by gold mining underground) and Juruti municipality - Tabatinga community (area without mining impact)). The region delimitate for rectangle correspond at Tapajós basin area.

## II.2 STUDIED POPULATION

Hair is made up primarily of a protein called Keratin. This protein includes a large amount of the sulfur-containing amino acid cysteine. Many of these cysteines are involved in disulfide bonds, which bind the individual keratin strands together and give hair its strength. Mercury has a great affinity for sulfur and binds to it avidly. As the hair is actively growing in the hair follicle, mercury in the blood can diffuse into the tissues and bind to the growing hair shaft [30]. The assumption that mercury concentration in hair is proportional to the amount of mercury in blood, which reflects the body load of the metal, has been extensively demonstrated [31]. For this reason, the mercury analysis in hair is widely used for monitoring the intake of Hg in exposed people [32]. Thus, human hair has been considered as a useful of mercury accumulation bioindicator (Hg) [33-34].

The riverine inhabitants belonging to Barreiras community (area impacted by gold mining underground) and Tabatinga community (area without mining impact) are peoples of high socio-economic deprivation, recruited between January 2010 and December 2012, and living for at least the 5 last years in the same location, whose daily diet consists of fish from the rivers and streams that surround them. So around 10 g of hair tissue were collected from 262 individuals, of whom 141 riverine inhabitants belonging to Barreiras community (83 were women and 58 men) and 121 riverine inhabitants belonging to Tabatinga community (73 were women and 48 men), with prior consent from all participants. In the case of children, authorization was granted by parents or guardians. The collection was done from the nape region in the form of locks, at a distance of around 1 cm from the scalp with stainless steel scissors and hair was placed in white envelopes that were duly identified and kept at room temperature [35-37]. In the laboratory they were washed with a neutral detergent and water solution, followed by washing with acetone and deionized water, dried under reduced pressure (vacuum dryer) or to room temperature protected from external contamination and transferred to amber-colored or transparent glass flasks 4 cm high by 2 cm in diameter. Finally, they were cut with stainless steel pink scissors, a procedure for reducing volume and facilitating the weighing process [35-37]. The study was performed in accordance with Brazilian norms for research involving human beings and approved by the ethics committee [38].

## II.3 BIOLOGICAL SPECIES

Fish were captured by local professional fishermen using nets in locations of the Tapajós river (Itaituba municipality - Barreiras community) and Amazon river (Juruti municipality - Tabatinga community). All of these species are commonly found along the Rivers, have a good market price and a significant commercial value. The manipulations of the collected fish species were: species identification, measurements of total length (cm) and weight (g) and removal of a sample of the dorsal muscle tissue without skin and bones with stainless steel scalpels. All samples were double-wrapped in clean PE bags, frozen at  $-20^{\circ}\text{C}$  and shipped immediately to the Institute Evandro Chagas - Health Ministry in Brazil, Environment Section, and analysed immediately by Akagi method [35-36]. In total 241 specimens of fish samples. The samples examined were divided into two groups depending on the feeding habits: carnivorous and non-carnivorous species. The carnivorous species: Filhote

(*Brachyplatystoma filamentosum*), Pescada Branca (*Plagisocion squamosissimus*), Piranambu (*Calophysus macropterus*), Tucunaré (*Cichla sp.*), Apapa (*Pellona castelnaeana*), Piranha (*Pygocentrus piraya*), Surubim (*Pseudoplatystoma fasciatum*) and non-carnivorous species: Aracu (*Leporinus sp.*), Caratinga (*Eugerus brasiliensis*), Acari (*Hypostomus affinis*), Pacu (*Mylossoma spp*), Curimatã (*Prochilodus scrofa*). The first group represented by seven carnivorous species amounted to 129 samples and for second group represented by five non-carnivorous species amounted to 112 samples.

## II.4 ANALYTICAL PROCEDURE

The glassware used in the analytical procedures was previously kept in a solution of 10%  $\text{HNO}_3$  for 24 h, and washed  $\text{KMnO}_4$  in a solution of  $\text{H}_2\text{SO}_4$  1N and  $\text{NH}_2\text{OH.HCl}$  10%, later in running water and distilled water, and dried in an oven to a temperature of approximately  $35^{\circ}\text{C}$  [35-37][39].

## II.5 MERCURY ANALYSIS

For determining Hg and  $\text{CH}_3\text{Hg}^+$ , the hair samples were submitted to analytical procedures from the method proposed by [35-36] and summarized as follows. For Hg, a mass of approximately 10mg was weighed in 50 mL digestions flasks. Next, 2mL of  $\text{HNO}_3$ ,  $\text{HClO}_4$  (1:1), 5 mL of  $\text{H}_2\text{SO}_4$  and 1 mL of deionized  $\text{H}_2\text{O}$  were added. After homogenization the flasks were placed on a hot plate at a temperature of  $220^{\circ}\text{C}$  for 20 minutes; the solutions were cooled to room temperature and transferred to 50 mL volumetric balloons and later compared with deionized water. Total Hg analyses were performed using Atomic Absorption Spectrophotometry with a cold vapor generation system, Model Analyzer Mercury Hg - 201, determinations of total mercury were registered through a registrar model 3102111, manufacturer YOKOGAWA.  $\text{CH}_3\text{Hg}^+$  was determined using the Gas Chromatograph (GC) method, with an electron capture detector, described as follows. Hair tissue of between 10 a 20 mg was weighed directly in 10 mL tubes with Teflon seal caps, to which were then added 2 drops of ethanol and 5 mL de Hal 2N. The solution was slowly heated at  $100^{\circ}\text{C}$  in *bain-marie* for 5 minutes, cooled to warm temperature and centrifuged for 3 minutes at 100rpm. Next, 1 mL of the HCl solution was extracted and transferred to 10 mL test tubes, the lower layer was discarded and a gas chromatography reading was performed [39]. However,  $\text{CH}_3\text{Hg}^+$  concentration to Juruti municipality it was not possible to analyze due to the low amount of sample obtained.

For determining Hg in fishes samples were submitted to analytical procedures from the method proposed by [35-36] and summarized as follows. Were weighed approximately 0.5 g - 1 g in a 50 mL volumetric flask, and the following reagents added: 2 mL of  $\text{HNO}_3$  -  $\text{HClO}_4$  (1:1), 5 mL of  $\text{H}_2\text{SO}_4$  and 1 mL of Milli-Q  $\text{H}_2\text{O}$ . The volumetric flasks containing the samples were left for a period of 12 hours, which were subsequently placed on a heating plate at a temperature of  $220^{\circ}\text{C}$  for 20 minutes time. After cooling to room temperature, the flasks were measured volume of 50 ml with Milli-Q water and mixed. Mercury is available in solution in ionic form. Total Hg analyses were performed using Atomic Absorption Spectrophotometry with a cold vapor generation system, Model Analyzer Mercury Hg - 201, determinations of total mercury were registered through a registrar model 3102111, manufacturer YOKOGAWA [39].

$\text{CH}_3\text{Hg}^+$  was determined using the Gas Chromatograph (GC) method, with an electron capture detector [39].

In this study of certified reference materials for analytical quality control (DORM 2), intercalibration exercises with Hg Analysis Laboratory of the National Institute for Minamata Disease (NIMD-Japan) and the Laboratory of the University of Quebec in Montreal were used (UQAM) in Canada beyond the Laboratory of Biochemistry, Federal University of Rondônia and the Laboratory of Food Analysis, Department of Pharmacy, USP. In fish, a satisfactory agreement between the analytical results ( $2.15 \pm 0.06 \mu\text{g/g}$  dry weight) and the certified values ( $2.91 \pm 0.15 \mu\text{g/g}$  dry weight) was obtained [39].

The results of the determining Hg and  $\text{CH}_3\text{Hg}^+$  concentrations in hair samples and fish samples, along with the epidemiological questionnaire with information on identification and nutritional habits were assessed by means of statistical treatment using Minitab Release 15 for Windows computer software, Revised Printing, inc. 2007, printed in the USA. All of the data and the concentrations of Hg and MeHg, are presented in terms of average  $\pm$  standard deviation and the level of significance was  $<0.05$ . Another important factor in this work was the implementation of population sub-division in terms of Age and Genus: Age (participants were divided into 14 groups: 0 to 2 years, 3 to 5 years, 6 to 10 years, 11 to 15 years, 16 to 20 years, 21 to 25 years, 26 to 30 years, 31 to 35 years, 36 to 40 years, 41 to 45 years, 46 to 50 years, 51 to 55 years, 56 to 60 years and above 60 years), Genus (Masculine and Feminine) and Fish Consumption Frequency (Times/Week).

## II.6 SELENIUM ANALYSIS

For determination of Se (Selenium) in capillary tissue was weighed about 10 to 20 mg in a test tube then are added 3 ml of 65%  $\text{HNO}_3$ , leaving at rest for  $\pm 12$  hours, and samples subsequently taken to a digestion block a temperature of up to  $100^\circ\text{C}$ , where a range of 15 to 15 minutes is homogenized and removed when the solution is clear. After removal of the digester block, the tubes are cooled at room temperature and then adding 0.3 ml of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), digestion being carried back to block for 30 min. to  $100^\circ\text{C}$ . The test tubes are thereafter removed from the digestion block and cooled to room

temperature and measured for a final volume of 10 ml using MilliQ water. If the analyzes were performed at the atomic absorption spectrometer coupled with graphite furnace (GF-AAS), Varian, AA220Z model, equipped with Zeeman broker and hollow cathode lamp elementary mono Selenium.

The method used for the determination of Se (Selenium) in fish was advocated by Dr. Andreas Martens, Institut für Anorganische Chemie und Analytische, Technische Universität Braunschweig, Braunschweig, Germany and modified in Institute Evandro Chagas. The method consists of weighing (0.3 - 0.5 g) of fish sample into test tubes, and then added 5 mL  $\text{HNO}_3$  of 65% and left to stand for 12 hours, and subsequently brought to a digestion block of samples a temperature of  $150^\circ\text{C}$ , where a range of 10 to 10 minutes is homogenized and removed when the solution is clear. After removal of the digester block, the tubes are cooled at room temperature and then adding 0.3 mL of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and taken back to the block digest for 30 min. to  $150^\circ\text{C}$ , and thereafter cooling the samples to room temperature, added 5 mL  $\text{HCl}$  1.2 N and placed back into the digestion block at a temperature in the range of  $100 - 120^\circ\text{C}$  for 30 minutes. The test tubes are taken after this period of digestion block and cooled to room temperature and measured for a final volume of 20 mL using MilliQ water. Analyzes of Se were performed at the Atomic Absorption Spectrometer coupled to graphite furnace (GFAAS- SPECTRAA 220Z) [39].

## III. RESULTS AND DISCUSSION

### III.1 MERCURY AND SELENIUM IN HUMAN HAIR, POPULATION OCCUPATION AND FISH CONSUMPTION

All of the values for Hg,  $\text{CH}_3\text{Hg}^+$ , Se and fish consumption frequency for the pre-established age intervals for the riverine inhabitants belonging to Itaituba municipality - Barreiras community (area impacted by gold mining underground) and Juruti municipality - Tabatinga community (area without mining impact) for the masculine and feminine Genus are described in Table 1 and Table 2, respectively. Thus, 28 groups of age classification were considered for the inhabitants, with 14 groups of the masculine Genus and 14 groups of the feminine Genus being considered.

Table 1: Results of Hg, CH<sub>3</sub>Hg<sup>+</sup>, Se concentration in hair samples and fish consume frequency, for age band; of people residents in Itaituba municipality - Barreiras community (area impacted by gold mining underground), belonging to the Tapajós basin, Pará state (Amazon, Brazil).

Age band (years)	N	<sup>a</sup> Fish consume frequency (time week <sup>-1</sup> )	<sup>a</sup> Hg (µg g <sup>-1</sup> )	<sup>a</sup> CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> )	<sup>a</sup> Se (µg g <sup>-1</sup> )	Hg (µg g <sup>-1</sup> ) Min-Max	CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) Min-Max	Se (µg g <sup>-1</sup> ) Min-Max
0 - 2 (M)	6	0 ± 0	6.85 ± 2.83	5.31 ± 2.21	3.38 ± 1.97	4.14 - 9.79	3.22 - 7.63	0.98 - 4.91
3 - 5 (M)	6	7 ± 1	19.57 ± 4.11	15.55 ± 3.53	3.73 ± 0.80	16.01 - 23.80	12.05 - 19.46	2.34 - 4.57
6 - 10 (M)	10	10 ± 1	18.58 ± 5.45	14.81 ± 4.13	3.34 ± 1.00	12.59 - 24.93	10.43 - 19.45	1.69 - 4.68
11 - 15 (M)	6	15 ± 1	13.08 ± 3.67	9.95 ± 2.98	4.10 ± 0.49	5.83 - 15.57	4.08 - 12.11	3.58 - 4.72
16 - 20 (M)	7	20 ± 2	20.87 ± 3.18	16.33 ± 3.12	4.14 ± 0.79	17.82 - 24.16	13.35 - 19.57	2.47 - 4.81
21 - 25 (M)	4	20 ± 1	15.52 ± 5.31	11.85 ± 4.47	3.69 ± 0.70	9.42 - 20.09	6.61 - 15.67	2.81 - 4.53
26 - 30 (M)	-	-	-	-	-	-	-	-
31 - 35 (M)	3	20 ± 2	9.69 ± 1.61	7.68 ± 1.54	3.46 ± 1.01	8.55 - 10.83	6.59 - 8.77	2.39 - 4.41
36 - 40 (M)	5	18 ± 1	15.29 ± 3.45	11.89 ± 3.05	3.68 ± 0.97	10.92 - 20.03	8.41 - 16.42	2.68 - 4.82
41 - 45 (M)	1	19 ± 0	14.81 ± 0.00	11.7 ± 0.00	3.47 ± 0.00	14.81 - 14.81	11.7 - 11.70	3.47 - 3.47
46 - 50 (M)	1	19 ± 0	2.07 ± 0.00	1.49 ± 0.00	1.58 ± 0.00	2.07 - 2.07	1.49 - 1.49	1.58 - 1.58
51 - 55 (M)	1	19 ± 0	13.89 ± 0.00	10.97 ± 0.00	3.47 ± 0.00	13.89 - 13.89	10.97 - 10.97	3.47 - 3.47
56 - 60 (M)	3	20 ± 1	14.08 ± 0.11	11.02 ± 0.02	3.51 ± 1.10	14.00 - 14.16	11.00 - 11.03	2.39 - 4.58
> 60 (M)	5	18 ± 1	14.57 ± 3.92	11.51 ± 3.69	3.81 ± 0.72	12.04 - 21.47	9.04 - 18.04	2.89 - 4.81
0 - 2 (F)	4	0 ± 0	10.38 ± 2.34	7.96 ± 2.15	3.06 ± 0.68	8.25 - 12.89	5.89 - 10.18	2.07 - 3.64
3 - 5 (F)	7	7 ± 2	15.83 ± 2.92	12.52 ± 1.73	3.59 ± 0.67	12.20 - 19.29	10.21 - 14.27	2.84 - 4.78
6 - 10 (F)	7	10 ± 2	15.84 ± 5.35	12.12 ± 3.91	3.11 ± 1.03	11.46 - 23.21	8.48 - 17.15	1.67 - 4.62
11 - 15 (F)	8	14 ± 1	13.12 ± 3.82	10.13 ± 3.02	3.47 ± 0.63	8.41 - 21.71	6.81 - 16.93	2.73 - 4.61
16 - 20 (F)	5	20 ± 1	16.74 ± 4.60	12.54 ± 3.28	4.01 ± 0.77	13.52 - 22.01	10.14 - 16.28	3.18 - 5.14
21 - 25 (F)	14	20 ± 3	11.22 ± 3.79	8.67 ± 2.89	3.96 ± 0.64	6.33 - 17.66	4.94 - 13.24	2.97 - 4.76
26 - 30 (F)	7	20 ± 0	7.16 ± 2.69	5.76 ± 2.45	3.02 ± 1.16	4.84 - 10.6	3.73 - 9.10	1.57 - 4.59
31 - 35 (F)	8	20 ± 0	13.52 ± 4.67	10.44 ± 3.75	3.92 ± 0.55	4.95 - 20.78	3.85 - 16.21	3.18 - 4.75
36 - 40 (F)	3	16 ± 1	11.09 ± 4.27	8.91 ± 3.89	3.69 ± 0.46	7.30 - 15.73	5.55 - 13.18	3.41 - 4.22
41 - 45 (F)	3	16 ± 0	18.62 ± 6.21	10.49 ± 5.27	3.53 ± 1.12	14.69 - 23.02	10.96 - 18.42	2.38 - 4.61
46 - 50 (F)	3	16 ± 0	10.27 ± 4.13	7.78 ± 3.16	3.34 ± 1.10	7.31 - 14.98	5.92 - 11.43	2.18 - 4.37
51 - 55 (F)	4	16 ± 0	17.02 ± 2.12	13.89 ± 2.11	2.81 ± 1.30	15.52 - 18.52	12.40 - 15.38	1.26 - 4.38
56 - 60 (F)	1	20 ± 0	14.29 ± 0.00	11.02 ± 0.00	3.74 ± 0.00	14.29 - 14.29	11.02 - 11.02	3.74 - 3.74
> 60 (F)	9	20 ± 2	20.39 ± 4.65	16.13 ± 4.01	4.13 ± 0.84	14.60 - 27.02	11.54 - 22.35	2.39 - 4.78

N: Individual number; M: Masculine genus; F: Feminine genus; <sup>a</sup>Mean and standard deviation; (-) individual not.

Table 2: Results of Hg, CH<sub>3</sub>Hg<sup>+</sup>, Se concentration in hair samples and fish consume frequency, for age band; of people residents in Juruti municipality - Tabatinga community (area without mining impact), belonging to the Tapajós basin, Pará state (Amazon, Brazil).

Age band (years)	N	<sup>a</sup> Fish consume frequency (time week <sup>-1</sup> )	<sup>a</sup> Hg (µg g <sup>-1</sup> )	<sup>a</sup> CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> )	<sup>a</sup> Se (µg g <sup>-1</sup> )	Hg (µg g <sup>-1</sup> ) Min-Max	CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) Min-Max	Se (µg g <sup>-1</sup> ) Min-Max
0 - 2 (M)	7	1 ± 1	1.23 ± 0.51	*	1.01 ± 0.40	0.49 - 2.07	*	0.57 - 1.69
3 - 5 (M)	1	2 ± 0	0.78 ± 0.00	*	1.34 ± 0.00	0.78 - 0.78	*	1.34 - 1.34
6 - 10 (M)	4	2 ± 1	1.34 ± 0.37	*	0.96 ± 0.32	1.03 - 1.84	*	0.63 - 1.40
11 - 15 (M)	6	3 ± 1	2.65 ± 0.96	*	1.52 ± 0.61	1.10 - 4.10	*	1.03 - 2.67
16 - 20 (M)	4	3 ± 0	3.43 ± 0.34	*	1.90 ± 0.19	3.11 - 3.85	*	1.67 - 2.13
21 - 25 (M)	6	3 ± 1	2.82 ± 0.32	*	1.46 ± 0.36	2.38 - 3.33	*	0.86 - 1.92
26 - 30 (M)	5	3 ± 1	2.94 ± 1.36	*	1.57 ± 0.42	1.19 - 4.76	*	0.98 - 2.09
31 - 35 (M)	2	3 ± 1	2.34 ± 1.82	*	0.99 ± 0.25	1.05 - 3.63	*	0.81 - 1.17
36 - 40 (M)	4	3 ± 2	3.69 ± 1.28	*	2.25 ± 0.94	2.34 - 5.07	*	1.54 - 3.61
41 - 45 (M)	2	4 ± 2	4.46 ± 2.05	*	2.13 ± 1.03	3.01 - 5.91	*	1.40 - 2.86
46 - 50 (M)	1	4 ± 0	4.38 ± 0.00	*	2.47 ± 0.00	4.38 - 4.38	*	2.47 - 2.47
51 - 55 (M)	3	4 ± 0	4.53 ± 1.30	*	1.96 ± 0.53	3.13 - 5.71	*	1.38 - 2.42
56 - 60 (M)	-	-	-	-	-	-	-	-
> 60 (M)	3	4 ± 1	3.57 ± 1.54	*	1.56 ± 0.92	2.13 - 5.20	*	0.75 - 2.55
0 - 2 (F)	7	2 ± 1	0.99 ± 0.35	*	0.74 ± 0.45	0.37 - 1.45	*	0.11 - 1.20
3 - 5 (F)	5	2 ± 0	1.48 ± 0.39	*	1.19 ± 0.28	0.91 - 1.89	*	0.82 - 1.48
6 - 10 (F)	6	3 ± 1	1.50 ± 0.17	*	1.12 ± 0.08	1.22 - 1.65	*	1.02 - 1.23
11 - 15 (F)	14	3 ± 1	2.08 ± 0.57	*	1.16 ± 0.37	1.21 - 3.43	*	0.42 - 1.79
16 - 20 (F)	3	2 ± 0	1.85 ± 0.34	*	1.20 ± 0.09	1.58 - 2.33	*	1.09 - 1.31
21 - 25 (F)	5	3 ± 1	2.55 ± 0.61	*	1.07 ± 0.29	1.62 - 3.29	*	0.67 - 1.38
26 - 30 (F)	3	3 ± 1	2.80 ± 0.70	*	1.36 ± 0.40	2.23 - 3.58	*	0.91 - 1.67
31 - 35 (F)	4	3 ± 1	2.67 ± 0.65	*	1.48 ± 0.56	1.94 - 3.48	*	0.79 - 2.01
36 - 40 (F)	2	3 ± 0	3.44 ± 0.62	*	0.83 ± 0.14	3.00 - 3.88	*	0.73 - 0.94
41 - 45 (F)	4	4 ± 1	4.98 ± 0.41	*	1.08 ± 0.32	4.52 - 5.52	*	0.71 - 1.43
46 - 50 (F)	5	4 ± 1	4.09 ± 0.83	*	1.11 ± 0.58	2.83 - 5.14	*	0.50 - 1.94
51 - 55 (F)	7	4 ± 0	5.47 ± 0.62	*	1.33 ± 0.48	4.34 - 6.23	*	0.82 - 2.08
56 - 60 (F)	1	3 ± 0	4.27 ± 0.00	*	1.47 ± 0.00	4.27 - 4.27	*	1.47 - 1.47
> 60 (F)	7	4 ± 1	4.00 ± 0.76	*	1.26 ± 0.31	3.03 - 5.24	*	0.72 - 1.64

N: Individual number; M: Masculine genus; F: Feminine genus; <sup>a</sup>Mean and standard deviation; < DL: (\*) Analyzed not by low amount of samples; (-) individual not.

The concentration average Hg and CH<sub>3</sub>Hg<sup>+</sup> in hair for the riverine inhabitants belonging to Itaituba Municipality was 13.87 µg g<sup>-1</sup> and 10.68 µg g<sup>-1</sup>, respectively (Table 1); values above the biological tolerance limit recommended by WHO, which is 6.00 µg g<sup>-1</sup> of Hg in hair of exposed persons [11-13], being the lowest Hg concentration 2.07 µg g<sup>-1</sup> and the largest 27.02 µg g<sup>-1</sup>; and with the lowest CH<sub>3</sub>Hg<sup>+</sup> concentration of 1.49 µg g<sup>-1</sup> and the largest 22.35 µg g<sup>-1</sup>. For Hg concentration among masculine genus there was a variation of 2.07 µg g<sup>-1</sup> to 24.93 µg g<sup>-1</sup>, with an average of 13.76 µg g<sup>-1</sup>, while among feminine the variation was from 4.84 µg g<sup>-1</sup> to 27.02 µg g<sup>-1</sup>, with an average of 13.96 µg g<sup>-1</sup>. For CH<sub>3</sub>Hg<sup>+</sup> concentration masculine genus there was a variation of 1.49 µg g<sup>-1</sup> to 19.57 µg g<sup>-1</sup>, with an average of 10.77 µg g<sup>-1</sup>, while among feminine the variation was from 3.73 µg g<sup>-1</sup> to 18.42 µg g<sup>-1</sup>, with an average of 10.59 µg g<sup>-1</sup>. However, the Hg and CH<sub>3</sub>Hg<sup>+</sup> concentration in hair no present significant differences between men and women groups (Figure 2). Similar findings are reported in other studies [27-29]. In implementation of population sub-division in terms of Age, the Hg and CH<sub>3</sub>Hg<sup>+</sup> concentration in hair are similar profiles and present a largest concentration in riverine inhabitants > 3 years and being the lowest concentration

in 0 to 2 years; but, considered values above the biological tolerance limit recommended by WHO [11-13]. These results are consistent with those from studies developed by [28] Rosa et al. (2000) who verified high levels of Hg in urine samples for inhabitants of Itaituba.

In contrast with results of Hg concentration found in hair for the riverine inhabitants belonging to Barreiras community, the concentration average Hg in hair for the riverine inhabitants belonging to Tabatinga community was 2.97 µg g<sup>-1</sup> (Table 2); values below the biological tolerance limit recommended by WHO [11-13], being the lowest Hg concentration 0.37 µg g<sup>-1</sup> and the largest 6.23 µg g<sup>-1</sup>. Among masculine genus there was a variation of Hg concentration of 0.49 µg g<sup>-1</sup> to 5.91 µg g<sup>-1</sup> with an average of 2.93 µg g<sup>-1</sup>, while among feminine genus the variation was from 0.37 µg g<sup>-1</sup> to 6.23 µg g<sup>-1</sup>, with an average of 3.01 µg g<sup>-1</sup>. However, the Hg concentration in hair no present significant differences between men and women groups (Figure 2). In implementation of population sub-division in terms of Age, the Hg and CH<sub>3</sub>Hg<sup>+</sup> concentration in hair are similar profiles and present a below concentration of WHO in all age years.

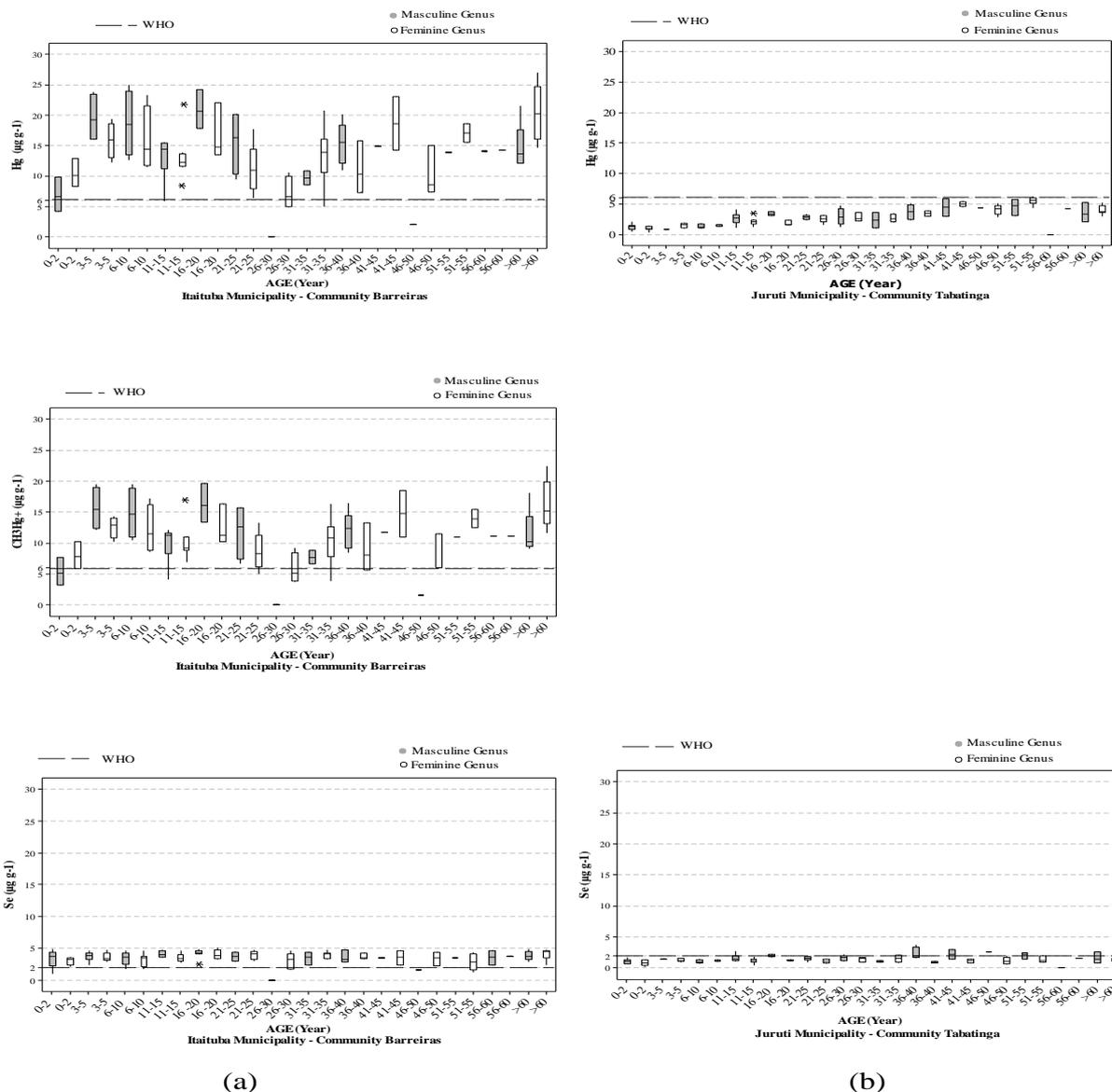


Figure 2: Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se concentration in hair samples of riverine inhabitants belonging to (a) Itaituba municipality - Barreiras community (area impacted by gold mining underground) and (b) Juruti municipality - Tabatinga community (area without mining impact) (masculine and feminine genus), belonging to the Tapajós basin, Pará state (Amazon, Brazil).

From the information of protective effect (or not) of selenium [24] [40-47], was accomplished Selenium analysis in hair. The concentration average Se in hair for the riverine inhabitants belonging to Barreiras community was  $3.51 \mu\text{g g}^{-1}$  (Table 1); values above the biological tolerance limit recommended by WHO, which is  $2.00 \mu\text{g g}^{-1}$  of Se in hair of exposed persons [11-13], being the lowest Se concentration  $0.98 \mu\text{g g}^{-1}$  and the largest  $5.14 \mu\text{g g}^{-1}$ . For Se concentration among masculine genus there was a variation of  $0.98 \mu\text{g g}^{-1}$  to  $3.58 \mu\text{g g}^{-1}$ , with an average of  $3.49 \mu\text{g g}^{-1}$ , while among feminine the variation was from  $1.26 \mu\text{g g}^{-1}$  to  $5.14 \mu\text{g g}^{-1}$ , with an average of  $3.53 \mu\text{g g}^{-1}$ . The concentration average Se in hair for the riverine inhabitants belonging to Tabatinga community was  $1.39 \mu\text{g g}^{-1}$  (Table 2); values below the biological tolerance limit recommended by WHO, which is  $2.00 \mu\text{g g}^{-1}$  of Se in hair of exposed persons [11-13], being the lowest Se concentration  $0.11 \mu\text{g g}^{-1}$  and the largest  $2.86 \mu\text{g g}^{-1}$ . For Se concentration among masculine genus there was a variation of  $0.57 \mu\text{g g}^{-1}$  to  $2.86 \mu\text{g g}^{-1}$ , with an average of  $1.62 \mu\text{g g}^{-1}$ , while among feminine the variation was from  $0.11 \mu\text{g g}^{-1}$  to  $2.55 \mu\text{g g}^{-1}$ , with an average of  $1.17 \mu\text{g g}^{-1}$ . However, the Se concentration in hair no present significant differences between men and women groups in both municipalities (Figure 2) and in implementation of population sub-division in terms of Age, the Se concentration in hair are similar profiles. Also, the Se concentration in hair for the riverine inhabitants belonging to Tabatinga community, present values below of Se concentration found in hair for the riverine inhabitants belonging to Barreiras community.

The levels of Hg and Se in hair above limit recommended by WHO [11-13], found in this study (Table 1, Table 2 and Figure 2) show that territorial occupation in areas with anthropic action (Barreiras community) can be responsible to possible human exposure. In fact, it is widely known that high quantities of mercury residues from gold mining activities are discarded annually without any control, constituting a potential risk to human health and environment [35][48-49]. However, it is also important to note that researchers have been trying to find an explanation for the absence of evident clinical symptoms in Amazonian populations who present high Hg levels. The presence of natural Se in the region has been considered a possible contributor to the apparent tolerance for chronic Hg intoxication [50-51]. Some studies suggested that adequate Se intake might provide effective protection against Hg and its toxic compounds [52]. It is believed that when Se is combined with other metals, it can produce inert compounds [53-54].

Given the variation in Se concentration and Hg exposition between the two populations, the molar ratio Hg/Se and Hg millimole (Figure 3) revealed a plausible correlation averaged for hair samples of riverine inhabitants belonging to Barreiras ( $R^2 = 0.86$ ,  $p < 0.05$ ) and Tabatinga ( $R^2 = 0.57$ ,  $p < 0.05$ ) community. As can be observed, there is a positive correlation between Hg/Se ratios and Hg concentration. The values of the molar ratio approach 1 it could be closely related to the formation of a Hg-Se-protein complex capable of decreasing the availability of Hg in the body [50]. This may mean that the protective mechanism of selenium, according [55-56], can be happening in the study area, which involves the formation of a complex Se and Hg. These authors observed that selenite ( $\text{SeO}_3^{2-}$ ) is reduced selenide ( $\text{Se}^{2-}$ )

and this binds to methylmercury ( $\text{CH}_3\text{Hg}^+$ ) forming the complex bis selenide (metilmercúrico) ( $\text{CH}_3\text{Hg})_2\text{Se}$ , neutralizing the toxic effects of mercury.

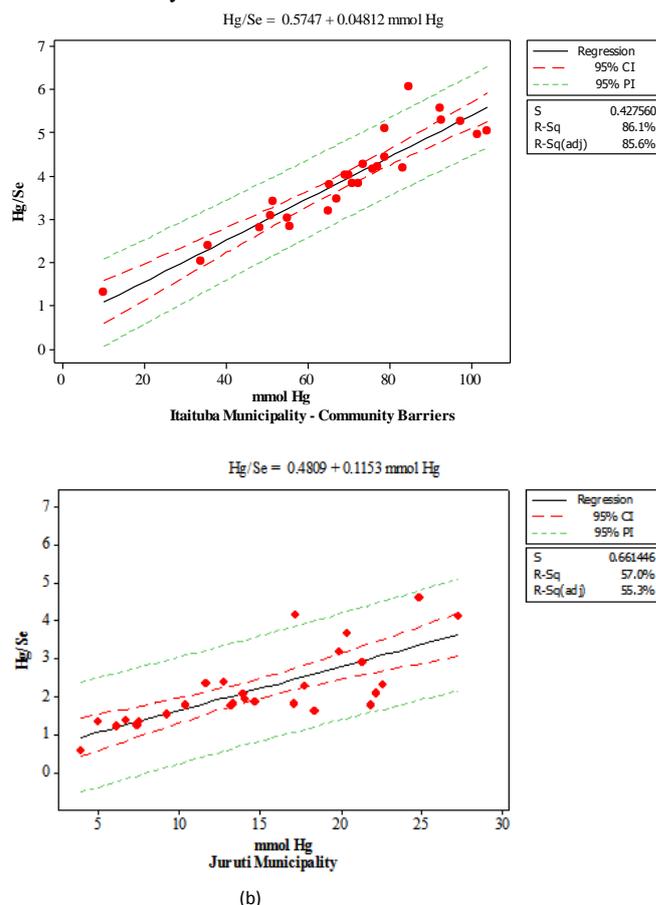


Figure 3: molar ratio Hg/Se and Hg millimole in hair samples of riverine inhabitants belonging to (a) Itaituba municipality - Barreiras community (area impacted by gold mining underground) and (b) Juruti municipality - Tabatinga community (area without mining impact) (masculine genus and feminine genus), belonging to the Tapajós basin, Pará state (Amazon, Brazil).

### III.2 MERCURY AND SELENIUM IN FISH

The Hg,  $\text{CH}_3\text{Hg}^+$  and Se concentrations in the edible portion of fish (carnivorous and non-carnivorous species) are given in Table 3. For each species, the mean and standard deviation within each zone are mentioned. Hg,  $\text{CH}_3\text{Hg}^+$  and Se concentrations in carnivorous species had an average of  $0.74 \mu\text{g g}^{-1}$ ,  $0.66 \mu\text{g g}^{-1}$  and  $0.37 \mu\text{g g}^{-1}$ , respectively; below recommended WHO that is of  $1.0 \mu\text{g g}^{-1}$  to Hg and  $\text{CH}_3\text{Hg}^+$ ,  $2.0 \mu\text{g g}^{-1}$  to Se [11-13], ranging Hg concentrations  $0.26$  to  $2.33 \mu\text{g g}^{-1}$ ,  $\text{CH}_3\text{Hg}^+$  concentrations  $0.20$  to  $1.98 \mu\text{g g}^{-1}$  and Se concentrations  $0.20$  to  $0.53 \mu\text{g g}^{-1}$ .

Table 4: Interaction between fish consumption frequency, Hg and CH<sub>3</sub>Hg<sup>+</sup> concentrations in hair samples of riverine inhabitants belonging to Barreiras community (area impacted by gold mining underground) and Tabatinga community (area without mining impact), masculine genus and feminine genus, belonging to the Tapajós basin, Pará state (Amazon, Brazil).

	FCF/ MG/IT	FCF/ FG/IT	FCF/ MG/JU	FCF/ FG/JU	Hg (µg g <sup>-1</sup> ) MG/IT	Hg (µg g <sup>-1</sup> ) FG/IT	Hg (µg g <sup>-1</sup> ) MG/JU	Hg (µg g <sup>-1</sup> ) FG/JU	CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) MG/IT	CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) FG/IT	Se (µg g <sup>-1</sup> ) MG/IT	Se (µg g <sup>-1</sup> ) FG/IT	Se (µg g <sup>-1</sup> ) MG/JU	Se (µg g <sup>-1</sup> ) FG/JU
FCF/ MG/IT	1													
FCF/ FG/IT	<b>0.970</b>	1												
FCF/ MG/JU	<b>0.847</b>	<b>0.779</b>	1											
FCF/ FG/JU	0.549	0.451	<b>0.796</b>	1										
Hg (µg g <sup>-1</sup> ) MG/IT	0.077	0.162	-0.107	-0.337	1									
Hg (µg g <sup>-1</sup> ) FG/IT	0.074	0.101	0.323	0.268	0.418	1								
Hg (µg g <sup>-1</sup> ) MG/JU	<b>0.745</b>	0.653	<b>0.894</b>	0.637	-0.172	0.160	1							
Hg (µg g <sup>-1</sup> ) FG/JU	0.648	0.529	<b>0.895</b>	<b>0.849</b>	-0.201	0.303	<b>0.870</b>	1						
CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) MG/IT	0.063	0.147	-0.110	-0.331	<b>0.999</b>	0.446	-0.178	-0.196	1					
CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) FG/IT	0.031	0.097	0.251	0.181	0.427	<b>0.923</b>	0.075	0.210	0.453	1				
Se (µg g <sup>-1</sup> ) MG/IT	-0.016	0.103	-0.207	-0.422	<b>0.752</b>	0.289	-0.221	-0.283	<b>0.741</b>	0.323	1			
Se (µg g <sup>-1</sup> ) FG/IT	0.375	0.497	0.244	-0.096	0.234	0.327	0.127	-0.038	0.231	0.311	0.302	1		
Se (µg g <sup>-1</sup> ) MG/JU	0.566	0.416	<b>0.718</b>	0.487	-0.153	0.023	<b>0.814</b>	<b>0.705</b>	-0.161	-0.075	-0.355	0.050	1	
Se (µg g <sup>-1</sup> ) FG/JU	0.561	0.625	0.511	0.238	0.164	0.233	0.321	0.330	0.168	0.305	0.099	0.158	-0.038	1

FCF: Fish Consume Frequency (time week<sup>-1</sup>), MG: Masculine Genus, FG: Feminine Genus, IT: Itaituba Municipality - Community barrier, JU: Juruti Municipality

The Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se concentrations in non-carnivorous species had an average of 0.10 µg g<sup>-1</sup>, 0.08 µg g<sup>-1</sup> and 0.24 µg g<sup>-1</sup>, respectively; below recommended WHO that is of 0.5 µg g<sup>-1</sup> to Hg and CH<sub>3</sub>Hg<sup>+</sup>, 2.0 µg g<sup>-1</sup> to Se [11-13], ranging Hg concentrations 0.01 to 0.26 µg g<sup>-1</sup>, CH<sub>3</sub>Hg<sup>+</sup> concentrations 0.01 to 0.20 µg g<sup>-1</sup> and Se concentrations 0.17 to 0.32 µg g<sup>-1</sup>. In this

study, fish species, generally classified as non-carnivores, showed significant differences in Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels in muscle tissue and are lower when compared with the species of carnivorous fish (Figure 4 and Figure 5).

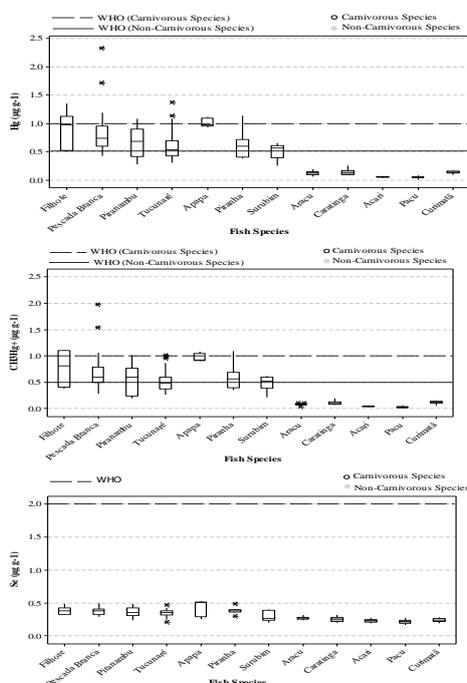


Figure 4: levels Hg, CH<sub>3</sub>Hg<sup>+</sup>, and Se in samples fish species (carnivorous and non-carnivorous) consumed by riverine inhabitants belonging to Barreiras community (area impacted by gold mining underground) and Tabatinga community (area without mining impact), both belonging to the Tapajós basin (Amazon, Brazil).

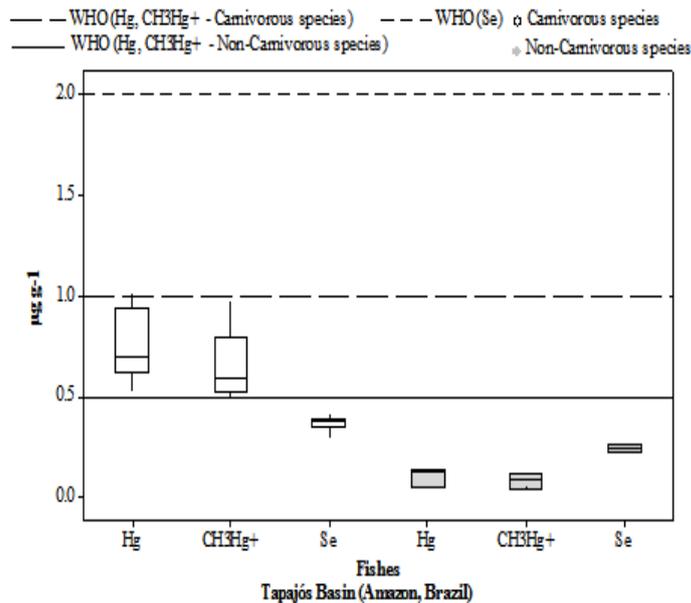


Figure 5: Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se concentration in fish species (Carnivorous and non-carnivorous).

This observation is consistent because carnivorous fish tend to have higher levels of Hg bioaccumulation process. So, this would indicate that consumption of these carnivorous fish at the high rate may be hazardous to people living in riverside community. However, the results shown in Table 4, Figure 5 and Figure 6 for carnivorous species, demonstrate that there is no difference significant between mercury levels in different species of fish carnivorous, or by weight or length of fish. Eg, Filhote is a freshwater fish of the Amazon region of South America can reach up to 2.50 meters in length and 300 kg in weight, however, Pescada branca is a South American species of fish can reach up to 60 cm in length and 2 kg in weight. Also, for non-carnivorous species no difference significant between mercury levels or by weight or length of fish. Eg, the Aracu reaches over 40 cm in length and 1.6 kg in weight, and Pacu can reach 1.0 m long and up to 20 kg. This might counter expectations of larger fish being older, and having eaten more prey, and thereby accumulated higher levels of Hg. The absence of statistically significant correlation could be explained by the fact that these species probably belonged to the same age classes. This is in conformity with the findings of several other investigators who have found no relation between Hg content in fish muscle tissue and body weight [57] or length [58].

Given the variation in Se concentration and Hg exposition between the two populations, the molar ratio Hg/Se and Hg millimole (Figure 6) revealed a plausible correlation averaged for fish samples carnivorous and non-carnivorous ( $R^2 = 0.97$ ,  $p < 0.05$ ). As can be observed, there is a positive correlation between Hg/Se ratios and Hg concentration. The values of the molar ratio approach 1 it could be closely related to the formation of a Hg-Se-protein complex capable of decreasing the availability of Hg in the body [50]. This may mean a protective mechanism of selenium, according what happened to Hg and Se results in hair.

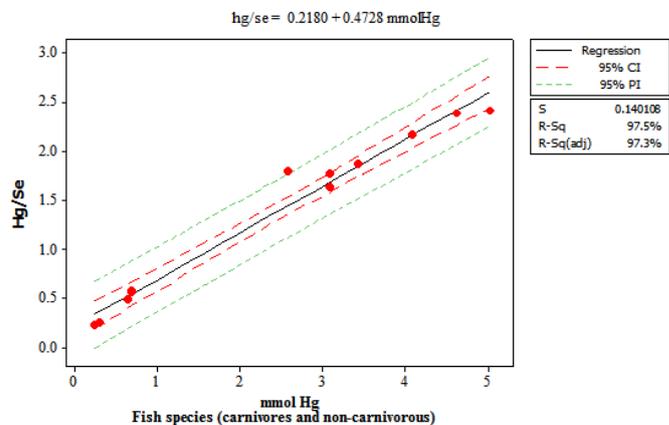


Figure 6: Molar ratio Hg/Se and Hg millimole in fish species (Carnivorous and non-carnivorous).

### III.3 RISK MANAGEMENT AND RISKS TO CONSUMERS

Important factor evaluated in this study was the relation between Hg concentrations present in the hair of people in the communities under study and fish consumption frequency due bioaccumulation and biomagnification of that Hg in aquatic food webs that is generally considered as a main pathway of Hg exposure to humans [59-61]. Because, Amazonian people who consume local fish as their main dietary protein source may be seriously threatened by Hg contamination [62]. This way, results show that although the fish consumes frequency between genders for each community this study was not significantly different showing median meals for men and women (time week-1) (Table 1, Table 2 and Figure 7), even when the whole group was assessed separately. However, despite consuming fish in a similar number of times as men, but in lower quantities, the women can be quite influenced by association with intra-uterine exposure second. The results the fish consumes frequency between Barreiras and Tabatinga community was significantly different (Figure 7) with much higher fish consumption to Barreiras community (Figure 7) and results obtained, can be connected to fish consumption that is generally considered as a main pathway of Hg exposure to humans [59-61] and/or residence time, as

individuals with age of 0 to 2 years that have low residence time at the site, have no significant fish consumption (Table 1) and maternal exposure to environmental Hg and  $\text{CH}_3\text{Hg}^+$  concentrations can impact in breast milk [63].

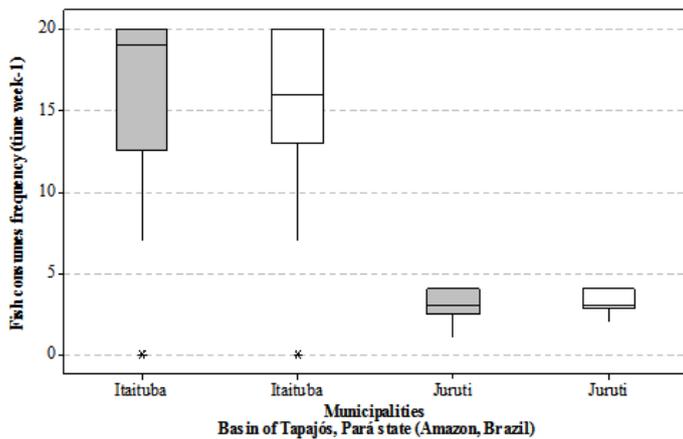


Figure 7: Fish consumes frequency of riverine inhabitants belonging to Barreiras community (area impacted by gold mining underground) and Tabatinga community (area without mining impact), masculine genus and feminine genus, belonging to the Tapajós basin, Pará state (Amazon, Brazil).

The interaction between fish consumption frequency (times/week), Hg,  $\text{CH}_3\text{Hg}^+$  and Se concentrations in hair samples by riverine inhabitants belonging to Barreiras and Tabatinga community, was evaluated through a correlation (Table 4). However,  $\text{CH}_3\text{Hg}^+$  concentration to Tabatinga community it was not possible to analyze due to the low amount of sample obtained and not introduced. This way, the results demonstrate that the correlations between the  $\text{CH}_3\text{Hg}^+$ - Hg variables in hair samples of riverine inhabitants belonging to Barreiras community for the masculine genus ( $r = 0.999$ ,  $p < 0.05$ ) and for the feminine genus ( $r = 0.923$ ,  $p < 0.05$ ) were greater with significant positive correlations and is in agreement with the ease of Hg methylation in the environment by population occupation in areas with anthropic action. On the other hand, the correlations to Hg variable in hair samples of riverine inhabitants belonging to Barreiras community for the masculine and feminine genus ( $r = 0.870$ ,  $p < 0.05$ ) with significant positive correlations, by low concentration in area without mining impact. Another positive and significant correlation between the Hg-Se ( $r = 0.752$ ,  $p < 0.05$ ) and  $\text{CH}_3\text{Hg}^+$ -Se ( $r = 0.741$ ,  $p < 0.05$ ) variables in hair samples of riverine inhabitants (masculine genus) belonging to Barreiras community, showed that production of methylmercury is controlled by the bioavailability of inorganic Hg (II) for methylation, microbial and antibiotic MeHg degradation, and the activity of resident methylmercury producing bacteria; and positive and significant correlation between the Hg-Se variables to masculine genus ( $r = 0.814$ ,  $p < 0.05$ ) and Hg (feminine genus) - Se (masculine genus) variables ( $r = 0.705$ ,  $p < 0.05$ ) in hair

samples of riverine inhabitants belonging to Tabatinga community. These positive correlations can be related to training of a Hg-Se-protein complex.

Also, positive and significant correlation ( $r = 0.970$ ,  $p < 0.05$ ) was considered between the variables of fish consumption frequencies (times/week) by masculine and feminine genus in hair samples of riverine inhabitants belonging to Barreiras community, also positive and significant correlation ( $r = 0.796$ ,  $p < 0.05$ ) was considered between the variables of fish consumption frequencies (times/week) by masculine and feminine genus in hair samples of riverine inhabitants belonging to Tabatinga community, and other positive correlations in the pairs fish consumption frequencies (Masculine Genus/Itaituba) – fish consumption frequencies (Masculine Genus/Juruti) ( $r = 0.847$ ,  $p < 0.05$ ), fish consumption frequencies (Feminine Genus/Itaituba) – fish consumption frequencies (Masculine Genus/Juruti) ( $r = 0.779$ ,  $p < 0.05$ ), which demonstrates that both masculine and feminine genus consume practically the same number of fish per week, with a difference in quantity. The positive correlations in the pairs fish consumption frequencies (Masculine Genus/Juruti) – Hg (Masculine Genus/Juruti) ( $r = 0.894$ ,  $p < 0.05$ ), fish consumption frequencies (Masculine Genus/Juruti) – Hg (Feminine Genus/Juruti) ( $r = 0.895$ ,  $p < 0.05$ ), fish consumption frequencies (Feminine Genus/Juruti) – Hg (Feminine Genus/Juruti) ( $r = 0.849$ ,  $p < 0.05$ ), fish consumption frequencies (Masculine Genus/Itaituba) – fish consumption frequencies (Masculine Genus/Juruti) ( $r = 0.745$ ,  $p < 0.05$ ) and fish consumption frequencies (Masculine Genus/Juruti) – Se (Masculine Genus/Juruti) ( $r = 0.718$ ,  $p < 0.05$ ), which demonstrates that the low mercury concentration can be related to the low consumption of fish in the Tabatinga community.

The advantages of fish consumption in large quantities (readily digestible protein, omega-3 fatty acids, minerals) by riverine inhabitants belonging to Barreiras community (area impacted by gold mining underground), while not conclusive, are strong indicators that despite not having been shown the relationship between the concentration of mercury in hair and feeding habits, along riverside communities, showed that a plausible correlation exists between levels of mercury and selenium in fish. This may indicate that possible changes in dietary habits such as increased consumption of herbivorous fish instead of carnivores, decrease consumption of river water by consumption of well water, increased consumption of fruits and vegetables can minimize contamination by mercury and maximize the selenium in health benefits through protective effect according to some authors [64].

Also, the levels of Hg,  $\text{CH}_3\text{Hg}^+$  and Se found in hair of riverine inhabitants belonging to Barreiras community, considered to be above the WHO limits, were compared to the amounts found in Tabatinga community (area without mining impact) and other studies carried out in different sites where gold mining occurs in Brazil (Table 5).

Table 5: Comparison between Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se concentrations in hair samples (µg g<sup>-1</sup>), of people residents on gold-mining areas in Brazil.

Local	N	<sup>a</sup> Hg (µg g <sup>-1</sup> )	<sup>a</sup> CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> )	<sup>a</sup> Se (µg g <sup>-1</sup> )	Hg (µg g <sup>-1</sup> ) Min- Max	CH <sub>3</sub> Hg <sup>+</sup> (µg g <sup>-1</sup> ) Min- Max	Se (µg g <sup>-1</sup> ) Min- Max	References
Barreiras community – Itaituba Municipality (area impacted by gold mining underground) - (Masculine genus)	58	15.21	10.48	3.64	2.07 – 24.93	1.49 – 19.57	0.98 – 4.91	This study
Barreiras community – Itaituba Municipality (area impacted by gold mining underground) - (Feminine genus)	83	13.89	10.68	3.60	4.84 – 27.02	3.73 – 22.35	1.26 – 5.14	This study
Tabatinga community – Juruti Municipality (area without mining impact) - (Masculine genus)	48	2.77	-	1.53	0.49 – 5.91	-	0.57 – 3.61	This study
Tabatinga community – Juruti Municipality (area without mining impact) - (Feminine genus)	73	2.84	-	1.15	0.37 – 6.23	-	0.11 – 2.08	This study
Women in fertile age from Amazon riverside communities: Pregnant Women	19	8.25	-	0.61	1.51– 19.43	-	0.40 – 2.33	[52]
Women in fertile age from Amazon riverside communities: Non-pregnant Women	21	9.39	-	2.46	5.25– 21.00	-	0.92–5.74	[52]
Wari Indians - Guajar-Mirim city, Rondnia State	13	6.06	-	2.65	1.41 – 11.70	-	1.49 - 4.62	[50]
Cumar gold-mining (PA)	16	5.2	-	-	1.5 – 13.7	-	-	[65]
Madeira river goldwasher (RO)	36	8.7	-	-	1.0 – 96.9	-	-	[48]
Madeira river riverine population (RO)	48	10.8	-	-	0.5 – 71.4	-	-	[48]
Pocon region riverine population	14	0.8	-	-	0.3 – 3.1	-	-	[66]
Pocon residents with activity out gold-mining	11	1.3	-	-	0.3 – 3.1	-	-	[67]

<sup>a</sup>Mean value, N: individual number.

Notable among these are the study by [52] that found an average of 8.25 µg g<sup>-1</sup> of Hg and 0.61 µg g<sup>-1</sup> of Se in the hair of pregnant women; and 9.39 µg g<sup>-1</sup> of Hg and 2.46 µg g<sup>-1</sup> of Se in the hair of non-Pregnant women. The studies by [50] that found an average of 6.06 µg g<sup>-1</sup> of Hg and 2.65 µg g<sup>-1</sup> of Se in the hair of Wari Indians. In the study by [65] that found an average of 5.2 µg g<sup>-1</sup> of Hg in the hair of persons living in the Garimpo (placer mine) at Cumar(PA), the studies by [35] who found an average of 8.7 µg g<sup>-1</sup> of Hg in the hair of persons living in the Madeira river Garimpo (RO) and 10.8 µg g<sup>-1</sup> of Hg in the hair of river bank inhabitants of the Madeira river (RO). On the other hand, levels of Hg below the WHO limit have also been found, as in the study by [66] who found an average of 0.8 µg g<sup>-1</sup> of Hg in hair among river bank inhabitants of the Pocon region, the studies by [67] who found on average 1.3 µg g<sup>-1</sup> of Hg in the hair of persons living in Pocon, working outside of the placer gold mining activity, 4.2 µg g<sup>-1</sup> of Hg in the hair of gold miners in Pocon (employees in ore gold mines and treatment of mine rejects, average amount) and 2.6 µg g<sup>-1</sup> of Hg in the hair of employees in gold-buying stores. Therefore, the levels of Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se found in the present study, which were an average of 15.21 µg g<sup>-1</sup> of Hg, 10.48 µg g<sup>-1</sup> of CH<sub>3</sub>Hg<sup>+</sup> and 3.64 µg g<sup>-1</sup> of Se for male inhabitants and an average of 13.89 µg g<sup>-1</sup> of Hg, 10.68 µg g<sup>-1</sup> of CH<sub>3</sub>Hg<sup>+</sup> and 3.60 µg g<sup>-1</sup> of Se for female inhabitants belonging to the Barreira community (area with mining impact), were higher than the Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels found in hair samples from inhabitants of Tabatinga community (area without mining impact) and other gold-producing areas, indicating risk for river side populations along river and for Barreira community. Another important point is to emphasize the complexity of this study to be able to do analysis of Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se in hair samples from riverine population and able to complement other studies for environmental management in the Amazon. However, factors

such as diet complexity, food habits, quality of information, age, body image, memory, beliefs, cultural behavior and socioeconomic status, as well as exposure factors, are variables that interfere and highly complicate the act of registering ingestion by an individual without exerting an influence on him or her [68].

#### IV. CONCLUSION

The levels of Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se found in the present study for hair samples of male and female inhabitants belonging to the Barreira community (area with mining impact); were higher than the Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels found in hair samples from inhabitants of Tabatinga community (area without mining impact). In the two groups were mainly related to age, gender and fish consumption. The results indicating that territorial occupation in areas with anthropic action can be responsible to possible human exposure. On the other hand, both of the studied riverine communities are likely to present an altered Hg status. Because, consumption of carnivores fish showed significant differences in Hg, CH<sub>3</sub>Hg<sup>+</sup> and Se levels in muscle tissue. This observation is consistent because carnivorous fish tend to have higher levels of Hg bioaccumulation process. So, this would indicate that consumption of these carnivorous fish at the high rate may be hazardous to people living in riverside community. However, it is also important to note that researchers have been trying to find an explanation for the absence of evident clinical symptoms in Amazonian populations who present high Hg levels. The presence of natural Se in the region has been considered a possible contributor to the apparent tolerance for chronic Hg intoxication [50-51]. Some studies suggested that adequate Se intake might provide effective protection against Hg and its toxic compounds [52]. It is believed that when Se is combined with other metals, it can produce inert compounds [53-54]. This information is

necessary in order to obtain a more complete portrait in order to test higher concentrations of selenium in the form of sodium selenite, or using other more sensitive biomarkers to relations between dietary Se in take and health of fish eating populations, because there are many limitations and shortcomings of the research with respect to the understanding of antagonism, synergism metals and fish consumption correlation with the concentration of Mercury in hair. In this regard, the results provided by this study, while not conclusive, provided reasonable estimates of trends associated with plausible correlation between levels of mercury and selenium in fish, where there is possibility of provide needed knowledge due to subsidy to research human health in Amazon.

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