Biomonitoring of Mercury, Cadmium and Selenium in Fish and the Population of Puerto Nariño, at the Southern Corner of the Colombian Amazon

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Abstract

Heavy metals threaten communities near biodiversity hotspots, as their protein sources come from the environment. This study assessed Hg, Cd, and Se concentrations in fish, as well as the magnitude of exposure and hematological conditions of adult citizens from Puerto Nariño (Colombian Amazon). Among fish samples, greater Hg concentrations were found in higher trophic level species, including *Rhaphiodon vulpinus* (880 ± 130 ng/g) and *Pseudoplatystoma tigrinum* (920 ± 87 ng/g). These species presented the highest hazard quotients and lowest Se:Hg molar ratios among those studied, showing their consumption represents a health risk to consumers. Moreover, some samples of *Mylossoma duriventre* and *Prochilodus magdalenae* had Cd levels greater than the regulated limit (100 ng/g). The average total Hg (T-Hg) concentrations in human hair and blood were 5.31 µg/g and 13.7 µg/L, respectively. All hair samples exceeded the 1.0 µg/g threshold set by the USEPA, whereas 93% of the volunteers had T-Hg blood levels greater than 5 µg/L, suggesting elevated exposure. The mean Cd level was 3.1 µg/L, with 21% of samples surpassing 5 µg/L, value at which mitigating actions should be taken. Eighty-four percent of participants presented Se deficiencies (<100 µg/L). There was a significant association between fish consumption and T-Hg in hair ($\rho = 0.323$; $p = 0.032$) and blood ($\rho = 0.381$; $p = 0.011$). In this last matrix, Se correlated with Cd content, whereas lymphocytes were inversely linked to Hg concentrations. The results of this study show that there is extensive exposure to Hg in fish, the consumption of which may promote detrimental impacts on hematology parameters within the community.

Environmental contamination is an issue of special concern for native populations, public policy decision-makers, and governmental and environmental agencies due to the potential negative effects of chemicals on human health (Burger et al. 2010). Heavy metals are nonbiodegradable elements that participate in the global life cycle, because they are dynamically transported through the atmosphere, soil, and water; where they are able to reach the trophic chain through natural erosion and anthropogenic sources, including urban activities, tourism, river transport, and mineral extraction processes (González-Merizalde et al. 2016; Li et al. 2017; Martínez-Salcido et al. 2018).

Exposure to heavy metals continues to be a worldwide public health issue, especially in biodiversity hotspots, such as the Amazon, home of the largest river system in the world, the Amazon River (Bonotto et al. 2018). This aquatic complex is being rapidly degraded and threatened by dams, deforestation, and mining activities that are also performed on its main tributaries, including the Caquetá (Peña-Venegas et al. 2014), Madeira (Balzino et al. 2015), Negro (Cabral et al. 2010), and Tapajós (Lobo et al. 2016; Oestreicher et al. 2017) Rivers. Unfortunately, artisanal small-scale gold mining (ASGM) is a key contributor to mercury (Hg) pollution in the Amazon (Olivero-Verbel et al. 2015), and as the rainforest is destroyed, other toxic elements present in the soil, such as cadmium (Cd), also are released into the aquatic environment (Basu et al. 2015; Lindell et al. 2010). Once in the water, these metals can be bioaccumulated by biota and reach humans through fish consumption. Thus, human

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exposure to heavy metals in the Amazon mostly derives from eating fish, which often are contaminated with these pollutants (Ferreira da Silva and de Oliveira Lima 2020), a reality not only linked to a lack of regular income, but to a traditional lifestyle that relies on the natural abundance of these organisms.

A chronological revision of countries with hydrographic zones and municipalities in the Amazon has shown that ASGM is a persistent problem. For instance, studies dating from the 1990s report high Hg contamination along the Madeira River, which is the main southern tributary of the Amazon River (Boischio and Barbosa 1993; Malm et al. 1990). Since these preliminary reports, high Hg concentrations have been observed in the hair of communities living along different hydrographic basins of the Brazilian (Castilhos et al. 2015), Bolivian (Maurice-Bourgoin et al. 2000), Ecuadorian (Counter et al. 2005), and Surinamese (Mohan et al. 2005) Amazon regions. In Colombia, elevated Hg levels also have been found in indigenous communities living along the Caqueta River (Olivero-Verbel et al. 2016), the Tarapaca village (Cotuhe and Putumayo Rivers) (Alcala-Orozco et al. 2019), and the Yaigojé Apaporis National Natural Park (Valdelamar-Villegas and Olivero-Verbel 2019). The issue is made even more complex due to the fact that it is known that in humans, Hg and Cd can affect many organs and tissues, such as the brain, the kidneys, and the blood (Gundacker et al. 2006). Thus, chronic exposure to these toxic trace metals can affect hematological functioning, causing a variety of effects, including increased burden of red blood cells, lowered hemoglobin concentrations, and inhibition of heme synthesis, among others (Chen et al. 2019; Roney et al. 2011).

In remote areas, such as the Colombian Amazon, health problems due to heavy metal exposure commonly co-exist with other factors due to social and economic aspects. As the clinical status of these rural communities worsens due to poverty and food insecurity, it often translates to micronutrient deficiencies and risks of presenting severe hematological conditions, including anemia. Because the load of essential elements, such as Selenium (Se) can, in turn, considerably affect the availability and, consequently, the hematotoxicity of multiple forms of these toxic metals (Roney et al. 2011), measurement of their concentrations is a key aspect for the identification of associations among them. Thus, it has been shown that the interactions of Se with Hg and Cd have profound effects on the fates of these two toxic elements, as has been observed in several organisms (Al-Saleh et al. 2015; Bjørklund et al. 2017; Chiera et al. 2017; Kosik-Bogacka et al. 2018; Spiller 2018). This mutual antagonism is due to the formation of insoluble salts of Hg and Cd with Se, and to the role that Se plays in glutathione peroxidase, an enzyme whose main function is to catalyze the reduction of peroxides to nontoxic compounds, a process that is an important element of the cellular reductive response (Cuypers et al. 2010; Pinheiro et al. 2005).

The evaluation of associations between Hg, Cd, and Se is of great significance for the communities of the Colombian Amazon, because these populations face situations that affect their survival, such as lack of government support and violence (Gómez-Restrepo et al. 2016). The concentrations of these elements, which are bioindicators of exposure; together with the sociodemographic characterization of these communities; their eating habits; and their specific symptomatology relating to exposure to these elements provide valuable information that can be used to assess health effects (e.g., hematological condition) (Faro et al. 2014). Although some studies have shown levels of contamination by both Hg and Cd in the Amazon region (Lechler et al. 2000; Moreno-Brush et al. 2016; Pfeiffer and de Lacerda 1988; Webb et al. 2016), few have focused on the relationships between such concentrations and health effects generated in individuals. Thus, the goals of this research were to (1) quantify the levels of Total-Hg (T-Hg), Cd, and Se, both in fish and in humans from Puerto Nariño; (2) to estimate health risks due to the exposure to Hg and Cd through fish consumption; (3) to characterize the hematological conditions of the study participants; and (4) to identify associations between levels of exposure to these elements and hematological markers in the communities.

Materials and Methods

Study Area and Sociodemographic Characteristics of the Population

Puerto Nariño (3°46′13″S and 70°22′59″W) is the second largest municipality of the Amazon Department of southern Colombia. It is located on the right bank upstream of the Loretoyacu River, approximately 1 km from the Amazon River, at a distance of 87 km from the city of Leticia, the capital of the department. Its population mostly consists of members of the Yaguas, Cocama, and Ticuna indigenous communities. It has an altitude of 159 m.a.s.l., and an average annual temperature of 30 °C (Peña-Venegas et al. 2009). The geographic location of the sampling site is shown in Fig. 1.

Ecotourism has increased substantially in the region in recent decades. In Puerto Nariño, the use of motorized vehicles is prohibited, and this has led it to be recognized as the first sustainable tourism destination in Colombia, as certified by the Tourism Quality Seal, granted by the Ministry of Commerce, Industry, and Tourism of the country (MCIT) (Gómez and Tabares 2007).

Local diet depends on the variety and availability of food harvested from a strategic combination of agriculture,

Fig. 1 Geographic location of Puerto Nariño, Colombian Amazon

harvest of wild fruits, and fishing. This diverse use of both wild and cultivated biological resources ensures a varied diet (Peña-Venegas et al. 2009).

A sociodemographic survey was performed in February 2019 to establish the characteristics of the population in terms of health habits: frequency of fish, fruit, vegetable, nut, and dried fruit consumption; and symptomatology related to Hg and Cd exposure (for details, see Data S1). For this purpose, the leaders of the community, together with representatives of the mayor's office and the secretariat of municipal health were invited to a meeting in which the scope of the research and the methods to be performed were explained. This study was approved by the Ethical Committee (Act No. 108-2018, Letter S1) of the University of Cartagena. A recruiting process for study participants was conducted in the urban area of the municipality using door-to-door invitations followed by the signing of an informed consent form. Once the survey was completed, volunteers were asked to donate hair and blood samples.

Fish Collection

A total of 102 fresh fish samples belonging to 24 species from the Amazon River (*Pseudoplatystoma tigrinum,*

Rhaphiodon vulpinus, Hemisorubim platyrhynchos, Hoplias malabaricus, Pseudoplatystoma fasciatum, Plagioscion squamosissimus, Osteoglossum bicirrhosum, Hoplerythrinus unitaeniatus, Calophysus macropterus, Caquetaia Kraussii, Hypophthalmus edentatus, Hoplosternum littorale, Pimelodus blochii, Mylossoma duriventre, Centrochir crocodili, Astronotus ocellatus, Pterodoras granulosus, Hypostomus plecostomus, Piaractus brachypomus, Brycon melanopterus, Cichlasoma amazonarum, Leporinus agassizii, Prochilodus magdalenae, and *Cyphocharax spiluropsis*) were purchased at the port of Puerto Nariño. As fish are highly perishable samples (Ashie et al. 1996), they were processed *on site* to obtain their morphometric variables and condition factors (Table S1). During processing, approximately 5 g of fish muscle sample was obtained from the dorsal area of each fish using a plastic knife, and then was placed in a plastic container and transported to the laboratory, where it was stored at −20 °C until sample analysis was conducted (Palacios-Torres et al. 2018).

Hair and Blood Sampling and Processing

Approximately 200 mg of hair was taken from the occipital region of the scalp from the participants. Samples were

placed in labeled envelopes according to national and international recommendations for the collection of this type of human matrix (Olivero-Verbel et al. 2011). Hair samples were washed according to the method described by Noreen et al. (2019), with minor modifications: briefly, samples were washed with 2% Triton X-100, rinsed three times with deionized water, and vortexed at 16 rpm for 10 s. Subsequently, 200 μL of acetone was added to the samples and then rinsed and dried to perform the analytical procedure.

Blood samples were collected by venipuncture of the arm, using vacuum tubes (Vacutainer®) with EDTA as an anticoagulant (682,030,202) (Alvarez-Ortega et al. 2016; McClure et al. 2016). From each participant, a total of 2 mL was obtained aseptically using sterile, disposable, pyrogen-free elements—keeping the system closed in accordance with the provisions of Article 40 of Decree 1571 of 1993. Samples were stored at 4 °C and transported to the laboratory, where they were preserved at − 20 °C and analyzed within 1 week after their collection.

Mercury Analysis in Fish, Human Hair, and Blood

Total Hg concentrations in fish, human hair, and blood were determined using a RA-915M Zeeman mercury analyzer with a pyrolysis unit (RP-91c) and RP-915P software (Lumex, St. Petersburg, Russia). Quantification was performed using calibration curves with an $R^2 > 0.99$ and were constructed with Certified Reference Materials (CRM), such as IAEA-085 and IAEA-086 (human hair) from the International Atomic Energy Agency; BE12-11,12,13,14, and 15 (blood lead and trace elements) from the Wadsworth Center, and DORM-3 (dogfish muscle) from the National Research Council of Canada for hair, blood, and fish, respectively. All samples were measured in duplicate (Palacios-Torres et al. 2018). The accuracy of the methods was assessed through the analysis of CRM DORM-2 (dogfish muscle) from the National Research Council of Canada and ERM-BB422 (fish muscle) from the European Commission (IAEA-086 and BE12-15), which returned recovery percentages of 93.8%, 99.6%, 99.7%, and 99.5%, respectively (Table S2). The limit of detection (0.001 μg/g) was calculated as three times the standard deviation of blanks (empty preheated combustion boats) (Long and Winefordner 1983). The precision of the method, estimated as the coefficient of variation between replicates of the same sample, ranged between 0.1 and 10.7%.

Determination of Cadmium and Selenium in Fish Samples

Approximately 0.2 g of fish muscle from the Amazon River was weighed out directly into microwave digester tubes with HNO_3, HCl, and H_2O_2. The temperature program of the microwave digestion system is shown in Table S3. Cadmium concentrations were determined using atomic absorption spectrophotometry (AA240 FS, Agilent Technologies) in a GTA-120 graphite furnace. Analytical conditions are shown in Table S4. In the case of Se quantification, analysis was performed with hydride generation atomic absorption spectroscopy using an Agilent Vapor Generation Accessory VGA 77. Analyses were performed at least in duplicate and with calibration curves with an $R^2 > 0.99$ and were constructed using Standard Reference Materials (SRM), such as Cd and Se Standard Solutions Certipur® traceable to SRM from NIST. The accuracy of the methods was assessed through analysis of the CRM ERM-BB422 (fish muscle) from the European Commission, the recovery percentage of which was 99.5%, for both Cd and Se (Table S5).

Risk-Based Consumption Limits and Molar Ratios

Because fish consumption is a determining risk factor for Hg and Cd poisoning, hazard quotients (HQ) were calculated as a measure of the ratio between the exposure level and the reference dose (RfD) of such elements (HQ = E/RfD). If the HQ value is above 1, systemic effects are expected (Alcala-Orozco et al. 2017). The RfD value used in this study was 0.1 μg/kg/day for both Hg and Cd (ATSDR (Agency for Toxic Substances and Disease Registry), 2008, USEPA 2000, 2014). For the general population, exposure level (E) was considered as $E = C*MS/W$; where C is the MeHg ($C = 0.80 \times$ T-Hg) or Cd mean level in fish, MS is the standard portion size of fish (230 g) for an average adult and W is the standard body weight for adults (70 kg). The MeHg percentage in this study was selected as a conservative value according to the range of percentages reported in the literature for fish samples collected in the Amazon region (Table S6) (Kehrig and Malm 1999; Martín-Doimeadios et al. 2014).

Risk assessment was also performed for pregnant/breastfeeding women and children, adjusting fish serving sizes and weights (USEPA-FDA 2018). Thus, fish serving sizes were 113, 28, 57, 85, and 113 g for pregnant/breastfeeding women and children aged 2, 6, 9, and 11 and up, respectively, whereas the standard weights were 75, 13, 24, 35.5, and 47.5 kg (CDC 2012). In addition, the maximum allowable fish consumption rate in meals per week (CRmw) that would not generate any significant adverse effect on humans was estimated as CRmw = 49/($C*MS$). The Acceptable Daily Intake (ADI) of Hg and Cd may be approximated as 7 μg/day/adult (49 μg/week) (Alonso et al. 2002; Olivero-Verbel et al. 2015). Selenium and Hg, as well as Se and Cd molar ratios were calculated to assess Hg and Cd toxicological hazards. Although this criterion is currently disputed in the scientific community, some studies have suggested that a

molar ratio exceeding 1 may potentially protect fish and their consumers against such toxicity (Albuquerque et al. 2020).

Cadmium and Selenium Analysis in Blood Samples

The treatment of blood samples for the analysis of Cd consisted of a dilution with Triton x-100 in an acid medium in order to induce the lysis of the cells and enhance the injection process necessary to perform the calcination stage in the graphite furnace. Solutions of $PdCl_2$, $Mg(NO_3)_2 \cdot 6H_2O$, and EDTA were employed as modifiers, whose function was Cd stabilization in the calcination process, allowing the process to be conducted with higher temperatures while eliminating interferences. Cadmium concentrations were determined through atomic absorption spectrophotometry (AA240 FS, Agilent Technologies) using a GTA-120 graphite furnace (Table S4).

Sample preparation for Se analysis consisted of carrying out a closed digestion at a temperature of 120 °C with HNO_3, HCl, and H_2O_2 for 50 min with a temperature increase of 10 °C every 10 min after reaching 80 °C. After finishing the digestion, Se was reduced over a period of 12 h with HCl and 2% urea. Then, sample analysis was performed through hydride generation atomic absorption spectroscopy using an Agilent Vapor Generation Accessory VGA 77. Analyses were performed employing calibration curves with an $R^2 > 0.99$ and were analyzed at least in duplicates. The accuracy of the methods was assessed through the analysis of the CRM BCR-634 (human blood) and ERM-BB422 (fish muscle) from the European Commission, the recovery percentages of which were 97.3% for Cd and 99.4% for Se, respectively (Table S5).

Hematological Analysis

Measurements were performed by impedance technology and photometry using an ABX Micros ES 60 (HORIBA Medical, Ltd). Blood controls and calibrators (high, normal, and low levels) were used to verify the proper operation of the equipment (Alvarez-Ortega et al. 2019). A tri-level multiparameter control designed for the monitoring of the accuracy and precision of hematology blood cell counters, the ABX Minotrol 16, was employed in this study. The same procedure was used as for the human blood samples. Briefly, they were brought to room temperature by rolling the vial between the palms of the hands until the red blood cell sediment was completely suspended. Immediately before sampling, the vials were then gently inverted and aspirated using the instrument sample probe. After use, the threads of the vials were wiped and caped with lint-free gauze. Then, the vials were promptly recapped and refrigerated. Assay values, based on replicate analyses using the ABX Minotrol 16, are shown in Supplementary Table S7. The hematological

parameters analyzed were: hemoglobin (HGB), hematocrit (HTC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), neutrophils (NEU), eosinophils (EOS), basophils (BAS), lymphocytes (LYM), monocytes (MONO), platelet count (PLT), and mean platelet volume (MPV) (Carranza-Lopez et al. 2020).

Statistical Analysis

Results are presented as mean ± standard error. Normality was verified using the Kolmogorov–Smirnov test. The Mann–Whitney test was used to compare Hg, Cd, and Se levels between sexes. Spearman correlation analysis was employed to find significant associations between element concentration and age, morphometric characteristics, feeding habits, and hematological parameters, as well as between blood and hair T-Hg levels. In addition, Spearman correlation was used to find significant associations between T-Hg concentrations in fish muscle and their mass and length. A gravimetric procedure that yielded Hg in blood values in µg/kg was used to aliquot the samples. For comparison with reference values and previous study reports in the discussion section, the measurements were converted to volumetric concentrations (µg/L) using 1.0506 as a specific gravity of whole blood at 37 °C (Nakayama et al. 2019). The multivariate relationships between the concentrations of each element and age, gender, feeding habits, hematological parameters, and general sociodemographic characteristics of the population were examined using a Principal Component Analysis. For all cases, the significance of variables was set at $p < 0.05$. GraphPad Prism 6.0 and IBM SPSS Statistics 25 software were utilized for statistical analysis.

Results

Morphometric Parameters and Condition Factors of Fish Samples

Morphometric parameters and condition factors of studied fish species are shown in Table S1. Fish size decreased in the following order: *P. tigrinum* > *P. fasciatum* > *P. granulosus* > *C. crocodile* > *H. platyrhynchos* > *C. macropterus* > *P. squamosissimus* > *B. melanopterus* > *O. bicirrhosum* > *A. ocellatus* > *R. vulpinus* > *H. edentatus* > *H. unitaeniatus* > *L. agassizii* > *H. malabaricus* > *P. magdalenae* > *H. littorale* > *P. brachypomus* > *H. plecostomus* > *C. amazonarum* > *C. spiluropsis* > *C. Kraussii* > *M. duriventre* > *P. blochii*. With the exception of *O. bicirrhosum, P. fasciatum, P. tigrinum, H. platyrhynchos, R. vulpinus, H. malabaricus,*

H. edentatus, and C. macropterus; fish physiological status was favorable as determined by the condition factors.

Mercury, Cadmium, and Selenium Concentrations in Fish

Total Hg, Cd, and Se levels in the edible portions of fish from the Amazon River (15 species, $n \geq 2$) are presented in Table 1 according to trophic levels. Results for fish samples whose n was equal to 1 (9 species) are shown in Table S8. Among the 102 fish samples, T-Hg levels varied between 10.8 and 1440 ng/g, with a mean value of 201 ± 30.1 ng/g. In general, the greatest concentrations (mean $\pm$ standard error) were found in the highest trophic species, *P. fasciatum, H. platyrhynchos, R. vulpinus,* and *P. tigrinum*, whose Hg concentrations were 1070, 630 ± 47, 880 ± 130, and 920 ± 87 ng/g, respectively. The Hg content found in these fish species exceeded the maximum allowable concentration of 500 ng/g. The species with the lowest T-Hg levels were *B. melanopterus* and *P. magdalenae*.

Cadmium levels ranged from 2.50 to 390 ng/g. *Mylossoma duriventre* (40 ± 31 ng/g), *P. magdalenae* (32 ± 22 ng/g), and *H. littorale* (20 ± 2 ng/g) were the species with the highest Cd content. In general, Cd concentrations were low, and only

two samples of *M. duriventre* and *P. magdalenae* exceeded the maximum limit of 100 ng/g established by the European Commission and the Colombian Ministry of Health and Social Protection.

Selenium concentrations in fish tissue ranged from 12.5 to 584 ng/g, with a mean value of 192 ± 12.2 ng/g. The species with the highest content of this essential element were *H. edentatus* (584 ng/g), *C. macropterus* (423 ± 115 ng/g), *C. Kraussii* (344 ± 55.5 ng/g), and *H. malabaricus* (305 ± 60.6 ng/g).

Correlations Between Element Levels in Tissue, Fish Morphometric Parameters, and Trophic Levels

Spearman correlation analysis for element concentrations in fish muscle and morphometric variables is shown in Table S9. Statistical relationships between T-Hg and both weight and length were detected only for *P. granulosus* and *M. duriventre*. There was a positive and significant association between T-Hg concentrations in fish and trophic levels ($\rho = 0.685$; $p < 0.0001$) (Fig. S1). In the case of Cd and Se levels, no significant associations were found with morphometric variables.

Table 1 Average and concentration range of measured elements in fish from the Amazon (ng/g), target hazard quotients (HQ), and maximum allowable fish consumption rate in meals/week (CRmw)

Scientific name	*n*	Trophic level*	Mercury	Cadmium	Selenium	HQ (Hg)	CRmw (Hg)	HQ (Cd)	CRmw (Cd)
Prochilodus magdalenae	17	2.1	49 ± 7.3 (11–120)	32 ± 22 (3–390)	223 ± 20.7 (102–357)	**1.29**	5	**1.05**	7
Leporinus agassizii	2	2.3	54 ± 24 (30–78)	18 ± 5 (13–23)	216 ± 45 (171–261)	**1.43**	5	0.58	12
Brycon melanopterus	3	2.5	23 ± 0.88 (22–25)	11 ± 2 (8–15)	28.3 ± 15.3 (13–59)	0.62	11	0.37	19
Hypostomus plecostomus	21	2.5	55 ± 10 (13–230)	14 ± 2.5 (3–42)	131 ± 17.2 (13–338)	**1.46**	5	0.45	15
Pterodoras granulosus	5	2.6	95 ± 3.2 (91–110)	8.6 ± 1.1 (5–11)	32.4 ± 19.4 (13–110)	**2.51**	3	0.28	25
Centrochir crocodili	3	2.8	140 ± 37 (73–200)	6.7 ± 0.88 (5–8)	298 ± 63.5 (194–413)	**3.78**	2	0.22	32
Mylossoma duriventre	8	3.0	46 ± 7.6 (12–66)	40 ± 31 (3–260)	47.1 ± 17.5 (13–132)	**1.20**	6	**1.32**	5
Hoplosternum littorale	2	3.0	140 ± 110 (32–250)	20 ± 2 (18–22)	210 ± 15 (195–225)	**3.75**	2	0.66	11
Caquetaia kraussii	2	3.0	490 ± 200 (290–690)	4 ± 1 (3–5)	344 ± 55.5 (288–399)	**12.79**	1	0.13	56
Calophysus macropterus	2	3.2	360 ± 84 (280–440)	13 ± 3.5 (9–16)	423 ± 115 (308–538)	**9.48**	1	0.40	17
Hoplerythrinus unitaeniatus	10	3.4	120 ± 20 (30–230)	14 ± 3.4 (5–41)	252 ± 24.6 (129–376)	**3.11**	2	0.47	15
Hoplias malabaricus	5	4.4	120 ± 20 (81–190)	14 ± 2 (10–21)	305 ± 60.6 (128–495)	**3.10**	2	0.48	15
Hemisorubim platyrhynchos	2	4.4	630 ± 47 (580–670)	14 ± 6 (8–20)	251 ± 93.5 (157–344)	**16.45**	0	0.46	15
Rhaphiodon vulpinus	9	4.5	880 ± 130 (320–1400)	7.4 ± 1.4 (3–14)	197 ± 25.1 (88–346)	**23.08**	0	0.24	29
Pseudoplatystoma tigrinum	2	4.5	920 ± 87 (830–1000)	12 ± 3.5 (8–15)	102 ± 47.5 (54–149)	**24.13**	0	0.38	19
Mean concentration (Total)			200 ± 30 (11–1400)	20 ± 5 (3–390)	180 ± 10 (13–538)				

*Trophic levels were obtained from FishBase (www.fishbase.org). HQs above 1 are indicated in bold

Risk-Based Consumption Limits and Molar Ratios

According to calculated Hg HQs for the general population (Tables 1, and S8), 96% of the fish species sampled may represent a risk to human health. Thus, Hg HQs > 1 were found in the analyzed samples. In the case of the risk assessment for both pregnant/breastfeeding women and children (Table S10), most fish species may represent a risk to their health, with the exception of *P. magdalenae*, *B. melanopterus*, and *M. duriventre*. In the case of Cd, potential risks to human health could be related to *P. magdalenae* and *M. duriventre*, given that their HQ values were 1.05 and 1.32, respectively. This information together with the CRmw results suggest that in general, the only fish species that could be eaten by the native communities from Puerto Nariño with no risk to their health is *B. melanopterus*, which could be consumed up to 11 times per week. With the exception of *P. granulosus*, *P. fasciatum, R. vulpinus*, and *P. tigrinum*; most specimens displayed a favorable Se:Hg molar ratio (> 1) (Table S11).
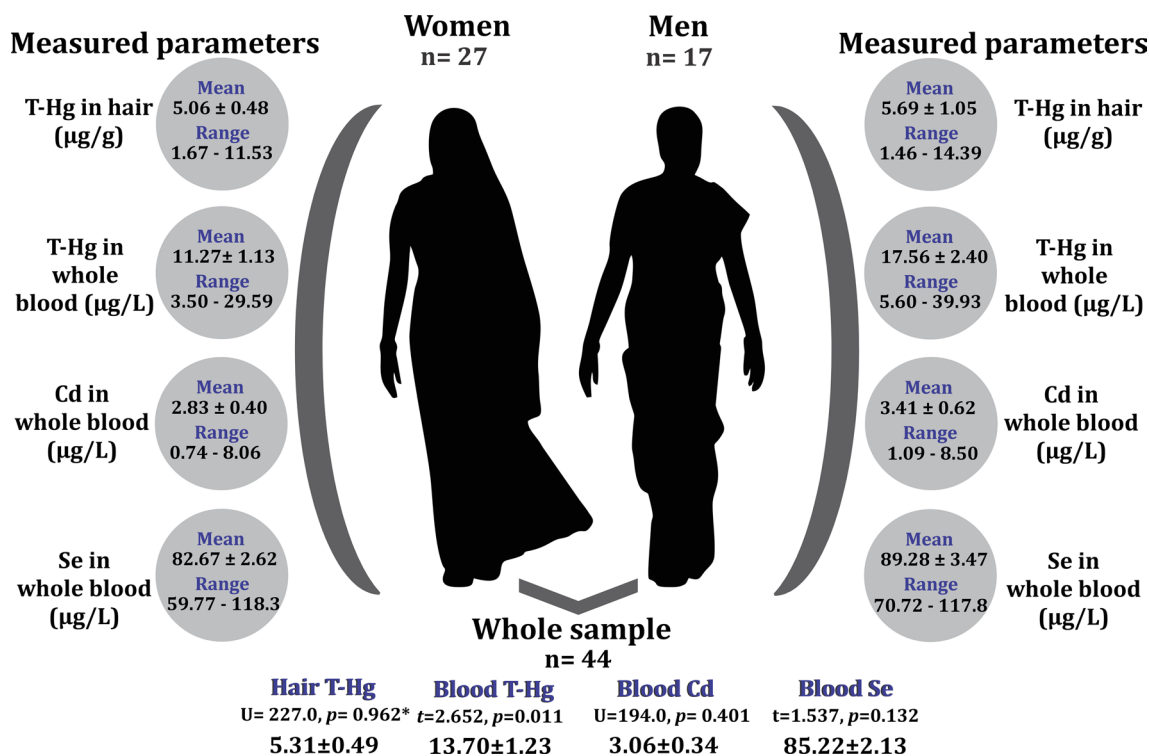
Sociodemographic Data of Inhabitants of Puerto Nariño

Sociodemographic information and other characteristics of the study volunteers living in Puerto Nariño is shown in Table S12. Mean age was similar (~ 35 years) for all volunteers. Fifty percent (50%) of participants included fish in their diet 5–10 times a week, whereas more than 40% of the study population consumed fruits and dairy products in at least one meal. A significant fraction of participants ate vegetables every day (47.7%). Nuts and dried fruits corresponded to the least consumed foods among the communities. In terms of educational status, a low percentage of the population had a college degree (4.5%). Although some had primary and secondary educations, many had completed technical school (> 40.9%). In addition, most people from Puerto Nariño were nonsmokers (88.6%) and consumed alcohol at least once a week. Several volunteers declared that they experienced signs and symptoms related to Hg and Cd poisoning (Table S13), with headache (63.5%), followed by changes in moods (40.9%), as the most frequently reported.

Mercury, Cadmium, and Selenium Levels

The distribution of hair and blood Hg levels in individuals from Puerto Nariño in the Colombian Amazon is presented in Fig. 2. The average T-Hg values found in the evaluated samples was 5.31 ± 0.49 µg/g (range 1.46–14.39) and 13.70 ± 1.23 µg/L (range 3.50–39.93) for hair and blood, respectively. For both matrices, T-Hg concentrations were higher in men than women; however, there were only

Measured parameters (Women n= 27)

Parameter	Mean	Range
T-Hg in hair (µg/g)	5.06 ± 0.48	1.67 - 11.53
T-Hg in whole blood (µg/L)	11.27 ± 1.13	3.50 - 29.59
Cd in whole blood (µg/L)	2.83 ± 0.40	0.74 - 8.06
Se in whole blood (µg/L)	82.67 ± 2.62	59.77 - 118.3

Measured parameters (Men n= 17)

Parameter	Mean	Range
T-Hg in hair (µg/g)	5.69 ± 1.05	1.46 - 14.39
T-Hg in whole blood (µg/L)	17.56 ± 2.40	5.60 - 39.93
Cd in whole blood (µg/L)	3.41 ± 0.62	1.09 - 8.50
Se in whole blood (µg/L)	89.28 ± 3.47	70.72 - 117.8

Whole sample n= 44

	Hair T-Hg	Blood T-Hg	Blood Cd	Blood Se
	U= 227.0, *p*= 0.962*	*t*=2.652, *p*=0.011	U=194.0, *p*= 0.401	*t*=1.537, *p*=0.132
	5.31±0.49	13.70±1.23	3.06±0.34	85.22±2.13

Fig. 2 Total mercury, cadmium and selenium concentrations according to gender in ethnic groups from Puerto Nariño, Colombian Amazon. *Statistical differences between mean values for women and men

statistically significant differences between the Hg content found in the blood for both sexes ($t = 2.652$, $p = 0.011$). In the case of hair, all samples exceeded the limit of 1.0 µg/g established by the United States Environmental Protection Agency, USEPA; and 36% of the population was above the maximum level of 5.0 µg/g set by the World Health Organization (WHO). In addition, 11.4% had values greater than 10 µg/g, and 93% of the participants had blood T-Hg concentrations above 5.0 µg/L. Spearman correlation analysis showed a strong and significant association between blood and hair T-Hg levels in the inhabitants of Puerto Nariño ($\rho = 0.635$; $p < 0.0001$; Fig. 3).

Cadmium concentrations in whole blood of participants from Puerto Nariño are presented in Fig. 2. Mean Cd concentration (3.06 ± 0.34 µg/L; range 0.74–8.50 µg/L) was below the CDC action level of 5 µg/L; however, 21% of samples were above this limit. As in the case of Hg, men had higher Cd concentrations than women, but these were not statistically different ($U = 194.0$, $p = 0.401$).

Selenium levels in whole blood displayed by gender are shown in Fig. 2. The mean Se concentration was 85.2 ± 2.1 µg/L, and concentrations ranged from 59.8 to 118.3 µg/L. Selenium concentrations were not statistically different between men and women ($t = 1.537$, $p = 0.132$). All samples were below the CDC action level of 500 µg/L. In addition, Se concentrations < 100 µg/L (deficiency), and from 100 to 340 µg/L (normal range) were found in 84% and 16% of participants, respectively.

Hematological Parameters

The results of the hematological analysis performed on citizens from Puerto Nariño are shown in Table S14. Average values of the evaluated parameters were within the reference data, with less than 5% of volunteers presenting low values for hemoglobin (< 11 g/dL), hematocrit percentage ($< 36\%$), and erythrocyte count ($< 4000/\text{mm}^3$). In addition, 20.5%,

9.1%, and 15.9% of the tested individuals had neutrophils, eosinophils, and lymphocytes percentages above their reference value, respectively.

Correlations Between Element Concentrations and Age, Morphometric Characteristics, Feeding Habits and Hematology
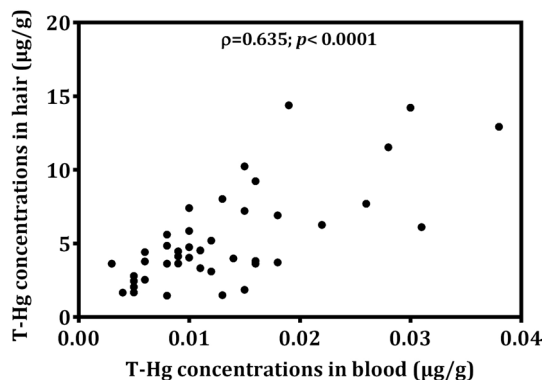
Spearman correlations for trace element levels and age, morphometric characteristics, feeding habits, and hematological variables are shown in Table 2. No correlations were found between hair and blood T-Hg levels and age, weight, or height of the participants. However, there was a positive and significant association between fish consumption and T-Hg concentrations in hair ($\rho = 0.323$; $p = 0.032$) and blood ($\rho = 0.381$; $p = 0.011$). In addition, correlation results suggested a statistical relationship between T-Hg levels and some hematological parameters. Thus, erythrocyte ($\rho = 0.317$; $p = 0.036$), eosinophil ($\rho = 0.333$; $p = 0.027$), and basophil ($\rho = 0.439$; $p = 0.022$) percentages had significant relationships with blood T-Hg, whereas neutrophils had them with hair T-Hg content ($\rho = 0.299$; $p = 0.049$). In contrast, lymphocytes presented an inverse association with the Hg concentrations found in both matrices.

In the case of Cd, no statistically significant associations were found between the levels found in blood for this element and the variables evaluated. In contrast, significant associations were observed between Se concentrations in whole blood and hematocrit ($\rho = 0.313$; $p = 0.039$) and erythrocyte ($\rho = 0.316$; $p = 0.037$) percentages.

Correlations among the three target elements are presented in Table S15. Associations were only observed for Cd and Se in whole blood (Spearman's $\rho = 0.524$, $p < 0.001$).

Principal component analysis (PCA) was employed to examine multivariate relationships and to explain variance in the data while reducing the number of variables. Hematological parameters that did not correlate with the elements in the Spearman correlation analyses were not considered in the model (e.g., medium corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, white blood cell count, platelet count, and mean platelet volume). An anti-image correlation matrix also was employed to exclude variables that may not be useful in the model. The main component analysis was used to explore the data matrix order in order to determine the weight (importance) of the evaluated parameters in the total variability of the data.

The Varimax PCA yielded five PCs (Table 3), which explained 79.78% of the variance. PC1 (31.72% of the variance) was clearly associated with hematocrit percentage, hemoglobin, and red blood cell percentage. These hematological parameters were below normal values in some samples. PC2 (17.05% of the variance) was mostly linked

Fig. 3 Spearman correlation between blood and hair T-Hg levels in inhabitants of Puerto Nariño, Colombian Amazon

Table 2 Spearman correlations between the elements concentrations and age, morphometric characteristics, feeding habits, and hematology

Parameters ($n=44$)	Hg in hair		Hg in blood		Cd in blood		Se in blood	
	Spearman correlation	p value	Spearman correlation	p value	Spearman correlation	p value	Spearman correlation	p value
Age ($n=44$)	0.091	0.558	0.073	0.636	0.267	0.080	0.075	0.628
Morphometric characteristics ($n=44$)								
Weight	−0.069	0.658	0.099	0.523	−0.083	0.592	0.141	0.360
Height	−0.040	0.794	0.210	0.171	0.088	0.570	0.065	0.678
Feeding habits ($n=44$)								
Fish intake	0.323	0.032*	0.381	0.011*	0.037	0.812	−0.116	0.452
Fruits consumption	−0.053	0.733	−0.136	0.378	0.006	0.968	0.024	0.877
Vegetables consumption	0.105	0.498	−0.017	0.912	−0.074	0.634	−0.057	0.714
Nuts and dried fruits consumption	0.008	0.958	0.012	0.939	0.025	0.872	−0.037	0.813
Dairy products consumption	0.068	0.659	−0.229	0.134	−0.293	0.057	−0.194	0.213
Hematological parameters ($n=44$)								
HGB	−0.076	0.624	0.253	0.097	0.087	0.573	0.274	0.072
HCT	−0.076	0.622	0.251	0.101	0.099	0.522	0.313	0.039*
ERI	−0.055	0.723	0.317	0.036*	0.138	0.371	0.316	0.037*
MCV	−0.113	0.467	−0.201	0.190	−0.119	0.442	−0.042	0.788
MCH	−0.088	0.571	−0.150	0.330	−0.109	0.482	−0.014	0.926
MCHC	0.053	0.731	0.139	0.367	−0.028	0.855	0.083	0.591
WBCs	0.233	0.129	0.153	0.321	−0.077	0.621	0.190	0.218
NEU	0.299	0.049*	0.228	0.137	−0.132	0.393	−0.151	0.328
EOS	0.129	0.403	0.333	0.027*	0.128	0.409	0.087	0.573
BAS*	0.242	0.224	0.439	0.022*	0.166	0.407	0.166	0.407
LYM	−0.428	0.004*	−0.418	0.005*	−0.054	0.727	−0.046	0.767
PLT	0.123	0.427	0.101	0.515	−0.036	0.818	0.195	0.205
MPV	−0.081	0.600	−0.012	0.941	0.044	0.777	−0.076	0.625

The [a]Number of *XY* pairs was 27

*Statistically significant correlations

with Hg concentration in hair and blood, which supports the fact that significant associations were observed between these two variables. PC3 (11.84% of the information) was mainly related with gender, height, and weight, whereas PC4 (10.83% of the variance) was associated with the hematological parameters and levels of Cd and Se (which had an inverse relationship). Finally, PC5 (8.34% of variance) was related with fish consumption and age of participants.

Discussion

Mercury and Cd are highly toxic elements with many adverse health effects, even at low concentrations. Given their impact on environmental health and their widespread use and release from activities, such as gold mining, they are of high concern, especially in areas of high cultural and environmental importance (Carranza-Lopez et al. 2019; Obiri et al. 2016). In this study, *O. bicirrhosum, P. fasciatum, P. tigrinum, H. platyrhynchos, R. vulpinus, H. malabaricus, H.*

edentatus, and C. macropterus were not found to be in good physiological condition, as indicated by the condition factors (<1). These results may imply that these fish species presented difficulty in their growth as a result of multiple issues, including nutrient deficits, environmental stress, and poor water quality (Famoofo & Abdul 2020). Fish of the highest trophic levels, such as *P. fasciatum, H. platyrhynchos, R. vulpinus,* and *P. tigrinum* displayed a mean T-Hg concentration above the maximum allowable limit of 500 ng/g recommended by the FAO/WHO (FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization), 1991) and the European Commission (EC (European Commission), 2006) for human consumption.

In addition, the average T-Hg level for *H. platyrhynchos* (630 ng/g) was higher than the values reported for this species in other Amazonian regions, including the Tapajós (490 ng/g) (Lino et al. 2018) and Bacajá (450 ng/g) Rivers (Souza-Araujo et al. 2016). Similarly, T-Hg content in *R. vulpinus* from the Amazon River was higher than that found for this fish in the Madeira River basin (Brazil)

Table 3 Results of principal component analysis for selected variables

	Components, eigenvalues and total variance explained (cumulative %)				
	1	2	3	4	5
Variables	4.448*	2.387	1.658	1.517	1.168
	31.72**	17.05	11.84	10.83	8.34
HTC	0.974	0.004	0.131	0.096	− 0.044
HGB	0.953	0.018	0.169	0.095	− 0.051
RBC	0.942	0.054	0.140	0.096	0.064
Gender	0.613	0.238	0.604	0.048	− 0.024
Hg Hair	− 0.099	0.857	− 0.042	− 0.147	0.135
Hg Blood	0.321	0.828	− 0.050	0.181	0.157
LYM	0.012	− 0.694	− 0.215	− 0.056	0.243
EOS	0.078	0.524	0.227	0.367	0.202
Height	0.227	0.168	0.844	− 0.019	− 0.076
Weight	0.148	− 0.043	0.835	0.097	0.235
Selenium	0.111	0.124	0.135	0.867	− 0.200
Cadmium	0.125	− 0.020	− 0.074	0.852	0.305
Fish	− 0.045	0.403	− 0.112	− 0.028	0.778
Age	− 0.012	− 0.183	0.394	0.150	0.733

Extraction method: principal component analysis

Rotation method: Varimax with Kaiser normalization

HTC hematocrit, *HGB* hemoglobin, *RBC* red blood cells, *LYM* lymphocytes, *EOS* eosinophils

*Eigenvalues

**Total variance explained

(Oliveira et al. 2010), whereas the mean Hg concentration reported for *P. tigrinum* (920 ng/g) was similar to that observed in the upper Madeira River (Bolivian Amazon) (980 ng/g) (Maurice-Bourgoin et al. 2000), but higher than the level found at the Caquetá River basin in Colombia (300 µg/g) (Olivero-Verbel et al. 2016).

In this study, statistically significant correlations between T-Hg and both weight and length were detected for *P. granulosus* and *M. duriventre*. Interestingly, in the case of *P. granulosus*, these morphometric variables were negatively correlated with T-Hg content. Previous studies have indicated that given the high Hg clearance rate through the food chain, in combination with rapid increases in the length and weight of fish specimens, whole fish Hg levels can be decreased through a "growth dilution" effect, yielding negative correlations between such parameters, especially in young specimens (Braune 1987; Ward et al. 2010). Lack of correlation for the other fish species analyzed could be the result of size homogeneity among samples (Olivero-Verbel et al. 2004). There also was a positive and significant relationship between the T-Hg concentrations and their trophic levels ($\rho = 0.685$; $p < 0.0001$) when data from all species were pooled, which is due to the fact that organisms that are higher on the trophic chain might be expected to present higher concentrations of bioaccumulative metals, as they may eat larger food items (Hosseini et al. 2013).

Given that Puerto Nariño inhabitants lead a traditional livelihood that depends on the availability of food provided by the surrounding rivers and forests, a risk assessment was performed to estimate the potential effects derived from fish consumption. Hazard quotient values suggested that this community may be subjected to potential hazards due to ingestion of Hg contaminated fish. Thus, the species with the highest trophic levels and Hg content, *R. vulpinus* (HQ = 23.08) and *P. tigrinum* (HQ = 24.13), had the highest Hg HQs. Hazard quotients obtained for these species were much higher than those found in the confluence of the Ventuari-Orinoco Rivers (Venezuela) (*R. vulpinus*, HQ = 9.1) (*P. tigrinum*, HQ = 1.0), an area highly impacted by illegal ASGM (Trujillo et al. 2010). Moreover, the HQ for *P. tigrinum* from the Amazon River also was much more elevated than that obtained for this fish in the Caquetá River, in the Colombian Amazon (HQ = 8.87) (Olivero-Verbel et al. 2016).

Unlike the trend observed with Hg concentrations in fish samples, Cd levels did not follow a trophic level-dependent relationship. *M. duriventre* and *P. magdalenae,* a detritivorous species, had the highest concentrations. Samples from these species exceeded the limit of 100 ng/g (EC (European Commission), 2006, MSPS (Ministerio de Salud y Protección Social), 2012) and presented an HQ value > 1, which suggests potential adverse effects due to this element for this species. Given that *P. magdalenae* feeds on detritus found on bottom sediments, an aquatic compartment that behaves as a sink for many contaminants released into the water column, Cd content may be reflecting the influence of anthropogenic activities carried out in the Amazon region, including mining operations and deforestation (Basu et al. 2015; Lindell et al. 2010). The average concentration of this element in *P. magdalenae* from the Amazon River was higher than that observed in specimens of this species collected in the Atrato River in Colombia, a biodiversity hotspot that also has been devastated by human activities, mostly linked to gold mining (Palacios-Torres et al. 2020).

Fish are not only responsible for human exposure to Hg in the Amazon basin but also are good sources of nutrients. Given their high accessibility, they are the main food option that may ameliorate deficiencies of micronutrients, such as Se (Dorea 2003) in the Amazonian forest environment. It is well known that Se is an essential trace element for the maintenance of organism homeostasis, as it participates in many biochemical functions and its deficiency generates a set of adverse effects in the body, including thyroid disorders, arthritis, mental confusion, muscle weakness, and hair loss (Roman et al. 2014). The range of Se concentrations found in fish from the Amazon River (13–538 ng/g) was

much lower than that observed in samples from the Tapajos River (50–1006 ng/g) (da Silva et al. 2013) but similar to the concentrations found by Lino et al. (2018) for specimens from the same hydrographic zone (20–440 ng/g).

Previously, it has been suggested that a molar excess of Se (molar ratio Se:Hg > 1) may confer protection from Hg toxicodynamic effects derived from fish consumption (Mirlean et al. 2019). Given that samples of *P. granulosus*, *P. fasciatum, R. vulpinus*, and *P. tigrinum*, had and unfavorable Se:Hg molar ratio (< 1), Se levels in these fish species are not expected to protect their consumers against Hg toxicity (Albuquerque et al. 2020), and since their Hg HQ values also were not acceptable, consumption of these specimens may not be recommended for citizens of Puerto Nariño, including pregnant/breastfeeding women and children. Low levels of essential Se in the food that represents the main diet of the inhabitants of this municipality would intensify the vulnerable condition in which they are currently found. It is important to highlight that for more precise human health fish consumption advisories in future studies, in addition to this information, it is required to have accurate data on how these elements actually interact and on the relationship between the molar ratios and health outcomes.

Besides element concentrations in fish, actual measurements in humans together with the sociodemographic characterization of populations allow for an integrated view of exposure levels. In this study, the age of participants was similar (~ 35 years) for all volunteers, and 50% of the total population consumed alcohol more than once per week, which is agreement with the consumption patterns in other populations from the Amazon basin (Grotto et al. 2010). In addition, the schooling level of the citizens of Puerto Nariño was higher than that reported for the inhabitants of other locales in the Colombian Amazon (Valdelamar-Villegas and Olivero-Verbel 2019). Similarly, the frequency of self-reported symptoms with greater recurrence, including headache and change in mood, was higher than that found in the communities of Nueva Unión, Caña Brava, and Pupuña in the Cotuhe Reservation of the Colombian Amazon (Alcala-Orozco et al. 2019).

Hair analysis is a well-established and preferred method for estimating Hg exposure (Nuttall 2006). All samples were above the limit of 1.0 µg/g set by the USEPA (USEPA 1997), and 36% of the population exceeded the maximum level of 5.0 µg/g established by the WHO (WHO (World Health Organization), 2003), suggesting high exposure to Hg in Puerto Nariño. Mean hair T-Hg concentration in participants (5.31 µg/g) was greater than that observed in populations from Campo Verde (Vereador) (0.268 µg/g), Campo Verde (Itaobi) (0.290 µg/g), Davinopolis (0.48 µg/g), and Sao Antonio Rio Verde (0.96 µg/g) (Gardner et al. 2010) in the Brazilian Amazon and from Mindanao (Philippines) (4.14 µg/g) (Drasch et al. 2001). In contrast, it was lower

than the mean level found in indigenous peoples from Tapajós River (13.7 µg/g) (Grotto et al. 2010) in Brazil and at Caquetá River basin (17.3 µg/g) (Olivero-Verbel et al. 2016), and Tarapaca Village (10.6 µg/g) (Alcala-Orozco et al. 2019) in the Colombian Amazon. In Colombian areas impacted by Hg from ASGM activities, hair T-Hg from Puerto Nariño was above that reported in Paimado (0.87 µg/g) (Palacios-Torres et al. (2018), the Atrato River basin (4.90 µg/g), Tadó (1.30 µg/g), and Union Panamericana (Choco) (3.07 µg/g) in the Choco biogeographic area (Salazar-Camacho et al. 2017), as well as that registered in San Martin de Loba (Bolivar) (2.1 µg/g) (Olivero-Verbel et al. 2015), Morales (Middle Magdalena) (1.50 µg/g) (Carranza-Lopez et al. 2019), La Raya (Bolívar) (5.27 µg/g), Achi (Bolívar) (2.44 µg/g), Montecristo (Bolívar) (2.20 µg/g) (Olivero-Verbel et al. 2011), and Caimito (Sucre) (4.91 µg/g) (Olivero et al. 2002).

Total-Hg content in blood is useful in describing recent or current exposure levels (Gibb and O'Leary 2014). Mean Hg levels found in this matrix (13.7 ± 1.2 µg/L) was lower than that reported by Grotto et al. (2010) (47.8 ± 36.3 µg/L) for riparian communities located on the banks of the Tapajós River and by Castilhos et al. (2015) for miners from São Chico (27.7 ± 23.2 µg/L) and Creporizinho (25.2 ± 25.6 µg/L) in the Brazilian Amazon. Even though the study volunteers from Puerto Nariño were not engaged in mining activities, their average blood T-Hg concentration was higher than the levels reported for miners from the Ecuadorian coast (5.3 µg/L) (Harari et al. 2012) and from Mindanao (Philippines) (11.5 µg/L) (Drasch et al. 2001). In addition, more than 90% of the sample studied had Hg levels in blood that exceeded 5.0 µg/L, which suggests elevated exposure. The finding that a significant association was found between blood and hair T-Hg levels ($\rho = 0.635$; $p < 0.0001$) is supported by the fact that at the time of the formation of the hair strand, Hg levels in hair are usually proportional to blood concentrations (Castilhos et al. 2015). In addition, PCA analysis suggested that approximately 17.1% of the data variance was associated with Hg concentrations in these matrices.

In the case of Cd, 21% of samples exceeded the recommended CDC action value of 5 µg/L (CDC 2014). Although this action level is highly used as an occupational trigger guideline, it was employed given that Cd concentrations in blood of healthy unexposed adults are usually in the range of 0.1–4 µg/L, as reported by WHO (1992) and CDC (2014). Based on the low smoking rates observed in this study, the variability of dietary habits and the lack of correlation between Cd levels and the frequency of fish consumption, future studies that take into account other sources of dietary exposure, such as organ meats, leafy vegetables, potatoes and grains, peanuts, and soybeans, are highly encouraged given the ability of these foods to bioaccumulate Cd from soil. In addition, the use of cadmium-plated utensils for food

processing and preparation, and enamel and pottery glazes with cadmium-based pigments may be evaluated as potential sources of exposure. Mean Cd concentration (3.06 µg/L) found here was higher than that observed in volunteers from the Rio Branco municipality (North of Brazil) (0.09 µg/L) (Freire et al. 2015), Korea (0.78 µg/L) (Kim et al. 2017), Sweden (0.6 µg/L) (Wennberg et al. 2006), and São Paulo (0.6 µg/L) (Kira et al. 2016) and below that observed for the inhabitants of mining areas in Ghana (Tarkwa Nsuaem Municipality and Prestea Huni Valley District) (303 µg/L) (Obiri et al. 2016) and in Porto Alegre, Brazil (8.52 µg/L) (Baierle et al. 2014).

Based on observed Se levels, 84% of participants had blood concentrations < 100 µg/L, indicating deficiencies of the micronutrient and highlighting the lack of protection by Se in fish and the possible food insecurity to which these inhabitants are exposed (USEPA 2002; Valentini et al. 2016). In addition, these values were below the 500 µg/L action level set by the CDC (CDC 2014). Mean Se concentration (85.22 µg/L) was similar to the levels reported by Nunes et al. (2010) for subjects from São Paulo, Minas Gerais, Goiás, Pará, and Rio Grande do Sul in Brazil (89.3 µg/L) but much lower than the concentrations found in communities living along the Tapajos River (361.7 µg/L) (Lemire et al. 2006) and in Uummannaq (488 µg/L), Ittoqqortoormiit (225 µg/L), and Tasiilaq (191 µg/L) in Greenland (Hansen et al. 2004).

As observed in other studies (Barbosa et al. 2001; Dix-Cooper & Kosatsky 2018; McKelvey et al. 2007; Palacios-Torres et al. 2018; Vega et al. 2017), there were no significant associations between age and blood ($p = 0.636$) or hair ($p = 0.558$) T-Hg levels, or Cd ($p = 0.080$) or Se ($p = 0.628$) concentrations, which suggests that in this group, this is not a determining factor for the accumulation of these elements in these matrices. Regarding feeding habits, Spearman correlation analysis showed that fish ingestion might have an influence on the Hg levels observed in Puerto Nariño ($p < 0.05$). Such a relationship is supported by the fact that the majority of the participants eat fish on a daily basis. This finding is in agreement with the results obtained by Dix-Cooper and Kosatsky (2018), Webb et al. (2004), and Carranza-Lopez et al. (2019).

Another significant association found for the citizens of Puerto Nariño corresponded to Cd and Se concentrations (Spearman's $\rho = 0.524$, $p < 0.001$). It has been reported that interactions between these two elements exert a protective effect mediated via Cd sequestration into biologically inert conjugates. In addition, Se-dependent antioxidant enzymes may play a significant role in element detoxification (Zwolak & Zaporowska 2012). Further studies need to be performed to evaluate, in addition to element concentrations, the activity of the selenoproteins in populations highly exposed to Cd.

In this study, the hematological condition of volunteers reflected that even though average hematological values were within the reference data, some volunteers had low hematocrit percentages (< 36–48%), hemoglobin concentrations (< 11 g/dL), and erythrocyte counts (< 4000/mm³), which may be indicative of anemia, and, as observed in the PC analysis, most of the variance of the data (31.72%) was clearly associated with these hematological parameters. Changes in hematocrit percentages have been determined to be a consequence of chronic Hg poisoning (Ekawanti and Krisnayanti 2015; SHokooh Saljooghi and Delavar Mendi 2012). In addition, reduction of hemoglobin levels due to Hg exposure may be the result of a decrease in renal function and its influence on iron (Fe) metabolism and hemolysis (Ekawanti and Krisnayanti 2015; Ribarov et al. 1983). As observed in animal models, the main effect of Hg on the hematological system results in inhibition of heme synthesis, leading to Fe deficiency, anemia, and increased absorption of Hg (SHokooh Saljooghi and Delavar Mendi 2012). Literature also suggests that MeHg can induce anemia by triggering red blood cell death secondary to oxidative damage (Lang et al. 2006) and by mimicking or exacerbating vitamin B12 or folate deficiency (Brito et al. 2015). Moreover, MeHg exposure has been associated with anemia in children living near ASGM sites in the Peruvian Amazon (Weinhouse et al. 2017). It is important to mention that hematocrits usually reflect the amount of red cell mass as a proportion of whole blood (Myers et al. 2002), and low hemoglobin is usually represented by a low hematocrit and vice versa, which may explain the decrease in the percentages of these parameters in some volunteers.

Blood and hair T-Hg concentrations from the population studied were negatively correlated with lymphocyte percentages. Although previous studies performed in the Amazon region have concluded that changes in this parameter mainly concern the individual's general nutritional status and the existence of inflammatory or infectious processes derived from viruses or intestinal parasites (Rodrigues et al. 2017), cytogenetic damage, reflected as an impairment of lymphocyte proliferation, has been observed as a result of MeHg exposure in a population living on the banks of the Tapajós River in the Brazilian Amazon (Amorim et al. 2000). Given that inhabitants of the Amazon region are highly exposed to this toxic element and have a high and constant prevalence of infectious diseases (Fernández-Niño et al. 2017), future studies should be designed to evaluate the general clinical conditions of the population, which may include the diagnosis of parasitosis.

There were some limitations in this study that should be mentioned. First, the complexity of entering the municipality and the climatic conditions were factors that made it difficult to collect a greater number of human samples; however, the concentrations of the elements evaluated made

it possible to establish a baseline in this population and to show its exposure to Hg and Cd, as well as to document Se deficiencies and their associations with a set of hematological parameters. The above was confirmed by the levels of these elements found in the fish samples and most notably in those of higher trophic levels, which constitute the most consumed species in the rural communities of Puerto Nariño in the Colombian Amazon, an ecosystem that is home to at least 10% of the known biodiversity in the world.

Conclusions

The results of this study indicate that due to the association between Hg concentrations and fish consumption patterns and the outcomes of the risk assessment, inhabitants of Puerto Nariño should restrict the intake of high trophic level fish species, particularly *R. vulpinus* and *P. tigrinum,* whose Hg HQs were > 1 and Se:Hg molar ratios < 1. The government could provide special training for these populations on consuming proteins from different sources. Concentrations of Hg and Cd in hair and blood exceeded the reference values established by the environmental and health authorities, confirming exposure to these toxicologically importance elements by the inhabitants of this municipality. In addition, 84% of volunteers presented Se deficiencies. Therefore, the use of fortified foods and supplements to reduce the risk of deficiency or its effects in the region is highly encouraged. Given the varied diet of the inhabitants of Puerto Nariño, and because plant foods generally obtain selenium from soil, the analysis of this element in biota and in plant-based products highly consumed by the communities also is recommended for future studies. Total Hg was significantly associated with fish consumption and blood Se was correlated with Cd content. Interestingly, lymphocyte count was inversely associated with Hg levels, suggesting this metal is impacting the hematology profile in the population.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics Approval The protocols for this study were approved by the Ethics Committee of the University of Cartagena (Act No. 108-2018) and conducted in accordance with the Helsinki declaration for studies involving human participants.

Informed Consent Participants consented to participate in this project after being informed of its procedures, risks, and benefits. Volunteers signed an informed consent form after fully comprehending the information provided by the investigators regarding the research. This study was performed with the approval of the health department of the municipality of Puerto Nariño, of the Colombian Amazon.

References

Al-Saleh I, Al-Rouqi R, Obsum CA, Shinwari N, Mashhour A, Billedo G, Al-Sarraj Y, Rabbah A (2015) Interaction between cadmium (Cd), selenium (Se) and oxidative stress biomarkers in healthy mothers and its impact on birth anthropometric measures. Int J Hyg Environ Health 218:66–90. https://doi.org/10.1016/j.ijheh.2014.08.001

Albuquerque FEA, Minervino AHH, Miranda M, Herrero-Latorre C, Barrêto Júnior RA, Oliveira FLC, Sucupira MCA, Ortolani EL, López-Alonso M (2020) 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, Morillo-Garcia Y, Caballero-Gallardo K, Olivero-Verbel J (2017) Mercury in canned tuna marketed in Cartagena, Colombia, and estimation of human exposure. Food Addit-Contam Part B 10:241–247. https://doi.org/10.1080/19393210.2017.1323803

Alcala-Orozco M, Caballero-Gallardo K, Olivero-Verbel J (2019) Mercury exposure assessment in indigenous communities from Tarapaca village, Cotuhe and Putumayo Rivers, Colombian Amazon. Environ Sci Pollut Res 26:36458–36467. https://doi.org/10.1007/s11356-019-06620-x

Alonso ML, Benedito J, Miranda M, Castillo C, Hernández J, Shore R (2002) Contribution of cattle products to dietary intake of trace and toxic elements in Galicia, Spain. Food Addit Contam 19:533–541. https://doi.org/10.1080/02652030110113744

Alvarez-Ortega N, Caballero-Gallardo K, Olivero-Verbel J (2016) Low blood lead levels impair intellectual and hematological function in children from a tropical city. Toxicol Lett 259:S165–S166. https://doi.org/10.1016/j.toxlet.2016.07.393

Alvarez-Ortega N, Caballero-Gallardo K, Olivero-Verbel J (2019) Toxicological effects in children exposed to lead: a cross-sectional study at the Colombian Caribbean coast. Environ Int 130:104809. https://doi.org/10.1016/j.envint.2019.05.003

Amorim MI, Mergler D, Bahia MO, Dubeau H, Miranda D, Lebel J, Burbano RR, Lucotte M (2000) Cytogenetic damage related to low levels of methyl mercury contamination in the Brazilian Amazon. An Acad Bras Ciênc 72:497–507. https://doi.org/10.1590/S0001-37652000000400004

Ashie I, Smith J, Simpson B, Haard NF (1996) Spoilage and shelf-life extension of fresh fish and shellfish. Crit Rev Food Sci Nutr 36:87–121. https://doi.org/10.1080/10408399609527720

ATSDR (2008) Agency for toxic substances and disease registry. Chronic durational oral minimal risk level (MRL) of 0.1 µg/kg/day of cadmium based on its renal effects. https://www.atsdr.cdc.gov/csem/csem.asp?csem=6&po=7

Baierle M, Charão MF, Göethel G, Barth A, Fracasso R, Bubols G, Sauer E, Campanharo SC, Rocha RC, Saint'Pierre TD (2014) Are delta-aminolevulinate dehydratase inhibition and metal concentrations additional factors for the age-related cognitive decline?

Int J Environ Res Public Health 11:10851–10867. https://doi.org/10.3390/ijerph111010851

Balzino M, Seccatore J, Marin T, De Tomi G, Veiga MM (2015) Gold losses and mercury recovery in artisanal gold mining on the Madeira River, Brazil. J Clean Prod 102:370–377. https://doi.org/10.1016/j.jclepro.2015.05.012

Barbosa A, Jardim W, Dorea J, Fosberg B, Souza J (2001) Hair mercury speciation as a function of gender, age, and body mass index in inhabitants of the Negro River basin, Amazon, Brazil. Arch Environ Contam Toxicol 40:439–444. https://doi.org/10.1007/s002440010195

Basu N, Clarke E, Green A, Calys-Tagoe B, Chan L, Dzodzomenyo M, Fobil J, Long R, Neitzel R, Obiri S (2015) Integrated assessment of artisanal and small-scale gold mining in Ghana—Part 1: human health review. Int J Environ Res Public Health 12:5143–5176. https://doi.org/10.3390/ijerph120505143

Bjørklund G, Aaseth J, Ajsuvakova OP, Nikonorov AA, Skalny AV, Skalnaya MG, Tinkov AA (2017) Molecular interaction between mercury and selenium in neurotoxicity. Coord Chem Rev 332:30–37. https://doi.org/10.1016/j.ccr.2016.10.009

Boischio AAP, Barbosa A (1993) Exposição ao mercúrio orgânico em populações ribeirinhas do Alto Madeira, Rondônia, 1991: resultados preliminares. Cad Saude Publica 9:155–160. https://doi.org/10.1590/S0102-311X1993000200006

Bonotto DM, Wijesiri B, Vergotti M, da Silveira EG, Goonetilleke A (2018) Assessing mercury pollution in Amazon River tributaries using a Bayesian network approach. Ecotoxicol Environ Saf 166:354–358. https://doi.org/10.1016/j.ecoenv.2018.09.099

Braune BM (1987) Mercury accumulation in relation to size and age of Atlantic herring (*Clupea harengus harengus*) from the southwestern Bay of Fundy, Canada. Arch Environ Contam Toxicol 16:311–320. https://doi.org/10.1007/BF01054948

Brito A, Mujica-Coopman MF, Olivares M, López de Romaña D, Cori H, Allen LH (2015) Folate and vitamin B12 status in Latin America and the Caribbean: an update. Food Nutr Bull 36:S109–S118. https://doi.org/10.1177/0379572115585772

Burger J, Jeitner C, Schneider L, Vogt R, Gochfeld M (2010) Arsenic, cadmium, chromium, lead, mercury, and selenium levels in blood of four species of turtles from the Amazon in Brazil. J Toxicol Environ Health A 73:33–40. https://doi.org/10.1080/15287390903248877

Cabral A, Fé N, Suárez-Mutis M, Bóia M, Carvalho-Costa F (2010) Increasing incidence of malaria in the Negro River basin, Brazilian Amazon. Trans R Soc Trop Med Hyg 104:556–562. https://doi.org/10.1016/j.trstmh.2010.03.008

Carranza-Lopez L, Caballero-Gallardo K, Cervantes-Ceballos L, Turizo-Tapia A, Olivero-Verbel J (2019) Multicompartment mercury contamination in major gold mining districts at the department of Bolivar, Colombia. Arch Environ Contam Toxicol 76:640–649. https://doi.org/10.1007/s00244-019-00609-w

Carranza-Lopez L, Alvarez-Ortega N, Caballero-Gallardo K, Gonzalez-Montes A, Olivero-Verbel J (2020) Biomonitoring of lead exposure in children from two fishing communities at Northern Colombia. Biol Trace Elem Res. https://doi.org/10.1007/s12011-020-02207-7

Castilhos Z, Rodrigues-Filho S, Cesar R, Rodrigues AP, Villas-Bôas R, de Jesus I, Lima M, Faial K, Miranda A, Brabo 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

CDC (2012) Centers for disease control and prevention. Anthropometric Reference Data for Children and Adults: United States, 2007–2010. Available at: https://www.cdc.gov/nchs/data/series/sr_11/sr11_252.pdf

CDC (2014) Centers for disease control and prevention. Laboratory procedure manual: cadmium, lead, manganese, mercury, and selenium. Available at https://www.cdc.gov/Nchs/Data/Nhanes/Nhanes_13_14/PbCd_H_MET.pdf

Counter SA, Buchanan LH, Ortega F (2005) Mercury levels in urine and hair of children in an Andean gold-mining settlement. Int J Occup Environ Health 11:132–137. https://doi.org/10.1179/oeh.2005.11.2.132

Chen Y, Xu X, Zeng Z, Lin X, Qin Q, Huo X (2019) Blood lead and cadmium levels associated with hematological and hepatic functions in patients from an e-waste-polluted area. Chemosphere 220:531–538. https://doi.org/10.1016/j.chemosphere.2018.12.129

Chiera N, Aksenov N, Albin Y, Bozhikov G, Chepigin V, Dmitriev S, Dressler R, Eichler R, Lebedev VY, Madumarov A (2017) Interaction of elemental mercury with selenium surfaces: model experiments for investigations of superheavy elements copernicium and flerovium. J Radioanal Nucl Chem 311:99–108. https://doi.org/10.1007/s10967-016-5018-8

Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ (2010) Cadmium stress: an oxidative challenge. Biometals 23:927–940. https://doi.org/10.1007/s10534-010-9329-x

da Silva DS, Lucotte M, Paquet S, Brux G, Lemire M (2013) Inverse mercury and selenium concentration patterns between herbivorous and piscivorous fish in the Tapajos River, Brazilian Amazon. Ecotoxicol Environ Saf 97:17–25. https://doi.org/10.1016/j.ecoenv.2013.06.025

Dix-Cooper L, Kosatsky T (2018) Blood mercury, lead and cadmium levels and determinants of exposure among newcomer South and East Asian women of reproductive age living in Vancouver, Canada. Sci Total Environ 619–620:1409–1419. https://doi.org/10.1016/j.ecoenv.2013.06.025

Dorea JG (2003) Fish are central in the diet of Amazonian riparians: Should we worry about their mercury concentrations? Environ Res 92:232–244. https://doi.org/10.1016/S0013-9351(02)00092-0

Drasch G, Böse-O'Reilly S, Beinhoff C, Roider G, Maydl S (2001) The Mt. Diwata study on the Philippines 1999—assessing mercury intoxication of the population by small scale gold mining. Sci Total Environ 267:151–168. https://doi.org/10.1016/S0013-9351(02)00092-0

EC (2006) European Commission. Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs, Brussels

Ekawanti A, Krisnayanti BD (2015) Effect of mercury exposure on renal function and hematological parameters among artisanal and small-scale gold miners at Sekotong, West Lombok, Indonesia. J Health Poll 5:25–32. https://doi.org/10.5696/2156-9614-5-9.25

Famoofo O, Abdul W (2020) Biometry, condition factors and length-weight relationships of sixteen fish species in Iwopin fresh-water ecotype of Lekki Lagoon, Ogun State, Southwest Nigeria. Heliyon 6:e02957. https://doi.org/10.1016/j.heliyon.2019.e02957

FAO/WHO (1991) Food and Agriculture Organization of the United Nations/World Health Organization. Codex Alimentarius: Guideline Levels for Mercury in Fish (CAC/GL 7-1991) Taked by the Commission at its Nineteenth Session in Italy 1–10 July 1991

Faro A, Pinto W, Ferreira A, Barbosa F, Souza V, Fujimoto D, Koifman R, Koifman S (2014) Serum cadmium levels in a sample of blood donors in the Western Amazon, Brazil, 2010–2011. Cad Saude Publica 30:403–414. https://doi.org/10.1590/0102-311X00087113

Fernández-Niño JA, Astudillo-García CI, Segura LM, Gómez N, Salazar ÁS, Tabares JH, Restrepo CA, Ruiz MÁ, López MC, Reyes P (2017) Profiles of intestinal polyparasitism in a community of

the Colombian Amazon region. Biomédica 37:368–377. https://doi.org/10.7705/biomedica.v34i2.3395

Ferreira da Silva S, de Oliveira LM (2020) Mercury in fish marketed in the Amazon triple frontier and health risk assessment. Chemosphere 248:125989. https://doi.org/10.1016/j.chemosphere.2020.125989

Freire C, Koifman RJ, Fujimoto D, de Oliveira Souza VC, Barbosa F Jr, Koifman S (2015) Reference values of cadmium, arsenic and manganese in blood and factors associated with exposure levels among adult population of Rio Branco, Acre, Brazil. Chemosphere 128:70–78. https://doi.org/10.1016/j.chemosphere.2014.12.083

Gardner RM, Nyland JF, Silva IA, Ventura AM, de Souza JM, Silbergeld EK (2010) Mercury exposure, serum antinuclear/antinucleolar antibodies, and serum cytokine levels in mining populations in Amazonian Brazil: a cross-sectional study. Environ Res 110:345–354. https://doi.org/10.1016/j.envres.2010.02.001

Gibb H, O'Leary KG (2014) Mercury exposure and health impacts among individuals in the artisanal and small-scale gold mining community: a comprehensive review. Environ Health Perspect 122:667–672. https://doi.org/10.1289/ehp.1307864

Gómez R, Tabares E (2007) Economía y usos de la biodiversidad. Diver Biol Cult AmazColombDiagnós 4:309–310

Gómez-Restrepo C, Rincón CJ, Urrego-Mendoza Z (2016) Salud mental, sufrimiento emocional, problemas y trastornos mentales de indígenas colombianos. Datos de la Encuesta Nacional de Salud Mental 2015. Rev Colomb Psiquiatr 45:119–126. https://doi.org/10.1016/j.rcp.2016.09.005

González-Merizalde MV, Menezes-Filho JA, Cruz-Erazo CT, Bermeo-Flores SA, Sánchez-Castillo MO, Hernández-Bonilla D, Mora A (2016) Manganese and mercury levels in water, sediments, and children living near gold-mining areas of the Nangaritza River basin, Ecuadorian Amazon. Arch Environ Contam Toxicol 71:171–182. https://doi.org/10.1007/s00244-016-0285-5

Grotto D, Valentini J, Fillion M, Passos CJS, Garcia SC, Mergler D, Barbosa F Jr (2010) Mercury exposure and oxidative stress in communities of the Brazilian Amazon. Sci Total Environ 408:806–811. https://doi.org/10.1016/j.scitotenv.2009.10.053

Gundacker C, Komarnicki G, Zödl B, Forster C, Schuster E, Wittmann K (2006) Whole blood mercury and selenium concentrations in a selected Austrian population: Does gender matter? Sci Total Environ 372:76–86. https://doi.org/10.1016/j.scitotenv.2006.08.006

Hansen JC, Deutch B, Pedersen HS (2004) Selenium status in Greenland Inuit. Sci Total Environ 331:207–214. https://doi.org/10.1016/j.scitotenv.2004.03.037

Harari R, Harari F, Gerhardsson L, Lundh T, Skerfving S, Strömberg U, Broberg K (2012) Exposure and toxic effects of elemental mercury in gold-mining activities in Ecuador. Toxicol Lett 213:75–82. https://doi.org/10.1016/j.toxlet.2011.09.006

Hosseini M, Nabavi SMB, Parsa Y (2013) Bioaccumulation of trace mercury in trophic levels of benthic, benthopelagic, pelagic fish species, and sea birds from Arvand River, Iran. Biol Trace Elem Res 156:175–180. https://doi.org/10.1007/s12011-013-9841-2

Kehrig HdA, Malm O (1999) Methylmercury in fish as a tool for understanding the Amazon mercury contamination. ApplOrganometall Chem 13:689–696

Kim H-J, Lim H-S, Lee K-R, Choi M-H, Kang NM, Lee CH, Oh E-J, Park H-K (2017) Determination of trace metal levels in the general population of Korea. Int J Environ Res Public Health 14:702. https://doi.org/10.3390/ijerph14070702

Kira CS, Sakuma AM, De Capitani EM, De Freitas CU, Cardoso MRA, Gouveia N (2016) Associated factors for higher lead and cadmium blood levels, and reference values derived from general population of São Paulo, Brazil. Sci Total Environ 543:628–635. https://doi.org/10.1016/j.scitotenv.2015.11.067

Kosik-Bogacka D, Łanocha-Arendarczyk N, Kot K, Malinowski W, Szymański S, Sipak-Szmigiel O, Pilarczyk B, Tomza-Marciniak A, Podlasińska J, Tomska N (2018) Concentrations of mercury (Hg) and selenium (Se) in afterbirth and their relations with various factors. Environ Geochem Health 40:1–13. https://doi.org/10.1007/s10653-018-0081-4

Lang F, Lang KS, Lang PA, Huber SM, Wieder T (2006) Mechanisms and significance of eryptosis. Antioxid Redox Signal 8:1183–1192. https://doi.org/10.1089/ars.2006.8.1183

Lechler P, Miller J, Lacerda L, Vinson D, Bonzongo J-C, Lyons W, Warwick J (2000) Elevated mercury concentrations in soils, sediments, water, and fish of the Madeira River basin, Brazilian Amazon: A function of natural enrichments? Sci Total Environ 260:87–96. https://doi.org/10.1016/S0048-9697(00)00543-X

Lemire M, Mergler D, Fillion M, Passos CJS, Guimaraes JRD, Davidson R, Lucotte M (2006) Elevated blood selenium levels in the Brazilian Amazon. Sci Total Environ 366:101–111. https://doi.org/10.1016/j.scitotenv.2005.08.057

Li X, Yang H, Zhang C, Zeng G, Liu Y, Xu W, Wu Y, Lan S (2017) Spatial distribution and transport characteristics of heavy metals around an antimony mine area in central China. Chemosphere 170:17–24. https://doi.org/10.1016/j.chemosphere.2016.12.011

Lindell L, Åström ME, Sarenbo S (2010) Effects of forest slash and burn on the distribution of trace elements in floodplain sediments and mountain soils of the Subandean Amazon, Peru. J Appl Geochem 25:1097–1106. https://doi.org/10.1016/j.apgeochem.2010.04.014

Lino A, Kasper D, Guida Y, Thomaz J, Malm O (2018) Mercury and selenium in fishes from the Tapajós River in the Brazilian Amazon: an evaluation of human exposure. J Trace Elem Med Biol 48:196–201. https://doi.org/10.1016/j.jtemb.2018.04.012

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

Long GL, Winefordner JD (1983) Limit of detection a closer look at the IUPAC definition. Anal Chem 55:712A–724A. https://doi.org/10.1021/ac00258a001

Malm O, Pfeiffer WC, Souza CM, Reuther R (1990) Mercury pollution due to gold mining in the Madeira River basin, Brazil. Ambio 19:11–15 **0044-7447**

Martín-Doimeadios RR, Nevado JB, Bernardo FG, Moreno MJ, Arrifano G, Herculano A, do Nascimento J, Crespo-López M (2014) Comparative study of mercury speciation in commercial fishes of the Brazilian Amazon. Environ Sci Pollut Res 21(7466–74797466):7479

Martínez-Salcido AI, Ruelas-Inzunza J, Gil-Manrique B, Nateras-Ramírez O, Amezcua F (2018) Mercury levels in fish for human consumption from the Southeast Gulf of California: tissue distribution and health risk assessment. Arch Environ Contam Toxicol 74:273–283. https://doi.org/10.1007/s00244-017-0495-5

Maurice-Bourgoin L, Quiroga I, Chincheros J, Courau P (2000) Mercury distribution in waters and fishes of the upper Madeira rivers and mercury exposure in riparian Amazonian populations. Sci Total Environ 260:73–86. https://doi.org/10.1016/S0048-9697(00)00542-8

McClure LF, Niles JK, Kaufman HW (2016) Blood lead levels in young children: US, 2009–2015. Pediatrics 175:173–181. https://doi.org/10.1016/j.jpeds.2016.05.005

McKelvey W, Gwynn RC, Jeffery N, Kass D, Thorpe LE, Garg RK, Palmer CD, Parsons PJ (2007) A biomonitoring study of lead, cadmium, and mercury in the blood of New York city adults. Environ Health Perspect 115:1435–1441. https://doi.org/10.1289/ehp.10056

Mirlean N, Ferraz AH, Seus-Arrache ER, Andrade CFF, Costa LP, Johannesson KH (2019) Mercury and selenium in the Brazilian

subtropical marine products: food composition and safety. J Food Compos Anal 84:103310. https://doi.org/10.1016/j.jfca.2019.103310

Mohan S, Tiller M, van der Voet G, Kanhai H (2005) Mercury exposure of mothers and newborns in Surinam: a pilot study. J Clin Toxicol 43:101–104. https://doi.org/10.1081/CLT-50404

Moreno-Brush M, Rydberg J, Gamboa N, Storch I, Biester H (2016) Is mercury from small-scale gold mining prevalent in the southeastern Peruvian Amazon? Environ Pollut 218:150–159. https://doi.org/10.1016/j.envpol.2016.08.038

MSPS (2012) Ministerio de Salud y Protección Social. Resolución número 122 de 2012

Myers JW, Neighbors M, Tannehill-Jones R (2002) Principles of pathophysiology and emergency medical care. Cengage Learning, Boston

Nakayama SF, Iwai-Shimada M, Oguri T, Isobe T, Takeuchi A, Kobayashi Y, Michikawa T, Yamazaki S, Nitta H, Kawamoto T (2019) Blood mercury, lead, cadmium, manganese and selenium levels in pregnant women and their determinants: the Japan Environment and Children's Study (JECS). J Exp Sci Environ Epidemiol 29:633–647. https://doi.org/10.1038/s41370-019-0139-0

Noreen F, Sajjad A, Mahmood K, Anwar M, Zahra M, Waseem A (2019) Human biomonitoring of trace elements in scalp hair from healthy population of Pakistan. Biol Trace Elem Res. https://doi.org/10.1007/s12011-019-01906-0

Nunes JA, Batista BL, Rodrigues JL, Caldas NM, Neto JA, Barbosa F Jr (2010) A simple method based on ICP-MS for estimation of background levels of arsenic, cadmium, copper, manganese, nickel, lead, and selenium in blood of the Brazilian population. J Toxicol Environ Health 73:878–887. https://doi.org/10.1080/15287391003744807

Nuttall KL (2006) Interpreting hair mercury levels in individual patients. Ann Clin Lab Sci 36:248–261 **0091-7370**

Obiri S, Yeboah PO, Osae S, Adu-Kumi S (2016) Levels of arsenic, mercury, cadmium, copper, lead, zinc and manganese in serum and whole blood of resident adults from mining and non-mining communities in Ghana. Environ Sci Pollut Res 23:16589–16597. https://doi.org/10.1007/s11356-016-6537-0

Oestreicher JS, Lucotte M, Moingt M, Bélanger É, Rozon C, Davidson R, Mertens F, Romaña CA (2017) Environmental and anthropogenic factors influencing mercury dynamics during the past century in floodplain lakes of the Tapajós River, Brazilian Amazon. Arch Environ Contam Toxicol 72:11–30. https://doi.org/10.1007/s00244-016-0325-1

Oliveira RC, Dórea JG, Bernardi JV, Bastos WR, Almeida R, Manzatto ÂG (2010) Fish consumption by traditional subsistence villagers of the Rio Madeira (Amazon): impact on hair mercury. Ann Hum Biol 37:629–642. https://doi.org/10.3109/03014460903525177

Olivero J, Johnson B, Arguello E (2002) Human exposure to mercury in San Jorge river basin, Colombia (South America). Sci Total Environ 289:41–47. https://doi.org/10.1016/S0048-9697(01)01018-X

Olivero-Verbel J, Johnson-Restrepo B, Mendoza-Marín C, Paz-Martinez R, Olivero-Verbel R (2004) Mercury in the aquatic environment of the village of Caimito at the Mojana region, north of Colombia. Water Air Soil Pollut 159:409–420. https://doi.org/10.1023/B:WATE.0000049162.54404.76

Olivero-Verbel J, Caballero-Gallardo K, Negrete-Marrugo J (2011) Relationship between localization of gold mining areas and hair mercury levels in people from Bolivar, north of Colombia. Biol Trace Elem Res 144:118–132. https://doi.org/10.1007/s12011-011-9120-z

Olivero-Verbel J, Caballero-Gallardo K, Turizo-Tapia A (2015) Mercury in the gold mining district of San Martin de Loba, South of Bolivar (Colombia). Environ Sci Pollut Res 22:5895–5907. https://doi.org/10.1007/s11356-014-3724-8

Olivero-Verbel J, Carranza-Lopez L, Caballero-Gallardo K, Ripoll-Arboleda A, Muñoz-Sosa D (2016) Human exposure and risk assessment associated with mercury pollution in the Caqueta River, Colombian Amazon. Environ Sci Pollut Res 23:20761–20771. https://doi.org/10.1007/s11356-016-7255-3

Palacios-Torres Y, Caballero-Gallardo K, Olivero-Verbel J (2018) Mercury pollution by gold mining in a global biodiversity hotspot, the Choco biogeographic region, Colombia. Chemosphere 193:421–430. https://doi.org/10.1016/j.chemosphere.2017.10.160

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

Peña-Venegas CP, Valderrama AM, Muñoz LEA, Rúa MNP (2009) Seguridad alimentaria en comunidades indígenas del Amazonas: ayer y hoy. Instituto Amazónico de Investigaciones Científicas "SINCHI"

Peña-Venegas CP, Eduardo AL, Gerard V, Esteban L-BC, Edwin A (2014) Mining threats to ancient anthropogenic soils and other resources associated to indigenous food security in the Middle Caquetá River, Colombia. J Earth Sci Eng 4:372–377 **2159-581X**

Pfeiffer WC, de Lacerda LD (1988) Mercury inputs into the Amazon region, Brazil. Environ Technol 9:325–330. https://doi.org/10.1080/09593338809384573

Pinheiro M, Müller R, Sarkis J, Vieira J, Oikawa T, Gomes M, Guimaraes G, Do Nascimento J, Silveira L (2005) Mercury and selenium concentrations in hair samples of women in fertile age from Amazon riverside communities. Sci Total Environ 349:284–288. https://doi.org/10.1016/j.scitotenv.2005.06.026

Ribarov S, Benov L, Benchev I (1983) On the mechanism of mercury induced hemolysis. Gen Physiol Biophys 2:81–84

Rodrigues PC, Ignotti E, Hacon SS (2017) Association between weather seasonality and blood parameters in riverine populations of the Brazilian Amazon. J Pediatr 93:482–489. https://doi.org/10.1016/j.jped.2016.11.012

Roman M, Jitaru P, Barbante C (2014) Selenium biochemistry and its role for human health. Metallomics 6:25–54. https://doi.org/10.1039/C3MT00185G

Roney N, Abadin HG, Fowler B, Pohl HR (2011) Metal ions affecting the hematological system. Met Ions Life Sci 8:143–155. https://doi.org/10.1039/9781849732116-00143

Salazar-Camacho C, Salas-Moreno M, Marrugo-Madrid S, Marrugo-Negrete J, Díez S (2017) Dietary human exposure to mercury in two artisanal small-scale gold mining communities of northwestern Colombia. Environ Int 107:47–54. https://doi.org/10.1016/j.envint.2017.06.011

ShokoohSaljooghi A, DelavarMendi F (2012) The effect of mercury in iron metabolism in rats. J ClinToxicol 3:1–5. https://doi.org/10.4172/2161-0495.S3-006

Souza-Araujo J, Giarrizzo T, Lima M, Souza M (2016) Mercury and methyl mercury in fishes from Bacaja River (Brazilian Amazon): evidence for bioaccumulation and biomagnification. J Fish Biol 89:249–263. https://doi.org/10.1111/jfb.13027

Spiller HA (2018) Rethinking mercury: the role of selenium in the pathophysiology of mercury toxicity. J Clin Toxicol 56:313–326. https://doi.org/10.1080/15563650.2017.1400555

Trujillo F, Lasso C, Diazgranados M, Farina O, Pérez L, Barbarino A, González M, Usma J (2010) Evaluación de la contaminación por mercurio en peces de interés comercial y de la concentración de organoclorados y organofosforados en el agua y sedimentos de la Orinoquia. Biodiversidad de la Cuenca del Orinoco: Bases Científicas para la Identificación de Áreas Prioritarias para la Conservación y Uso Sostenible de la Biodiversidad 340–355

USEPA (1997) United States Environmental Protection Agency. Mercury study report to Congress. Volume 1. Executive summary. Research Triangle Park, NC (United States). Office of Air Quality Planning and Standards

USEPA (2000) United States Environmental Protection Agency. Reference Dose for Methylmercury. Washington, D.C., NCEA-S-0930. Available at https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=20873

USEPA (2002) United States Environmental Protection Agency. Integrated risk information systems (IRIS) for selenium. Washington: National Centre for Environmental Assessment, Office of Research and Development. 444 p.

USEPA (2014) United States Environmental Protection Agency. Risk-Based Concentrations Table. Regional screening level (RSL) summary table.

USEPA-FDA (2018) United States Environmental Protection Agency. Food and Drug Administration. EPA-FDA Fish Advice: Technical Information. Available at https://www.epa.gov/fish-tech/epa-fda-fish-advice-technical-information

Valdelamar-Villegas J, Olivero-Verbel J (2019) High mercury levels in the indigenous population of the Yaigojé Apaporis National Natural Park, Colombian Amazon. Biol Trace Elem Res. https://doi.org/10.1007/s12011-019-01760-0

Valentini J, Passos CJS, Garcia SC, Davidson R, Lucotte M, Mertens F, Romana C, Valadão LM, Charão MF, Baierle M (2016) Blood antioxidant nutrients in riparian villagers of the Brazilian Amazon: its associations with wet/dry seasons and modulation by sociodemographic determinants. Cadernos Saúde Coletiva 24:21–31

Vega CM, Godoy JM, Barrocas PR, Gonçalves RA, De Oliveira BF, Jacobson LV, Mourão DS, Hacon SS (2017) Selenium levels in the whole blood of children and teenagers from two riparian communities at the Madeira River Basin in the Western

Brazilian Amazon. Biol Trace Elem Res 175:87–97. https://doi.org/10.1007/s12011-016-0741-0

Ward DM, Nislow KH, Chen CY, Folt CL (2010) Rapid, efficient growth reduces mercury concentrations in stream-dwelling Atlantic salmon. Trans Am Fish Soc 139:1–10. https://doi.org/10.1577/T09-032.1

Webb J, Mainville N, Mergler D, Lucotte M, Betancourt O, Davidson R, Cueva E, Quizhpe E (2004) Mercury in fish-eating communities of the Andean Amazon, Napo River Valley, Ecuador. EcoHealth 1:SU59–SU71. https://doi.org/10.1007/s10393-004-0063-0

Webb J, Coomes OT, Ross N, Mergler D (2016) Mercury concentrations in urine of amerindian populations near oil fields in the peruvian and ecuadorian amazon. Environ Res 151:344–350. https://doi.org/10.1016/j.envres.2016.07.040

Weinhouse C, Ortiz EJ, Berky AJ, Bullins P, Hare-Grogg J, Rogers L, Morales A-M, Hsu-Kim H, Pan WK (2017) Hair mercury level is associated with anemia and micronutrient status in children living near artisanal and small-scale gold mining in the Peruvian Amazon. Am J Trop Med Hyg 97:1886–1897. https://doi.org/10.4269/ajtmh.17-0269

Wennberg M, Lundh T, Bergdahl IA, Hallmans G, Jansson J-H, Stegmayr B, Custodio HM, Skerfving S (2006) Time trends in burdens of cadmium, lead, and mercury in the population of northern Sweden. Environ Res 100:330–338. https://doi.org/10.1016/j.envres.2005.08.013

WHO (1992) World Health Organization. Environmental Health Criteria 134: Cadmium

WHO (2003) World Health Organization. Elemental mercury and inorganic mercury compounds: human health aspects

Zwolak I, Zaporowska H (2012) Selenium interactions and toxicity: a review. Cell Biol Toxicol 28:31–46. https://doi.org/10.1007/s10565-011-9203-9