



Research article

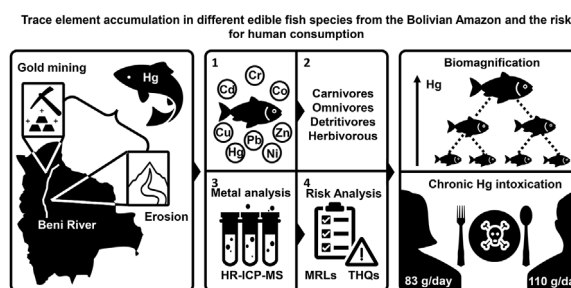
Trace element accumulation in different edible fish species from the Bolivian Amazon and the risk for human consumption

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HIGHLIGHTS

- Concentration of selected trace elements were examined in edible fish.
- Carnivorous fish species showed the highest concentrations of mercury.
- Biomagnification of methylmercury is present in the trophic webs of the Beni River.
- Only Mercury presented a potential health risk to humans.
- Chronic Hg poisoning can occur at low rates of fish ingestion for females and males.

GRAPHICAL ABSTRACT



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ABSTRACT

Artisanal mining and erosion of metalbearing soils can contaminate aquatic ecosystems and affect the health of riparian human populations, through metal bio-accumulation processes and fish consumption. Concentrations of eight trace metals (Cd, Cr, Co, Cu, Pb, Hg, Ni, Zn) and a metalloid (As) were measured in the muscle tissue of different edible fish species collected from markets of two cities along the Beni River banks, in the Bolivian Amazon. Relationships between the size of fish belonging to different trophic levels (carnivores, omnivores, detritivores and herbivorous) from four different fishing zones were analyzed. The most relevant results corresponded to the detritivore group, whose members exhibited significant positive correlations between the fish size and the concentration of three metals (cadmium, cobalt and nickel).

Furthermore, a 3×3 scenario-risk analysis was performed to assess local risk for human health. This was done by relating three different scenarios of local fish consumption collected from literature (maximum, average and minimum) and three different levels of trace element concentrations (95th, 50th and 5th percentile) derived from the present study and the Minimal Risk Levels suggested by the Agency for Toxic Substances and Disease Registry. Results of these calculations determined the amount of fish muscle per contaminant that could be consumed per day without risking human's health. Finally, Target Hazard Quotients were calculated for each trace element, aiming to indicate the potential exposure to each one and the concentration at which no adverse effects are expected.

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The obtained results made clear that mercury is the only trace element that represents an important health risk to humans within the studied region, considering most of the combinations in the 3×3 analysis. Chronic mercury intoxication could occur when consumption of fish in Riberalta or Rurrenabaque exceeded 83 g/day in females and 110 g/day in males.

1. Introduction

Despite having an important fishing potential, the annual per capita consumption of fish in Bolivia is low. Compared to the rest of South American countries, Bolivian's fish consumption is four times lower, with an average of 2 kg of fish per year (Wiefels 2006). This value is also well below the 12 kg of fish that FAO (2020) recommends for people to consume per year. The low fish demand and consumption in Bolivia results from the peculiarities of the country, including being a landlocked territory, the lack of fishery infrastructure and the lack of fish-eating tradition in the big cities (Camburn 2011).

Nonetheless, in some regions of the north and east part of the Bolivian Amazon, local indigenous communities consume considerably more fish than the rest of the country, as fish is their most important source of protein (Lee et al., 2021). As a matter of fact, in some of these communities, fish consumption can reach up to 40 kg of fish per capita per year (Camburn 2011; Van Damme et al., 2014). However, the degradation of aquatic ecosystems and the disruption of fish migration routes in Bolivia are threatening the contribution of fish to food security for these communities (Camburn 2011; Van Damme et al., 2019). In this framework, one of the most significant threats to aquatic ecosystems in Bolivia is the pollution by trace elements, caused by the country's long mining history and the erosion of natural metal-bearing soils (Maurice-Bourgoin and Quiroga 2002; Guzmán-Uria et al., 2020).

Among these contaminants, mercury is debatably the most important and hazardous one in the Bolivian territory, since the monomethylated form ($\text{CH}_3\text{-Hg}^+$ or MeHg) is able to enter the aquatic food webs on which Bolivian Amazonian communities are part of (Maurice-Bourgoin and Quiroga 2002; Benefice et al., 2010; Molina et al., 2010; Tschirhart 2011). The toxic mechanism of MeHg involves the increase of intracellular Ca^{2+} , the inhibition of protein synthesis, microtubule disruption, and overproduction of free radicals by the formation of reactive oxygen species (ROS) in kidney, liver and brain (Castro-González and Méndez-Armenta 2008; Lacerda et al., 2020). These impacts on human health are generally chronic, so the effects are long-lasting and can affect entire generations (de Bakker et al., 2021).

Monomethyl-mercury is closely related to fish consumption since it is highly available in aquatic environments, despite constituting only around 1 % of the total mercury pool. This happens because of its highly persistent and lipid-soluble nature, being very bioavailable to fish and easily accumulated especially in fatty tissue, accruing in aquatic food webs and biota in general (Mataba et al., 2016; Govaerts et al., 2018; Meneses et al., 2022). The consequences of this have been reported in several studies (Lee et al., 2021; Vasconcellos et al., 2021; Meneses et al., 2022) where people from indigenous communities of the Amazon and, more specifically, the Beni River (Lee et al., 2021) have shown high concentrations of mercury in their bodies.

Although there is still much uncertainty in this regard (Arrifano et al., 2018; Guzmán-Uria et al., 2020; Vasconcellos et al., 2021; Serra et al., 2021), it is probable that a significant part of the mercury in Amazonian ecosystems is the result of artisanal and small-scale gold mining (ASGM) (Gerson et al., 2022; Mestanza-Ramón et al., 2022). Mercury is a trace metal used to extract gold from rocks through an amalgamation process and it has been banned worldwide since the Minamata convention, due to the negative effects it causes on human health and aquatic ecosystems (Maurice-Bourgoin and Quiroga 2002). However, its use is still common in the Amazon, causing pollution of water bodies, soil and the atmosphere (Gerson et al., 2022).

Hence, in the case of the Bolivian Amazon, where fish is considered of paramount importance for local nutritional demand and an important income-generating good (Van Damme et al., 2008; Barbieri and Gardon 2009; Coca Méndez et al., 2012), trace element pollution is a direct infringement to the principle of food security. This situation is especially critical for indigenous communities, since their socioeconomic conditions do not allow them to have access to many other food alternatives (Guerrero 2001; Godfray et al., 2010; Camburn 2011).

Some efforts have been made to quantify and analyze the environmental situation related to trace contaminants in the Bolivian territory resulting in valuable information (Van Damme et al., 2008; Benefice et al., 2010; Pouilly and Pérez 2014; Guzmán-Uria et al., 2020). Nevertheless, characterizing trace element pollution in Bolivian aquatic ecosystems deserves continuing efforts to obtain environmental data that can show objectively the whole impact of these pollutants.

In this sense, the present study investigates the level of trace element found in fish sold at local markets of two important cities in the lowlands of the Bolivian Amazon (Riberalta and Rurrenabaque), where the commercialized fish is caught in rivers and lagoons from all the Beni river basin. This research also investigated the risks posed by consuming this protein source. Thus, the specific objectives are (1) to assess the human health risk associated with concentration of trace elements in the fish caught for sale, (2) to determine and compare the different risk values associated to different fish trophic levels, and (3) to determine and compare the different risk values associated to the different fishing sites where the sold fish originate from.

2. Materials and methods

2.1. Study area

All samples in this study were collected from local markets in Riberalta and Rurrenabaque, Amazonian towns located in the Beni River basin (part of the Bolivian Amazon basin). Rurrenabaque and Riberalta, are located in the upper and lower parts of the basin, respectively. Rurrenabaque is situated at the lowest portion of the Bolivian Andean piedmont, in the extreme west part of the Beni department. On the other hand, Riberalta is situated in northern Bolivia, more than 400 km downstream from Rurrenabaque, at the confluence of the Madre de Dios and the Beni rivers (Figure 1). Both towns are major regional commercial centers, where fish collected from all over the basin are gathered and sold (Maurice-Bourgoin et al., 2000; Maurice-Bourgoin and Quiroga 2002).

In order to organize collected data, four zones were defined by grouping several nearby capture fish sites, according to the information given by merchants. The different zones were determined as follows:

Zone 1: Included all collected samples from fishing sites upstream of the Beni River (around Rurrenabaque): Lower Beni River, Upper Beni River and Rurrenabaque surroundings.

Zone 2: Comprised fish samples caught in the Biata River and around Triunfo, an indigenous community settled close to the conjunction of the Biata and Beni Rivers.

Zone 3: Included all samples caught in the Yata River.

Zone 4: Consisted of all fish samples caught at the downstream part of the Beni River, including the surroundings of indigenous communities of 27 de Mayo and Nazareth.

Due to distance reasons, Rurrenabaque and Riberalta are completely independent markets, so there is no possibility of having Riberalta fish in Rurrenabaque, and vice versa.

2.2. Fish sampling, processing and transportation

The study was conducted during the dry season, between July and August of 2015, coinciding with the moment of major fish migrations and highest fishing production (Camburn 2011). In Riberalta, the sampling was done at the Mercado Central market and in Rurrenabaque, at the Los Sauces market. Overall, a total of 106 individual fish of different origin, trophic levels and sizes were collected (Table A1). Among these samples, 35 fish species were spread over 33 genera, 11 families and four orders. These species corresponded to four different trophic levels: carnivores (46 samples), detritivores (16 samples), herbivorous (20 samples) and omnivores (24 samples). Twenty-two samples were collected from Zone 1 (FB Coding), 27 samples in Zone 2 (IR Coding), 24 samples in Zone 3 (IR Coding) and 29 samples in Zone 4 (IR Coding).

Although it was intended to measure all specimens for standard length, some of them were already beheaded by the merchants at the moment of the collection. This was the case for 14 samples, most of which were collected at the Rurrenabaque market (11 specimens) and, therefore, were excluded from the calculation of the effect of size on trace element accumulation. Additionally, four samples corresponding to the species *Arapaima gigas* were impossible to measure according to the protocol, since the species can reach up to three or four meters and a weight up to 200 kg (Berra 2001; Nelson 2006; Coca Méndez et al., 2012) which is why vendors sell smaller portions (fillets). In this case, a length value of 123 cm was used for the five samples corresponding to this species, as an average value extracted from literature (Martinelli and Petrere, 1999).

Although muscle tissue in fish tends to accumulate lower concentrations of trace elements than liver and gills (with the exception of mercury

and arsenic) (Uysal et al., 2008; Lü et al., 2011; Mohan et al., 2012; Van Ael et al., 2017), only muscle samples were considered in this study. This decision was made as a more direct approach to a trace element risk analysis for human health, since people tend to only consume fish muscle. Evidence of this is the fact that merchants at both sampled markets often sell the fish previously beheaded and completely eviscerated in almost all cases.

Once collected, samples from Rurrenabaque and Riberalta were transported in cooler boxes to the laboratories of the Center for Food and Natural Products of the Mayor de San Simon University, in the city of Cochabamba, where they were stored in a freezer at -20 °C for two days and then freeze dried for 24 h. The freeze-drying process was carried on using an Alpha 2–4 LD Freeze dryer plus, with initial values of -45 to -40 °C and a reduced pressure of 0.07–0.12 mbar, during the first 22 h of drying. The second and final step of the process used a temperature of -76 °C and 0.001 mbar of pressure for the final 2 h.

Once freeze dried, fish samples were weighted again on an OHAUS Scout Pro SP x 4001 semi-analytical balance (with an accuracy of 0.1 g), in order to calculate wet weight/dry weight ratios (ww/dw). Finally, all samples were transferred to individual Ziploc bags and then, transported to the laboratories of the SPHERE group (Systemic Physiological and Ecotoxicological Research) in the University of Antwerp, to be further analyzed.

2.3. Sample analysis

In the SPHERE lab, fish samples were transferred to pre-weighed 10 ml polyethylene vials. In each case, a maximum of 0.5 g of sample were

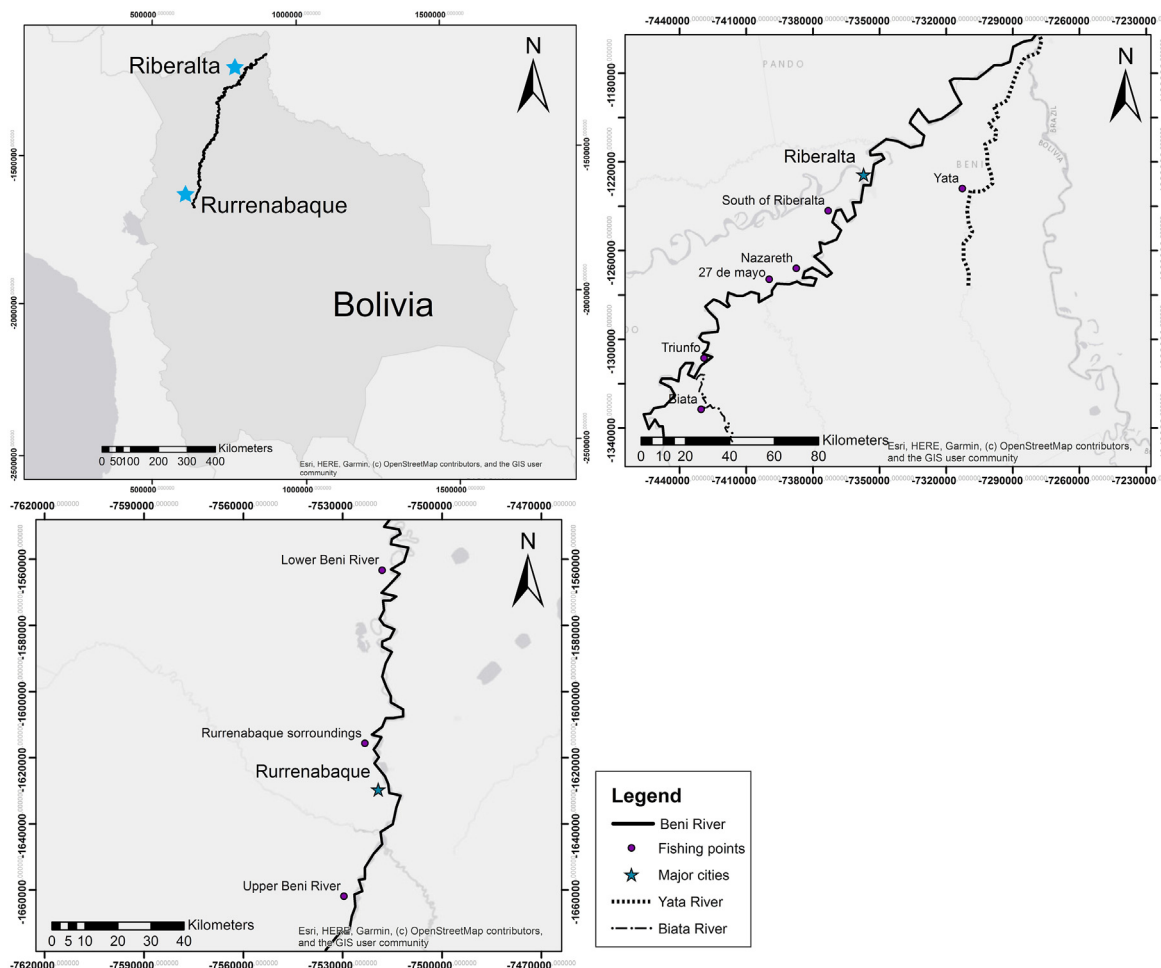


Figure 1. The Beni river basin with its respective tributaries, the reference cities (Riberalta and Rurrenabaque) and the fishing points.

transferred to the vials using a Mettler AT261 DeltaRange® (± 0.001 g) sensitive balance as a weighing instrument. Once weighed, samples were digested in two steps applying a SP-Discover Microwave (CEM, USA), as described by [Mataba et al. \(2016\)](#). For that, each freeze-dried sample was transferred from the polyethylene tubes to 35 ml glass vials (SPD-Discover) to be digested, as a previous step to the trace element analysis. In this stage, 4.5 ml of high purity of hydrochloric acid (HCl, 37 %) and 1.5 of high purity nitric acid (HNO₃, 69 %) were added to the samples and left to rest at least 24 h at room temperature. After that time lapse, 600 μ l

of high-purity hydrogen peroxide (H₂O₂, 29 %) was added to each sample, along with a magnetic stirrer.

Subsequently, the samples were digested according to the standard method described by [Mataba et al. \(2016\)](#). In this sense, all samples were digested in pre-cleaned glass vials using the SP-D-microwave digester under the following conditions: 120 °C, ramp time of 5 min, hold time of 5 min, pressure of 34 bars, power of 300 W and low stirring. As reference material, freeze-dried cod muscle (BCR 422) from the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium) was used.

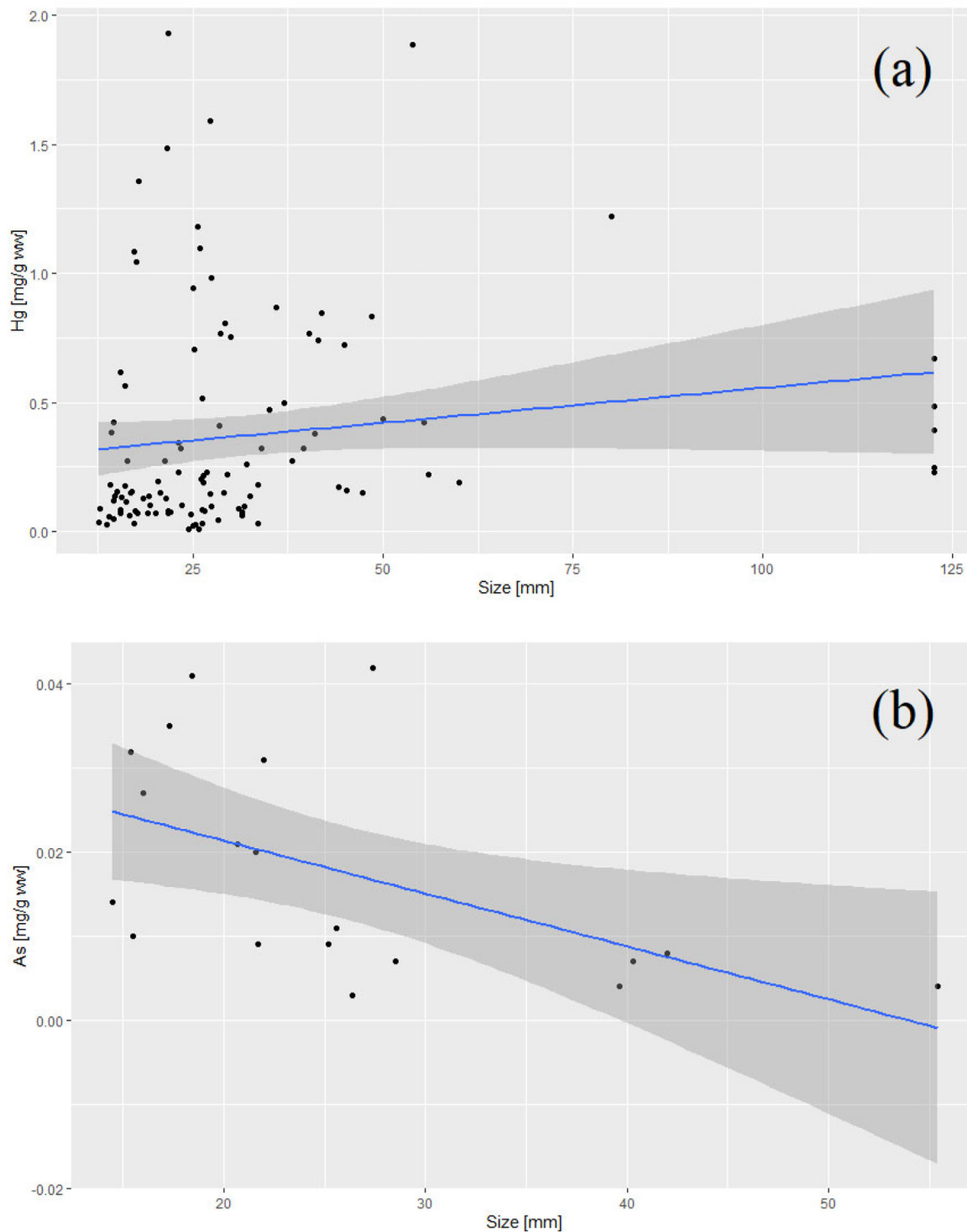


Figure 2. Correlations among: (a) Fish size and mercury concentrations throughout all the samples obtained from Rurrenabaque and Riberalta fish markets (Bolivian Amazon). (b) Concentration of arsenic in the samples of Zone 3.

After each vial had passed through the first digestion process in the SP-D microwave, all samples were opened and left to cool down under the fume hood.

During the second step, samples were digested at 160 °C, ramp time of 5 min, hold time of 5 min, pressure of 34 bars, power of 300 W and low stirring. Once again, afterwards, samples were left to cool down under the fume hood. Once all were digested, samples were transferred to 50 ml polypropylene vials and then, diluted with ultra-pure water (Milli-Q, Millipore, MA, USA) up to 30 ml, to finally be stored in freezers until the trace element analysis.

2.4. Trace element analysis

Trace element analysis of arsenic (As); cadmium (Cd); lead (Pb), chromium (Cr); cobalt (Co); nickel (Ni); copper (Cu); zinc (Zn) and mercury (Hg) were performed by using High Resolution Inductively Coupled Mass Spectrometry (HR-ICP-MS) in cold plasma mode (Thermo scientific Finnigan element 2, Waltham, MA, USA), with an instrumental detection limit of 0.001 µg/l. To obtain trace element concentration values in the fish (in µg of pollutant/g wet weight), calculations were done using the ww/dw ratio from the corresponding samples. The recovery percentages from the analysis of reference material were between 90 and 110 % for all trace elements.

In all the cases normality conditions were not fulfilled ($p < 0.050$), being that the observed data were closer to a Beta Distribution, which was determined through the *Descdist* function (Cullen and Frey Graph) using the *Fitdistrplus* program on the RStudio Platform (Delignette-Muller and Dutang 2015).

2.5. Data analysis

All obtained values below the detection limit, were replaced with DL/2 in order to further process the obtained data. All instrumental analyses were always performed in triplicate and in none of the cases the relative standard deviation of the replicates was more than 10%. Therefore, the mean of the three replicates was always used as results.

Initially, the observed data were log transformed but they biased from normal distribution. Therefore, in a first attempt, a beta correction was used in the correlation, as data were best described by this kind of distribution. However, it was decided to normalize the data for a better comparison and interpretation in relation with other studies that were able to normalize data. Hence, the observed data were normalized according to residual distribution, using the formula “ $1/\text{square root}(X + 1)$ ” in order to later, assess possible positive or negative correlations between the concentrations of different trace elements and the registered sizes, grouping samples by zones and by trophic levels). This calculation was made with the 0.5.3 program under the Kolmogorov-Smirnov test program in the RStudio platform, Build 554.

2.6. Risk analysis

Minimum Risk Levels (MRL) for oral intake of trace pollutants in the environment from a US standard (ATSDR, 2016), were calculated for different trace elements concentrations and the average body weight of a person: 70 kg was the average value used for males and 53 kg for females (Monroy et al., 2014).

Information extracted from ATSDR (2016) included MRL values for arsenic, cadmium, chromium, cobalt, copper, methyl-mercury and zinc. Whereas for lead and nickel, exposure values were extracted from the World Health Organization-Provisional Tolerable Weekly Intake list (WHO-PTWI) (WHO 2002, 2011). Also, chronic values for MRL's (corresponding to more than a one year of exposition to trace elements) were used whenever they were available (arsenic, cadmium, chromium, mercury and zinc).

Since MRL value for mercury is designed specifically for methyl-mercury, and during this particular study only total mercury was

measured, a conversion factor of 1.0 was used based on the methyl-mercury/total mercury proportion, assuming that 80–100 % of total mercury is in its methylated form (Malm et al., 1990; EFSA 2012).

The maximum amount (g) of trace element-polluted fish meat that a person (of 70 kg if male or 53 kg if female) expected to eat per day was calculated as follows (Verhaert et al., 2013, 2019; Mataba et al., 2016):

$$Q = Y/C \text{ where } Y = (W * M) * 1000$$

$$\text{Therefore: } Q = (W * M) * 1000/C$$

Where:

M = Minimum Risk Level (MRL) for oral intake of a trace element (mg/kg body weight/day).

W = 70 kg/53 kg (Average male weight/average female weight).

Y = Maximum amount of trace element (µg) a 70 kg/53 kg person can consume per day without posing health risk.

C = Trace element concentration in fish muscle (µg/g ww), using three different values: 5th, 50th and 95th percentile for each pollutant.

Q = Maximum amount (g) of contaminated fish muscle a 70 kg/53 kg person can consume per day without posing health risks.

Once the maximum edible amount of fish per day (g) was calculated, a 3 × 3 scenario risk analysis was performed using a combination of sampled fish data and information related to fish consumption in the Bolivian Amazon. This included values from some other nearby territories found in recent scientific literature. Once the above steps were done, Target Hazard Quotients (THQ) were calculated, aiming to determine the potential exposure to each trace element and the concentration at which no adverse effects are expected. This method was selected due to their many advantages:

- It is easy to interpret as a single numerical value explains a complex situation.
- It is easy to visualize, so it is possible to understand which combinations of scenarios pose more risk than others.
- The type of format in which the results are displayed is handy to compare data all over the world.
- It is a handy instrument to transmit scientific data and complex environmental situations to the nonscientific part of society.
- Therefore, it is an ideal method to help environmental authorities to take faster decisions. This is a key point since Bolivia is a ratifying state of the Minamata Convention on Mercury.

Target Hazard Quotients are calculated by dividing all the consumption values by the maximum amount (g) of contaminated fish muscle that a 70 kg/53 kg person can consume per day without posing health risks (Q). So:

$$\text{THQ} = \text{Consumption value}/Q$$

On the other hand, the 3 × 3 scenarios analysis considered three contamination scenarios, including the lowest (5th percentile), the median (50th percentile) and the highest (95th percentile) concentration values obtained for each trace element. Also, three different consumption values were taken into account, based on 11 case studies related to the fish consumption of indigenous communities from the Bolivian Amazon (Camburn 2011). In this sense, the lowest, average and highest consumption values considered in this study were: 22 g per person per day, 108.84 g per person per day and 222.93 g per person per day, respectively.

Moreover, for comparative purposes, the average value of fish consumption corresponding to three of the biggest cities in Bolivia was also considered in the risk analysis. Certainly, only the consumed percentage of fish originating in the Bolivian Amazon was considered for these cities (0.68 g per person per day) (Wiefels 2006).

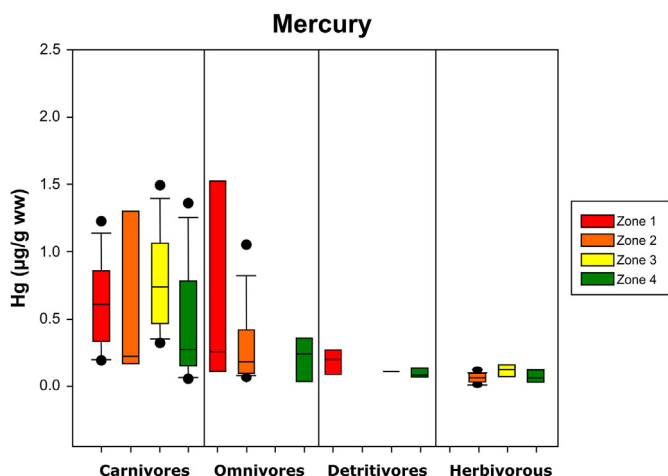


Figure 3. Median (0.19 µg/g ww), 5th (0.03 µg/g ww), and 95th (1.30 µg/g ww), percentile concentrations of mercury among 106 samples obtained from Rurrenabaque and Riberalta fish markets (Bolivian Amazon), taking into account the trophic level and the catching zone of each specimen.

3. Results

3.1. Correlation between fish size and trace element concentrations

In general, few statistically significant correlations were found between the size of the sampled fishes and the concentrations of the nine studied trace elements, both while grouping the samples by trophic level and while grouping them by zones.

Among the significant correlations found in this study, mercury concentrations presented a positive correlation with respect to the size of the fish, considering all 106 sampled individuals ($r = 0.28, p = 0.004$) (Figure 2, a).

On the other hand, when samples were grouped by zones, the highest calculated correlation was a negative one related to arsenic concentrations in Zone 3 ($p = 0.02, r = -0.53$) (Figure 2, b).

Finally, when grouping the samples by trophic levels, significant positive associations were found within detritivores and nickel ($p > 0.001$), cadmium ($p > 0.001$) and cobalt ($p = 0.003$). In the case of omnivores, significant negative correlations were found for copper ($p = 0.002$) and zinc ($p = 0.028$). No significant correlations were found for carnivores or herbivorous.

As seen in Figure 3, when considering all study areas, grouping the data by trophic levels revealed that the only significant correlation between trace element accumulation and fish size was the case mercury.

3.2. Risk analysis

The first step of the risk analysis consisted of calculating the maximum amount of fish from the Beni river basin, which can still be consumed without representing a risk to health. Taking as reference 70 kg of weight for a male person and 53 kg for females, mercury was the only trace element to exceed MRL values found in the literature (0.0003 µg/g ww/day) (WHO 2002, 2011; ATSDR 2016), considering the concentration data corresponding to the median, 95th and 5th percentile (Table 1).

Similarly, by calculating the target hazard quotients (THQ) for the average consumption scenario in Bolivia (109 g per person per day), mercury was the only trace element that represented a risk for human health for both male and female populations (Table 2). This occurred when the median and the 95th percentile of the data were considered (THQ >1, means that adverse health effects are possible, while THQ <1 indicates that no adverse health effects are expected as a result of exposure).

While performing the calculations under the minimum consumption scenario (22 g per person per day) once again, mercury was the only element to show a risk for human health (Table 2), but only under the maximum contamination scenario (95th percentile).

Values for maximum consumption scenario showed the same trend as the other scenarios (Table 2), positioning mercury as the most hazardous trace element of this study, while taking in to account the worst and medium contamination scenarios.

Used only as a comparison in this study, the calculation of THQ values related to the biggest three cities in Bolivia showed very low values for all the trace elements (Table 3), even under the highest contamination scenarios, due to the low levels of amazon fish consumption. Nevertheless, mercury values were the only ones higher than 0.01 while using high and medium contamination scenarios, both for males and females.

4. Discussion

4.1. Trace element levels in fish muscle

Trace element concentrations and its relation to human health risk can be expressed in terms of Minimum Risk Level (MRL) for oral intake (mg/kg body weight per day), indicating the risk of a pollutant by considering the intake levels (Verhaert et al., 2013, 2019; Geng et al., 2015; Mataba et al., 2016). In the following paragraphs an analysis of the obtained concentrations is made, mainly comparing mercury concentration (the most important pollutant in this study) to the corresponding standard in order to have a prior rough examination, before performing the correspondent risk analysis.

Table 1. Quantiles of the concentrations of the trace elements and the amount of fish muscle (in gram ww) per trace element that could be consumed per day without risking health, taking into account three different contamination scenarios (median, 95th, 5th percentile (µg/g ww)) and 70 kg of body weight for males and 53 kg for females.

5 th percentile (µg/g ww)									
	As (<0.01)	Cd (<0.01)	Cr (<0.01)	Co (<0.01)	Cu (0.09)	Pb (0.01)	Hg (0.03)	Ni (0.02)	Zn (2.20)
Q Male (g ww/day)	4.35×10^5	2.53×10^3	1.34×10^6	1.83×10^7	7.99×10^3	3.93×10^3	7.18×10^2	1.13×10^6	9.53×10^3
Q Female (g ww/day)	3.30×10^5	1.91×10^3	1.01×10^6	1.38×10^7	6.05×10^3	2.98×10^3	5.43×10^2	8.52×10^5	7.21×10^3
Median (µg/g ww)									
	As (0.01)	Cd (0.01)	Cr (0.01)	Co (<0.01)	Cu (0.18)	Pb (0.01)	Hg (0.19)	Ni (0.03)	Zn (2.84)
Q Male (g ww/day)	1.89×10^3	1.26×10^3	4.94×10^3	2.17×10^5	4.04×10^3	2.05×10^3	1.10×10^2	5.87×10^5	7.40×10^3
Q Female (g ww/day)	1.43×10^3	9.54×10^2	3.74×10^3	1.64×10^5	3.06×10^3	1.55×10^3	83	4.45×10^5	5.60×10^3
95 th percentile (µg/g ww)									
	As (0.04)	Cd (0.02)	Cr (0.22)	Co (0.06)	Cu (1.23)	Pb (0.05)	Hg (1.30)	Ni (0.10)	Zn (6.57)
Q Male (g ww/day)	4.98×10^2	5.05×10^2	3.56×10^2	1.49×10^4	6.06×10^2	5.44×10^2	16	2.49×10^5	3.93×10^3
Q Female (g ww/day)	3.77×10^2	3.83×10^2	2.69×10^2	1.13×10^3	4.59×10^2	4.12×10^2	12	1.89×10^5	2.97×10^3

Table 2. Hazard quotient for the nine trace elements under three different consumption scenarios for both the median and 95 percentile trace element concentrations in fish muscle: average consumption (109 g per person per day), minimum consumption (22 g per person per day) and maximum consumption (223 g per person per day).

5 th percentile		As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Zn
Minimum consumption scenario	THQ Male	<0.01	0.01	<0.01	<0.01	<0.01	0.01	0.03	<0.01	<0.01
	THQ Female	<0.01	0.01	<0.01	0.01	<0.01	0.01	0.04	<0.01	<0.01
Average consumption scenario	THQ Male	<0.01	0.04	<0.01	<0.01	0.01	0.03	0.15	<0.01	0.01
	THQ Female	<0.01	0.06	<0.01	<0.01	0.02	0.04	0.20	<0.01	0.02
Maximum consumption scenario	THQ Male	<0.01	0.09	<0.01	<0.01	0.03	0.06	0.31	<0.01	0.02
	THQ Female	<0.01	0.12	<0.01	<0.01	0.04	0.07	0.41	<0.01	0.03
Median		As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Zn
Minimum consumption scenario	THQ Male	0.01	0.02	<0.01	<0.01	0.01	0.01	0.20	<0.01	<0.01
	THQ Female	0.02	0.02	0.01	<0.01	0.01	0.01	0.26	<0.01	<0.01
Average consumption scenario	THQ Male	0.06	0.09	0.02	<0.01	0.03	0.05	0.99	<0.01	0.01
	THQ Female	0.08	0.11	0.03	<0.01	0.04	0.07	1.31	<0.01	0.02
Maximum consumption scenario	THQ Male	0.12	0.18	0.05	<0.01	0.06	0.11	2.03	<0.01	0.03
	THQ Female	0.16	0.23	0.06	<0.01	0.07	0.14	2.68	<0.01	0.04
95 th percentile		As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Zn
Minimum consumption scenario	THQ Male	0.04	0.04	0.06	<0.01	0.04	0.04	1.36	<0.01	0.01
	THQ Female	0.06	0.06	0.08	<0.01	0.05	0.05	1.79	<0.01	0.01
Average consumption scenario	THQ Male	0.22	0.22	0.31	0.01	0.18	0.20	6.72	<0.01	0.03
	THQ Female	0.29	0.28	0.40	0.01	0.24	0.26	8.87	<0.01	0.04
Maximum consumption scenario	THQ Male	0.45	0.44	0.63	0.01	0.37	0.41	13.76	<0.01	0.06
	THQ Female	0.59	0.58	0.83	0.02	0.49	0.54	18.17	<0.01	0.07

Table 3. Target hazard quotients (THQ) for an average consumption scenario in the three biggest cities in Bolivia (0.68 g per person per day).

5 th percentile		As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Zn
THQ Male (city)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
THQ Female (city)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Median		As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Zn
THQ Male (city)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01
THQ Female (city)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.01	<0.01	<0.01
95 th percentile		As	Cd	Cr	Co	Cu	Pb	Hg	Ni	Zn
THQ Male (city)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.04	<0.01	<0.01
THQ Female (city)		<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	0.06	<0.01	<0.01

In this sense, most of the consulted literature related to the presence of trace metals in the Bolivia Amazon, focus primarily on the study of mercury concentrations due to potential health risk this metal represents and the magnitude of gold mining in this area (Maurice-Bourgoin et al., 2000; Maurice-Bourgoin and Quiroga 2002; Benefice et al., 2010). However, we tried as much as possible to compare all trace element results to national experiences or experiences in the Amazon basin.

In the present study, analyzed samples seemed to be free of arsenic contamination as the median value (0.01 µg/g ww) and the highest concentration registered (0.06 µg/g ww) were lower than the international limit of 0.1 µg/g ww (Burger and Gochfeld 2005). This happens despite the fact that arsenic is commonly enriched in gold-bearing mineral deposits which is the case of the study area (Lechler et al., 2000). Given the vast mining history of Bolivia, arsenic pollution is a common documented situation. Nevertheless, most studies have fixated on the Altiplano side of the country and usually focusing on soil, ground and surface water samples (Mercado et al., 2009; Archer et al., 2014; Muñoz et al., 2015). Compared to similar studies in South America, the values found in the Beni River are relatively low. For example, maximum arsenic concentrations have been found in muscle of various commercial

fish species ranging from 0.76 mg/kg ww in Buenos Aires markets, Argentina (Avigliano et al., 2015); to 23.50 mg/kg in certain estuaries of the Argentine South Atlantic Ocean. Taking into account some studies from Brazil, the average arsenic concentrations in fish from naturally contaminated rivers reached 1.76 µg/g ww (Rosso et al., 2013) and 1.90 µg/g dw (Juncos et al., 2016) whereas up to 19.40 µg/g dw in Taim wetlands (Quintela et al., 2019).

Chromium and cobalt were the trace metals with the highest number of samples under the detection limit (17 and 27 respectively) and they both presented median and maximum concentration values below the standard thresholds for edible fish (1.00 µg/g ww) (Burger and Gochfeld 2005). One of the only studies held in the Bolivian Amazon basin, related to chromium pollution in biota (edible turtles) showed also low concentrations of chromium in comparison to other elements (Burger et al., 2009). This could be explained due to the fact that relatively acidic environments with high organic content, like the one of the present study, promote the reduction of Cr (VI) to nonhazardous Cr (III), making this element to be even less risky to humans (Zhitkovich 2011).

Nickel concentrations registered in the present study were below the standard limit in all cases, with a maximum measured value of 0.18 µg/g

ww, where the limit suggested by the USFDA (1993) is almost 400 times higher. By comparing these results to others in the Amazon basin, they seem to be consistent with what was found in the Madeira River by Sousa et al. (2015), where nickel levels registered in fish did not exceed 0.19 µg/g ww.

This study did not show signs of lead contamination in fish for human consumption, as just one sample reached the strictest international limits (0.20 mg/kg ww (European Union 2008)). Although not referred to the study area, but also within the Bolivian territory, the study of Stassen et al. (2012) in the Pilcomayo and Bermejo rivers, showed similar results, since EU limits for lead were not exceeded. Reported cases showing actual lead contamination of edible fish are usually related to heavy industrial pollution and oil tankers (Andreji et al., 2005, 2006; Rahimi and Gheysari 2016). In the case of the Beni River basin, these kinds of scenarios do not represent an ecological problem, since there are not present in the area.

Copper was one of the only micro nutrients analyzed in this research, essential for fish in relatively important amounts (Clearwater et al., 2002) which is why it was expected to find relatively higher concentrations in comparison to the others. In this sense, the copper median concentration value was one of the highest in this study (0.17 µg/g ww), but still below the most conservative standard limit for this metal found in the literature (20.0 µg/g ww; Uysal et al., 2008). The highest registered concentration value for the present study was 4.65 µg/g ww. While comparing this research to similar ones in Brazil, results here showed lower levels in comparison to the study of de Carvalho Costa and Hartz (2009), for example, where *Leporinus obtusidens* samples registered mean values in muscle between 0.56 µg/g and 1.65 µg/g ww. On the other hand, in the research of Medeiros et al. (2012) edible fishes exhibited median copper values between 1.00 µg/g and 2.80 µg/g among different species, surpassing what was obtained for the Beni River. Moreover, literature suggest levels of copper found in the present study (less than 1 µg/g) would pose no threat to humans (Burger et al., 2002; Clearwater et al., 2002; Medeiros et al., 2012).

Similar to copper, zinc is an essential element, so it was expected to be naturally present in high concentrations among the samples, as it is also an essential element. Nevertheless, in this study, zinc levels reached 10.00 µg/g of wet weight (maximum value found in a carnivore species), still not representing a real threat to human health, especially when compared to the values of maximum standards for edible fish (Burger and Gochfeld 2005). In this sense, results obtained in Riberalta and Rurrenabaque are consistent with what was found by Molina et al. (2012), in the Poopó Lake (western part of Bolivia) where zinc levels of fish were even 50 times higher than the other assessed elements. This is also shown by Mataba et al. (2016) and de Carvalho Costa and Hartz (2009) for the Thigithe River of Tanzania and the Guaíba Lake of Brazil, respectively, where zinc (and copper) concentrations in edible fish samples were the highest in comparison to all the assessed trace elements.

In an opposite trend to the rest of trace elements, cadmium concentrations measured in this study were closer to what is established as a limit for metal pollutants standards in edible fish, with median and maximum values of 0.01 and 0.03 µg/g ww respectively, whereas the maximum threshold for human health risk is 0.05 µg/g ww (European Union 2008). Moreover, these results are higher than the ones registered in another study in Bolivian territory (Stassen et al., 2012) where cadmium concentrations on the muscle tissue of *Pimelodus clarias* specimens, for example, showed median values of 0.02 µg/g and 0.01 µg/g ww for the Pilcomayo and Bermejo rivers respectively. Nevertheless, as will be evident in the risk analysis (by using THQs), these apparently high levels of cadmium in fish from Riberalta and Rurrenabaque, are actually not threatening to human health. This happens because, for that specific type of calculation, the standard limit (minimal risk levels) differed from the parameters used in this part of the analysis.

Finally, mercury values in this study ranged from a minimum of 0.009 µg/g ww to a maximum of 1.93 µg/g ww, with a median value of 0.19 µg/g ww. These results were the most alarming, since they are way higher

than the standard limit of 0.02 µg/g ww (European Union 2008), with most of the samples above this value. This seems to be an indicator of significant mercury contamination in fish for human consumption for the study area. These results coincide with what was documented in the studies of Maurice-Bourgoin et al. (1999, 2000) for the Beni River, and the results of (Rivera et al., 2016) who found similar values for the same basin, in fish and *Caiman yacare* specimens (0.21 ± 0.22 µg/g ww), also consumed by indigenous people of the Amazon.

Furthermore, by overlaying the results of mercury concentrations in this research to other local studies that examined the levels of this metal in the abiotic compartments of the ecosystem (water, suspended particles and sediment) (Maurice-Bourgoin et al., 1999, 2000; Guzmán-Uria et al., 2020) and/or other links in the food web (invertebrates) (Molina et al., 2010), it seems that mercury pollution has an important presence, especially in certain areas of the Beni River related to gold mining (Maurice-Bourgoin et al., 1999).

Most of the samples with mercury concentrations above the European Union Commission biota quality standard of 0.02 µg/g ww (European Union 2008) belonged to carnivorous species. This differentiation in mercury concentrations according to trophic levels in fish has not only been widely documented at an international level (Gammons et al., 2006; Mohan et al., 2012; Mosquera-Guerra et al., 2019; Albuquerque et al., 2020) but also previous studies in the Beni River basin have shown similar results. In the study of Maurice-Bourgoin et al. (1999) mercury in muscle of fish from Rurrenabaque carnivore species ranged from 0.70 to 1.80 µg/g ww, whereas concentrations in herbivorous species ranged low enough to be considered edible. The same tendency was observed by Maurice-Bourgoin et al. (2000), for sampling sites around Riberalta, with mercury concentrations in non-carnivorous fish ranging from 0.01 to 0.20 µg/g ww, while carnivorous species reached a maximum level of 2.30 µg/g ww and an average of 1.00 µg/g ww. This trend does not appear to have diminished over time, as Rivera et al. (2016) study for the same basin, showed that muscular tissues of carnivorous fish harbored the highest total mercury concentrations, ranging from 0.35 to 1.27 Hg µg/g ww.

Both the present study and the ones found in the literature (Maurice-Bourgoin et al., 1999, 2000; Rivera et al., 2016) could be an indication of a mercury bioamplification process in the food web of the Beni River. This can be explained by the fact that methyl-mercury is highly lipophilic (hydrophobic) and will mainly accumulate via the food. In fact, this pollutant acts as an organic pollutant rather than a metal (Malm et al., 1990). Yet, due to the methodology followed in the present research, the obtained results only express values of total mercury in the samples and do not express separate methyl-mercury content. Nevertheless, literature has suggested that depending on the species, methyl-mercury can constitute an important percentage of total mercury in fish tissues, especially when muscle tissue is analyzed, constituting 80–100 % of total mercury in some cases (Lino et al., 2019). Among different fish tissues tested in literature, muscle contained the highest ratio of methyl-mercury/total mercury in most fish species (Bloom 1992; Peng et al., 2016; Yoon et al., 2018; de Queiroz et al., 2019), since muscle is a major tissue for high metal storage, being methyl-mercury an important one due to its negligible elimination (Peng et al., 2016).

Although it appears that anthropogenic pollution related to gold mining has had an important impact on certain areas of the Beni River, it is important to point out that not all mercury present in the Bolivian Amazon basin has an anthropogenic origin. There are several places in this basin's soils, where pre-anthropogenic mercury represents more than 97% of the total concentration of this metal (Maurice-Bourgoin et al., 1999). In some Andean tributaries of the Amazon River these values are the result of rainy season-soil erosion processes (weathering of mercury-bearing rocks, contaminated through natural crustal degasification and dissolution) and other natural sources such as volcanism (Maurice-Bourgoin and Quiroga 2002) which could also have caused mercury concentrations in other biotic and abiotic compartments of the ecosystem including water, suspended particles, sediment and

invertebrates (Maurice-Bourgoin et al., 1999, 2000; Molina et al., 2010). Globally, it is estimated that only 30% of mercury in the environment is the result of human activities (de Bakker et al., 2021).

However, no clear differences in accumulation among areas were found for any of the trace elements including mercury. This contrasts with the results of Maurice-Bourgoin et al. (1999) for the same river, who found clear differences in mercury concentrations throughout the waterbody, both in particulate matter and water samples, where it seemed that mercury emitted by gold mining activities does not contaminate surface water directly in the outputs of the mines but pollution is worst in the discharge system of those sub-basins. This could be an indication that distribution of mercury in fish in this particular area, depends mainly on their dietary habits and less so on environmental factors.

4.2. Correlation between fish size and trace element concentrations

In general, few statistically significant correlations were registered between the size of the sampled fishes and the concentrations of the nine studied elements, both when grouping the samples by trophic level as when grouping them by zones. Yet it is important to mention that, it still possible that the few significant correlations found could have been by coincidence. The effect of size on trace element concentration levels can only be assessed correctly if environmental concentrations data is also known and is constant during the exposure of the fish (Burger et al., 2002; Merciai et al., 2014). Since we have no information on the environmental concentrations in the present study, the studied relationships are only indicative and should be interpreted with caution.

Overall, the positive correlation found between mercury concentrations and the size of individuals (Figure 2, a) turns out to be an expected finding. This particular relationship has been studied and identified in several studies in various species of fish, over time (Kraepiel et al., 2003; Campbell et al., 2004; Bosch et al., 2016). This phenomenon is explained by the fact that mercury bioaccumulates in the body of the fish throughout their life cycle. Therefore, the larger (older) the fish, the more contaminant accumulated (Costa et al., 2020). Nevertheless, it is worth considering that, although Hg accumulates, it could also tend to be diluted by the increased fish weight (growth dilution), balancing out the concentrations (Mailman et al., 2006).

Therefore, a more likely component to contemplate in this case, is that in most of the aquatic food webs, the guilds with the largest specimens tend to be the groups of carnivores and omnivores (Dorea et al., 2006; Souza-Araujo et al., 2016), this correlation would be confirming once again the bioaccumulation of this trace metal through the food web in the Beni River.

In this sense, taking into account the different trophic levels, most of the significant positive correlations corresponded to the detritivores, when associating cadmium, cobalt and nickel to the size of the fish. Nevertheless, it is also important to mention, that unlike other trophic levels in this particular study, one species (*Prochilodus nigricans*) constitute more than 80% of the samples of the group. In that sense, it is explained by several authors that species like this, who feed on bottom mud detritus, are generally considered to be very susceptible to accumulate high levels of trace elements on their tissues (Lombardi et al., 2010; Jarić et al., 2011; Schenone et al., 2014).

In the case of omnivores, a negative association was observed between zinc and the specimen's size. These results seem to disagree with some studies found in the literature, where the correlations between the concentration of this element and the fish size showed a positive trend (de Carvalho and Hartz 2009; Naeem et al., 2011; Yi and Zhang 2012), with the difference that most of these examples of positive correlations are related to liver concentrations and not to muscle tissue.

Nevertheless, all around the world the vast majority of the study cases (held in different type of aquatic environments) seem to match the results of negative correlations between zinc and the size obtained for fish samples of the Bolivian Amazon (Canli and Atli 2003; McKinley et al.,

2012; Merciai et al., 2014). The explanation behind this type of correlation may be related to the fact that essential metals like zinc typically do not rise in concentration with age or size since they are thought to be under homeostatic control. Also, concentration might decrease due to growth dilution (Evans et al., 1993).

On the other hand, in the case of copper within the guild of omnivores, when comparing this correlation to the literature, it seems to exist more ambiguity than with the case of zinc, since in a number of cases copper/size correlations proved to be not even statistically significant (Evans et al., 1993; Burger et al., 2002; Merciai et al., 2014).

4.3. Risk analysis

From the calculated THQs in samples from markets at Rurrenabaque and Riberalta, it was evident that mercury is the most hazardous element in the study. This was also found in other studies performed in the same area (Benefice et al., 2010; Molina et al., 2010; Tschirhart 2011). Unlike other studies, this one did analyze the presence of other trace elements in addition to mercury, although no risk was found from the other measured analytes. This is not surprising since most trace elements, even at high concentrations in other tissues such as liver and kidney, do not tend to accumulate up to high concentrations in the muscle (Bervoets and Blust 2003; Van Ael et al., 2017). On the contrary, due to its lipophilic properties, mercury will accumulate in the muscle tissue (Mataba et al., 2016).

In a similar analysis, Mataba et al. (2016) concluded that a person who feeds from fish at the Thigithe River in Tanzania would be able to eat up to 179 g per day without risking their health due to mercury presence. Under the same premise, when the median mercury concentration was used in the calculations for the present study, a female who eat fish from Riberalta or Rurrenabaque markets would be only capable of eating 83 g per day without putting their health under the risks of chronic mercury intoxication, while men could consume up to 110 g per day. However, in the worst-case scenario, when the most contaminated fish are consumed, ingestion of 16 and 12 g for respectively males and females may already pose a health risk.

In this sense, a remarkable outcome of the present analysis has to do with the differentiation of results between female and male population, by showing women's particular vulnerability to trace element poisoning. For the three tested pollution scenarios (5th, 50th and 95th percentile), women are always more susceptible to risk their health with 25% less fish to be consumed. It is important to mention, though, that this situation is based on the assumed weight of males and females. Therefore, following this analysis, a woman that weighs the same as a man should experience the same risk. Comparing "safe" intake level or Provisional Tolerable Weekly Intake (PTWI) of 5 µg/kg of body weight Hg, proposed by the World Health Organization, risk values for the Beni River basin are high, especially taking into account the 95th and the 50th percentile scenarios.

These results are better explained by using the 3 × 3 risk analysis approach and target hazard quotients (THQ) to explain the dangers that population is subject to. Although this kind of methodology does not include quantitative estimates on the probability of an exposed population to a negative effect, it has the advantage of offering a signal of risk level caused by an exposure to a contaminant (Storelli 2008). The matrix in Table 4 gives an overview of the risk analysis for mercury.

The THQ values reported in the above matrix, are high compared with literature in some scenarios. For example, Storelli (2008) found THQ values for mercury lower than 1.00 for most of the studied fish species in the Adriatic Sea. Moreover, the only fish species that were related to THQs greater than 1.00 (Albacore and Rosefish) only got a value of 1.87 and 1.50, respectively.

Mataba et al. (2016) reported THQ values for mercury of 0.20 in the 95th percentile. However, these important differences are not only related to high mercury concentrations in Rurrenabaque and Riberalta fish, but also to the high fish consumption that some of the indigenous people of the Bolivian Amazon exhibit. This becomes apparent when

Table 4. Target Hazard Quotients for mercury under the different consumption and contamination scenarios for the study area.

		Consumption scenarios			
		Maximum (222.93 g per person per day)	Average (108.84 g per person per day)	Minimum (22 g per person per day)	Big cities (0.68 g per person per day)
Pollution scenarios	95 th percentile (Male)	13.76	6.72	1.36	0.04
	95 th percentile (Female)	18.17	8.87	1.79	0.06
	50 th percentile (Male)	2.03	0.99	0.20	0.01
	50 th percentile (Female)	2.68	1.31	0.26	0.01
	5 th percentile (Male)	0.31	0.15	0.03	<0.01
	5 th percentile (Female)	0.41	0.20	0.04	<0.01

comparing the THQs for the Bolivian Amazon people with the THQs for the three most important cities of the country, where even fish with the highest mercury concentrations result in very low THQs due to the low levels of fish consumption). Because indigenous people of the Beni River basin depend on fish as a principal protein source (Guerrero 2001; Camburn 2011), mercury pollution in the area poses a serious risk to human health particularly to woman, and probably to children as well.

Although some early studies on mercury in hair samples from indigenous communities in the Beni River basin have shown relatively low concentrations of mercury compared to what was found in neighboring inhabitants from Brazil (Barbosa et al., 2001), more recent studies have found the opposite. For example, the research made by Lee et al. (2021) found that women of the Bolivian indigenous Portachuelo and Eyiyo Quibo people (inhabitants of the Beni river basin) were shown to have the highest levels of mercury in their bodies, in comparison to other Amazonian communities from three other countries. In their research, Lee et al. (2021) found average levels of $7.58 \mu\text{g/g} \pm 4.75 \mu\text{g/g}$ (fw) in hair samples that would be the product of fish consumption, as these communities are not directly involved in gold mining. Thus, whether from small-scale illegal gold mining or from natural occurrence in sediment, elevated mercury concentrations in fish appear to be the main source of mercury in the body of the Beni River indigenous inhabitants.

5. Conclusions and recommendations

Trace element pollution in biota from Beni River was evaluated by analyzing fish samples collected at Rurrenabaque and Riberalta markets. No significant differences were detected, when comparing different fishing zones along the river basin. However, it is necessary to recognize that there are insufficient comparisons of the sites to conclude there was no anthropogenic influence, so further studies regarding the influence of the different fishing areas would be worthwhile. Only mercury showed potentially problematic concentrations among the 106 samples, especially in carnivore and omnivore fish species. This situation appears to show that mercury contamination only depends on the fish-eating habits and not so much on the environmental conditions of their habitat. However, we can't dismiss the fact that the information about fishing sites was given by market vendors who provided the samples, so in some occasion's information could have been inaccurate, causing mix of data. Also, results from the correlations between size and concentrations seem to confirm bioaccumulation and magnification of mercury along the food webs, previously studied by other authors in the past decades. Therefore,

large species, widely consumed in these markets (such as *Arapaima gigas*, especially), would represent a greater risk through their consumption, also considering the trophic habits of these species and the consequent accumulation of mercury in their organisms.

The 3×3 scenario risk analysis highlighted, one more time, that mercury is the most important trace pollutant in the Beni River basin, since it was the only element to exceed the safe intake levels (PTWI of $5 \mu\text{g/kg}$ of body weight) established by the World Health Organization. This situation was also evident when Target Hazard Quotients (THQ) were calculated, being that mercury was the only element to present an imminent risk to human health, under most of the possible combinations in the 3×3 analysis. Furthermore, this study proved, that women are 25% more susceptible than men to suffer mercury poisoning by fish consumption, raising the possible implications related to the pregnancy "transmission" of mercury pollution from mothers to unborn babies.

In terms of mercury poisoning risk related to fish consumption, by comparing the Bolivian Amazon to the rest of the country, it is clear from this study that the Amazonian indigenous groups who are almost completely dependent on fish for their protein, are more vulnerable to mercury poisoning than people from the cities.

As was evident in this study, using a varied selection of fish species seems to be an advantage for this kind of research, since it offers a global analysis of local health risks. Nonetheless, by using this methodology it is also more difficult to reach a balanced design, with similar sizes of the samples for each trophic level.

Declarations

Author contribution statement

Inti E. Rodríguez-Levy: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Paul A. Van Damme; Lieven Bervoets: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Fernando M. Carvajal-Vallejos: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

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