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Research Article

Human health risks associated to trace elements and metals in commercial fish from the Brazilian Amazon

Andrea V. Waichman[1], *Gabriel Silva de Souza Nunes*[2], *Rhaul de Oliveira*[3], *Isabel López-Heras*[4], *Andreu Rico*[4,5,*]

[1] *Federal University of the Amazon, Institute of Biological Sciences, Av. Rodrigo Ramos 3000, Manaus 69077-000, Brazil*

[2] *Federal University of Pernambuco, Department of Zoology, Av. Prof Moraes Rego 1235, Cidade Universitária, Recife 50670-901, Brazil*

[3] *University of Campinas, School of Technology, Rua Paschoal Marmo 1888 - Jd. Nova Itália, Limeira 13484-332, Brazil*

[4] *IMDEA Water Institute, Science and Technology Campus of the University of Alcalá, Av. Punto Com 2, Alcalá de Henares 28805, Madrid, Spain*

[5] *Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, c/ Catedrático José Beltrán 2, 46980, Paterna, Valencia, Spain*

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ABSTRACT

Fish constitutes the main protein source for the Amazonian population. However, the impact of different anthropogenic activities on trace element and metal accumulation in fish and their risks for human health at a regional scale remain largely unexplored. Here we assessed exposure levels of 10 trace elements and metals (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Pb, and Hg) in 56 samples belonging to 11 different species of fish from the Brazilian Amazon. We studied the relationship between exposure levels, fish origin, and fish feeding habits, and assessed toxicological and carcinogenic risks for the Amazonian population. No significant correlation was found between sampling site and exposure levels to the studied elements, but a significant difference was found between the accumulation of some metals and the position of the fish species in the food chain. The concentrations of Cr and Hg in fish flesh were found to exceed the Brazilian limits for human consumption. This study shows that current fish consumption patterns can lead to estimated daily intakes of Hg, As and Cr that exceed the oral reference dose, thus posing a toxicological concern. Furthermore, carcinogenic risks may be expected due to the continued exposure to Cr and As. The results of this study show that the consumption of wild caught fish in the Amazon region should be controlled. Moreover, continued monitoring of trace element and metal contamination in fish

* Corresponding author.
E-mail: andreu.rico@uv.es (A. Rico).

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and on the health of the Amazonian population is recommended, particularly for riverine and indigenous communities.
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Introduction

Fish is widely acknowledged as a healthy food item due to its essential nutrients, such as long-chain omega-3 fatty acids, essential amino acids, vitamins (notably A, B, and D), and trace elements including Fe, Zn, and Se (FAO, 2021). Among the list of trace elements, metals are particularly important as they tend to accumulate in fish tissues, especially in carnivorous species, through processes of bioaccumulation and biomagnification (Ali and Khan, 2019). Humans who regularly consume contaminated fish may be chronically exposed to these contaminants. Excessive consumption of contaminated fish can have adverse effects on human health, particularly among vulnerable groups such as pregnant women and children (Palmieri et al., 2019). Furthermore, continued exposure to some elements such as As, Cr, Pb and Cd can contribute to an increase in the risk of developing cancer and cancer-related diseases (Cao et al., 2010).

The Amazon region is home to a myriad of freshwater fish species, which do not only serve as a food source for human populations but also contribute significantly to the local economy (Junk et al., 2007; Nobre et al., 2021). In the Brazilian Amazon, fish is highly important for the diet of the riverine and indigenous populations (Begossi et al., 2019). Studies have shown that fish comprises 64%–76% of the animal-derived items and 79%–87% of the total animal protein weight consumed. On average, the fish consumption per capita by riverine populations of the Amazon region amounts to 462 g of fish per day or 169 kg per year (Isaac et al., 2015), which is about 8 times higher than the world average fish consumption rate established by the Food and Agriculture Organization of the United Nations (FAO, 2021).

Numerous studies reveal that fish captured in the Amazonian rivers exceed the limits established by the Brazilian legislation and the World Health Organization for Hg exposure (Albuquerque et al., 2020; Azevedo et al., 2020; Dorea et al., 2006; Ferreira da Silva and de Oliveira Lima, 2020; Lima et al., 2015). High Hg exposure levels in freshwater ecosystems of the Amazon region have been attributed to gold mining activities, which contribute to its direct environmental release during the gold amalgamation process (Arrifano et al., 2018; Barbosa et al., 2003; Boischio and Henshel, 2000; Dorea et al., 2006; Fadini and Jardim, 2001; Marshall et al., 2016; Vieira et al., 2018). The practices of mining, but also deforestation, agricultural expansion, and the construction of hydroelectric dams in the Amazon have indirectly aggravated problems associated to environmental pollution (Albuquerque et al., 2020; Castello et al., 2013; Fearnside, 2015), and contributed to the accumulation of Hg and other trace elements in areas that were originally considered pristine and that constitute important reservoirs for fish diversity (Galarza et al., 2023; Nascimento et al., 2022; Capparelli et al., 2020; Ribeiro et al., 2017). Additionally, the urbanization process and the establishment of new industries, coupled with the lack of basic sanitation systems, further contributes to the presence of these contaminants in Amazonian surface waters and sediments (Viana et al., 2016).

To date, most studies assessing the human health risks of trace element and metal contamination in the Amazon region have focused on Hg contamination from gold mining activities, or on a wider list of trace elements analyzed in water, sediment or fish samples collected in a specific river or location (Moulatlet et al., 2023). Therefore, the aim of this study was to provide a large-scale assessment of trace element contamination in various fish species collected from different regions of the Amazon River basin and to evaluate the potential health risks faced by the population that consume them. To achieve this, we analyzed the concentration of 10 trace elements (Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Pb, and Hg) in fish flesh samples belonging to 11 different species obtained from local markets and studied the relationship between their contamination levels and their size, origin and feeding habits. Then, we calculated toxicological and carcinogenic risks for the local population and provided key information as to what are the elements and fish species that contribute most to human health risks in the Amazon region.

1. Materials and methods

1.1. Fish sampling

A total of 56 fish individuals belonging to 11 species were purchased from local markets in the urban areas of Nova Airão, Manacapuru, Iranduba, Manaus, Santarém, Belém and Macapá, between the 16th of November and the 8th of December of 2019 (Table 1). The species chosen were the most appreciated and consumed by the Amazonian population. At the time of purchase, a brief interview was carried out to inquire about the origin of the fish individuals. According to such interviews, fish were found to be caught from 21 different locations in the Brazilian Amazon (Fig. 1). The fish samples were dissected and a fish flesh sample of approximately 10-20 g was wrapped in aluminum foil and frozen (−20°C) until further analysis.

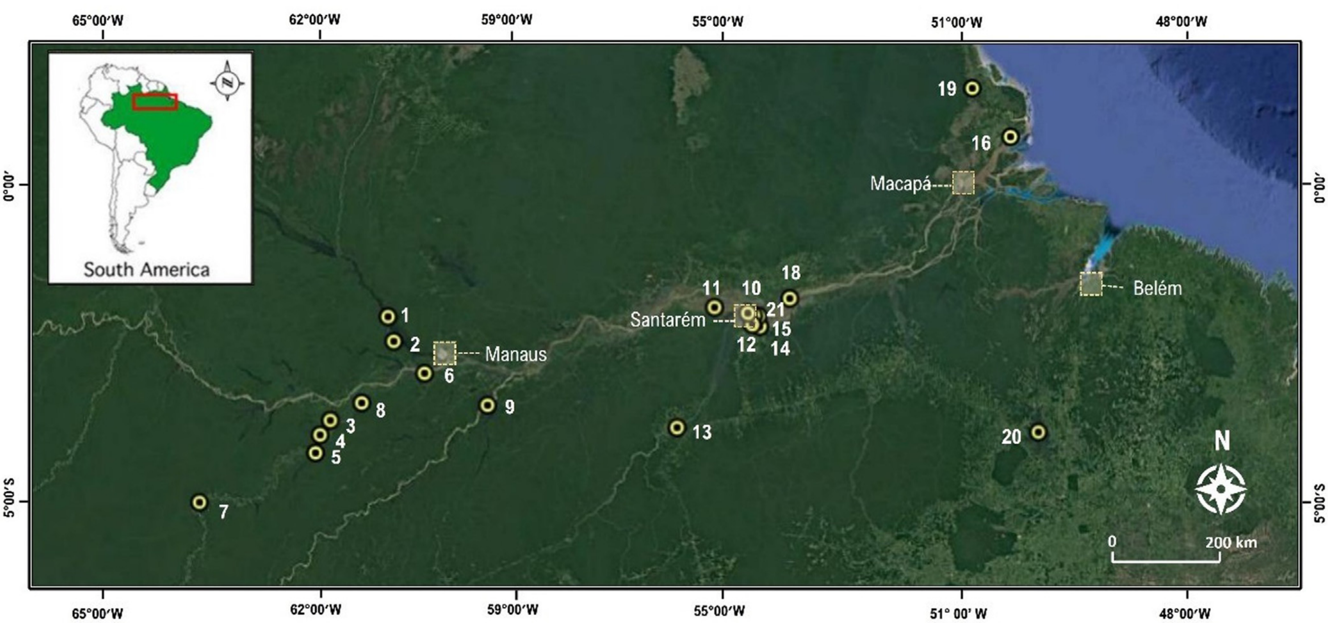
1.2. Trace element analysis

The concentration of Cr, Mn, Fe, Ni, Cu, Zn, As, Cd, Pb and Hg in the fish samples was analyzed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the chemical analysis laboratory of the IMDEA Water Institute (Spain) following

Table 1 – Species name, feeding habits, number of samples analyzed, fish weight, fish length, and fishing site numbers*.

Scientific name	Local name	Feeding habits	Number of samples	Weight (g) (mean ± SD)	Length (cm) (mean ± SD)	Fishing sites
Arapaimas gigas	Pirarucu	Carnivorous/Piscivorous	5	[a]	[a]	4, 20
Astronotus ocellatus	Cara	Omnivorous	8	274 ± 168	18.7 ± 4.24	1, 2, 3, 10, 20
Cichla monoculus	Tucunare	Carnivorous/Piscivorous	6	809 ± 443	29.9 ± 4.15	2, 3, 10, 13, 17
Colossoma macropomum	Tambaqui	Omnivorous	3	1074 ± 618	31.7 ± 8.44	3, 8, 15
Hoplias malabaricus	Traira	Carnivorous/Piscivorous	4	780 ± 120	34.8 ± 3.37	2, 5, 14, 20
Myleus sp.	Pacu	Herbivorous	5	144 ±151	15.8 ± 7.75	1, 5, 7, 10, 13, 20
Osteoglossum bicirrhosum	Aruana	Omnivorous	5	1020 ± 318	54.4 ± 4.18	2, 3, 6, 7, 13
Prochilodus nigricans	Curimata	Detritivorous	4	646 ± 190	24. 3 ± 7.38	6, 10, 16, 18, 20
Pseudoplatystoma fasciatum	Surubim	Carnivorous/Piscivorous	5	1031 ±708	34.8 ± 17.2	2, 9, 11, 12, 21
Pygocentrum nattereri	Piranha	Carnivorous/Piscivorous	7	388 ±30.7	21.1 ± 5.01	1, 2, 5, 14, 19
Semaprochilodus taeniurus	Jaraqui	Detritivorous	4	294 ±57.3	22.8 ± 0.85	1, 5, 7

* The name and location corresponding to the different fishing site numbers are shown in Fig. 1.
[a] Only a portion of the fish was purchased from local markets.



1	Coridiqui Lake	8	Purus River - Beruri	15	Amazon River - Tapará
2	Tumbira	9	Madeira River	16	Amazon River - Baeliqui
3	Macaquinho Lake	10	Amazon River -Aritapera	17	Amazon River - Prainha
4	Supiá Channel	11	Grande Lake	18	Amazon River - Monte Alegre
5	Piranha Lake	12	Pacoval Lake	19	Calçoene River
6	Janauacá Lake	13	Bom Jardim Lake	20	Tucuruí Dam
7	Purus River - Tapauá	14	Maicá Lake	21	Amazon River - Santarem

Fig. 1 – Sampling sites where the fish individuals were collected.

de Santiago-Martín et al. (2020). Fish samples were lyophilized (Lyomicrom, Coolvacuum Technologies, Barcelona, Spain). Then, the samples were digested at 190°C during 30 min (Microwave Digestion System Ethos One, Milestone, Bergamo, Italy), with a mixture of HNO_3:H_2O_2 (3:1, V/V). The samples were filtered through a 0.45 µm PVDF filter membrane. Finally, the samples were diluted by a 1:5 or 1:50 volume ratio depending on the quantification requirements.

The quantification of trace elements in the fish samples was carried out by using a quadrupole ICP-MS 7700x series from Agilent Technologies (Santa Clara, USA). Nitric acid (65%), chlorohydric acid (35%), and ICP-MS standard solutions of 1000 mg/L (Fluka, St Louis, USA) for each of the analyzed elements were used. A multielement stock solution of 10 mg/L was prepared in ultra-pure water containing nitric acid at 1% (V/V). The Hg stock solution was prepared in 1% (V/V) nitric acid + 0.5% (V/V) chlorohydric acid. Stock solutions were stored at 4°C, while working standard solutions used for external calibration were prepared daily. Additionally, an internal standard solution containing 10 mg/L of Li, Sc, Ge, Y, In, Tb and

Bi was obtained from Agilent Technologies (Santa Clara, USA). The mass calibration of the ICP-MS instrument was tuned daily with a solution containing 1 µg/L of Ce, Co, Li, Mg, Tl and Y in 1% (*V/V*) HNO_3 (Agilent Technologies, Santa Clara, USA). The operating conditions for the ICP-MS used in this study are provided in Appendix A Table S1.

1.3. Human health risk assessment

The estimated daily intake (EDI), target hazard quotient (THQ) and the carcinogenic risk (CR) indicator were used as endpoints to estimate the potential human health risks of the analyzed trace elements (USEPA, 1989). The EDI, THQ and CR were calculated using the Regional Screen Levels Calculator from the USEPA (https://epa-prgs.ornl.gov/cgi-bin/chemicals/csl_search/) and the following equations:

$$\mathrm{EDI} = \frac{\mathrm{C} \times \mathrm{IR}}{\mathrm{BW}} \tag{1}$$

The EDI (mg/(kg·day)) was calculated based on the mean trace element concentration (*C*, mg/kg wet weight (ww)) in fish, the fish ingestion rate (IR, 0.497 kg/day) and the average human body weight (BW, 60 kg). The EDI was compared with the oral reference dose (R*f*D) for each element (USEPA, 2000).

$$\mathrm{THQ} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{IR} \times \mathrm{C}}{\mathrm{R}f\mathrm{D} \times \mathrm{BW} \times \mathrm{AT}} \times 10^{-3} \tag{2}$$

To assess chronic toxicological risks, the THQ was calculated assuming that the ingestion dose was equal to the absorbed dose and that cooking had no effect on the chemical availability (Chien et al., 2002; USEPA, 1991). The THQ was calculated considering an exposure frequency (EF) of 365 days/year, during an average lifetime of 70 years (ED), the above-described IR, C, R*f*D and BW parameters, and AT, which is the average exposure time for non-carcinogens (EF $\times$ ED). When the THQ value was lower than 1, the exposed population was not expected to experience any adverse health risk. Conversely, if the THQ was equal to or higher than 1, health risks were assumed to be likely, with an increasing probability as the value increases. To assess the overall potential risk posed by the exposure to all elements found in the fish sample, the hazard index (HI) approach was used (USEPA, 1991):

$$\mathrm{HI} = \sum \mathrm{THQs} \tag{3}$$

This approach assumes that simultaneous (sub-threshold) exposure to several chemicals could result in an adverse health effect. The magnitude of the adverse effect is proportional to the sum of the ratios between the (sub-threshold) exposures and the acceptable exposures. Thus, the HIs for each species were calculated as the sum of the THQs for each trace element. HIs larger than one indicate a potential risk by the trace element mixture, while values lower than one indicate a low risk.

$$\mathrm{CR} = \frac{\mathrm{EF} \times \mathrm{ED} \times \mathrm{EDI} \times \mathrm{CSF_0}}{\mathrm{AT}} \times 10^{-3} \tag{4}$$

For potential carcinogenic risks, the CR was estimated using a slope factor (CSF_0) for As, Cd, Cr and Pb. This factor is used to estimate the probability of a person developing cancer because of exposure to a particular level of these potential carcinogen metals. Following the USEPA (1991), calculated CRs values greater than 1×10^{-4} were considered to pose high risks, CR values in the range of 1×10^{-6} to 1×10^{-4} were considered to pose potential risks, and CR values below 1×10^{-6} were considered to pose low risks. Finally, the HI was calculated by summing up all the CR values for a given species.

1.4. Statistical analysis

A Pearson correlation analysis was done between the trace element concentrations found in the different locations to identify potential common sources and origin, and with the size of the fish individuals. A Kruskal-Wallis test was used to determine differences in trace element concentrations among the different fish feeding groups, followed by a Mann-Whitney contrast test. Statistical analyses were done using the statistical software Mystat 12 version 12.02.0 and a significance level (alpha) of 0.05.

2. Results and discussion

2.1. Trace element concentrations in fish samples

Although other studies have investigated trace element concentrations in fish from different Amazon locations (Albuquerque et al., 2020; Alcala-Orozco et al., 2020; Lino et al., 2018; Barros et al., 2010), this is the first work providing a wide coverage of sites along the Amazon Basin. In addition, previous studies have mainly focused on the risks posed by Hg contamination, while we extended our monitoring to 10 elements that may pose potential (mixture) toxicity and risks for human health.

The mean, maximum and minimum concentration of the 10 trace elements in fish flesh samples are shown in Table 2. A comparison of our results with other studies in the Amazon indicated that the measured levels of trace elements are within the range of the concentrations found in the same or similar species, except for Cr and Ni, which show higher concentrations (Table 3). The Cr and Ni values found in our study are comparable with those found in the Piracicaba River, in the state of São Paulo, which drains areas with intensive agricultural and forestry activity (Meche et al., 2010). Moreover, similar Cr levels have been found in fish collected along the Tramadaí River, in the south of Brazil, which receive industrial and agricultural effluents (Tesser et al., 2021), or in areas of the Paraopeba and Abaeté rivers (Northeastern Brazil), which are impacted by a broken mining tailings dam (Savassi et al., 2020).

The fishing areas from which the fish were obtained are generally distant from urban areas, which indicates that industrial contamination was not likely to be the main pollution source. Fishing areas such as the Coridiqui Lake, the Macaquinho Lake and the Piranha Lake, where fish had high levels of Cr and Ni contamination, are far from agricultural areas too. Therefore, possible factors that contribute to a higher concentration of trace elements in fish are the geology of

Table 2 – Mean, minimum and maximum trace element concentrations in muscle of the fish individuals (mg/kg wet weight) and maximum level (MLs) established by the Brazilian Agency of Health Surveillance (ANVISA) (Brasil, 2013).

Fish species	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Arapaimas gigas	0.034	ND	0.450 (0.375–0.525)	0.142 (0.117–0.166)	3.873 (3.770–3.976)	0.560 (0.238–0.883)	0.205 (0.173– 0.237)	0.283 (0.235– 0.331)	0.148 (0.010– 0.285)	2.522 (0.010– 0.285)
Astronotus ocellatus	0.033 (0.030–0.035)	0.0025 (0.0023–0.0027)	0.635 (0.188–1.114)	0.328 (0.053–0.480)	8.055 (2.127–11.868)	0.148 (0.028–0.360)	0.415 (0.205–1.009)	0.301 (0.065– 0.550	0.017 (0.008– 0.053)	2.340 (0.010– 0.285)
Cichla monoculus	ND	0.0035 (0.0023–0.0033)	0.484 (0.292–1.189)	0.151 (0.103–0.208)	3.890 (2.411–7.607)	0.448 (0.078–1.056)	0.162 (0.075–0.274)	0.243 (0.144– 0.595)	0.010 (0.010– 0.011)	2.751 (0.010– 0.285)
Colossoma macropomum	0.036	ND	0.446 (0.265–0.598)	0.174 (0.158–0.205)	4.880 (4.084–5.590)	0.046 (0.026–0.074)	0.258 (0.093–0.4768)	0.204 (0.121– 0.268)	0.010 (0.009– 0.010)	1.868 (0.010– 0.285)
Hoplias malabaricus	0.033 (0.032–0.034)	0.0035 (0.0023–0.0049)	0.597 (0.2333–1.054)	0.184 (0.136–0.280)	6.376 (3.800–9.193)	0.463 (0.163–0.653)	0.228 (0.156–0.378)	0.247 (0.133– 0.345)	0.016 (0.009– 0.035)	3.072 (0.010– 0.285)
Myleus sp.	0.040 (0.033–0.047)	0.0026 (0.0022–0.0032)	0.658 (0.220–1.341)	0.157 (0.064–0.239)	4.880 (2.389–8.768)	0.083 (0.025–0.149)	0.248 (0.086–0.727)	0.315 (0.109– 0.658)	0.011 (0.009– 0.013)	3.262 (0.010– 0.285)
Osteoglossum bicirrhosum	0.036	0.0024 (0.0024–0.0025)	0.563 (0.263–1.039)	0.227 (0.199–0.260)	5.287 (3.448–13.679)	0.151 (0.013–0.488)	0.389 (0.131–0.598)	0.270 (0.096– 0.520)	0.011 (0.010– 0.013)	2.640 (0.010– 0.285)
Prochilodus nigricans	0.037	0.0026 (0.0025–0.0030)	0.734 (0.327–1.801)	0.232 (0.100–0.376)	6.245 (2.476–6.026)	0.081 (0.009–0.196)	0.235 (0.0105–0.449)	0.355 (0.168– 0.906)	0.011 (0.011– 0.011)	2.463 (0.010– 0.285)
Pseudoplatystoma fasciatum	ND	0.0030 (0.0025–0.0048)	0.288 (0.174–0.464)	0.214 (0.138–0.362)	4.127 (2.486–10.255)	0.312 (0.013–1.189)	0.135 (0.086–0.249)	0.127 (0.092– 0.172)	0.011 (0.009– 0.015)	2.116 (0.010– 0.285)
Pygocentrum nattereri	0.0040	0.0027 (0.0025–0.0028)	0.593 (0.340–1.592)	0.165 (0.115–0.217)	5.784 (3.714–0.166)	0.363 (0.065–0.597)	0.224 (0.1578–0.304)	0.278 (0.177– 0.628)	0.014 (0.011– 0.018)	2.770 (0.010– 0.285)
Semaprochilodus taeniurus	0.036	0.0025 (0.0024–0.0025)	0.521 (0.437–0.611)	0.297 (0.196–0.460)	5.961 (5.003–8.885)	0.084 (0.039–0.143)	0.640 (0.230–1.101)	0.254 (0.215– 0.28)	0.017 (0.010– 0.027)	2.653 (0.010– 0.285)
MLs	**1.00**	**0.05**	**0.10**	**30.00**		**0.50**		**5.00**	**0.30**	**50.00**

ND: not detected. The detection limits for As and Cd were 0.015 and 0.0008 mg/kg wet weight, respectively.

Table 3 – Trace element concentrations in fish (mg/kg wet weight) from different Amazon regions.

Fish species	Location	Region	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	Reference
Arapaima sp.	Santarém	East Amazon	0.008	0.00065	0.012	0.119	3.05	0.05	0.111	0.007	0.0015	3.41	Albuquerque et al. (2020)
Cichla monoculus	Santarém	East Amazon	0.005	0.0005	0.013	0.113	3.15	0.18	0.123	0.008	0.0017	2.79	Albuquerque et al. (2020)
	Itaituba	East Amazon				1.2	28					7	Lino et al. (2018)
	Buburé	East Amazon				1.1	203					11	Lino et al. (2018)
	Manacapurú Lake	Middle Amazon						0.125					Beltran-Pedreros et al. (2011)
	Gelado River	East Amazon		1.67	1.45								Barros et al. (2010)
Colossoma	Santarém	East Amazon				1.5	24					4	Lino et al. (2018)
macropomum	Belém	East Amazon				<0.52	193		<0.81		0.54	7.46	Cruz et al. (2015)
	Manacapurú Lake	Middle Amazon						0.063					Beltran-Pedreros et al. (2011)
Hoplias malabaricus	Cassiporé	East Amazon		0.033	0.026	0.048		0.329			0.141	0.286	Lima et al. (2015)
	Javarí River	West Amazon						0.112					Alcala-Orozco et al. (2020)
	Loracayu River	West Amazon		0.014				0.12					Alcala-Orozco et al. (2020)
Myleus sp.	Javarí River	West Amazon						0.027					Ferreira da Silva et al. (2020)
	Santarém	East Amazon				0.7	8.0					7.0	Lino et al. (2018)
Prochilodus nigricans	Manacapurú Lake	Middle Amazon						0.08					Beltran-Pedreros et al. (2011)
	Javarí River	West Amazon						0.048					Ferreira da Silva and de Oliveira Lima (2020)
	Loracayu River	West Amazon		0.032				0.049					Alcala-Orozco et al. (2020)
Pseudoplatystoma	Santarém	East Amazon	0.011	0.00035	0.017	0.084	3.05	0.025	0.081	0.008	0.0007	2.73	Albuquerque et al. (2020)
fasciatum	Cassiporé River	East Amazon		0.034	0.006	0.061	0.175	0.53				0.099	Lima et al. (2015)
	Santarém	East Amazon				1.3	17					7	Lino et al. (2018)
	Loracayu River	West Amazon		0.012				0.012					Alcala-Orozco et al. (2020)
Pygocentrus nattereri	Cassiporé River	East Amazon		0.046	0.056	0.036		0.365			0.078	0.182	Lima et al. (2015)
	Santarém	East Amazon	0.0038	0.0005	0.012	0.096	3.22	0.11	0.079	0.007	0.0001	3.34	Albuquerque et al. (2020)
	Cassiporé River	East Amazon		0.046	0.056	0.036		0.365			0.078	0.182	Lima et al. (2015)
Semaprochilodus	Santarém	East Amazon				2	23					5	Lino et al. (2018)
taeniurus	Manacapurú Lake	Middle Amazon						0.063					Beltran-Pedreros et al. (2011)

the region, deforestation and the expansion of (illegal) artisanal mining in the Amazon (Pontes et al., 2022). Studies of Cr concentrations in Amazonian soils show that the main soils of the region (i.e., ultissols and oxisoils) have low Cr content (Dos Santos and Alleoni, 2013; Gonçalves et al., 2022; Rebêlo et al., 2020). However, soils derived from mafic and ultramafic rocks may present high Cr and Ni concentrations, which can be up to 2346 mg/kg for Cr (Moreira et al., 2018) and 3894 mg/kg for Ni (Martins et al., 2021). High potential for Cr availability has been found in lowlands, which are subject to seasonal flooding (Rebêlo et al., 2020), and where Cr is leached due to high rainfall events (do Nascimento et al., 2018; Gonçalves et al., 2022; Moreira et al., 2018). Other fractions adsorbed on clay particles of the soil can reach water bodies through deforestation and mining processes, transported during the flood season, and accumulated in lakes due to the deposition of suspended sediment particles (Nascimento et al., 2022). Indeed, previous studies indicated that Cr and Ni were deposited in the sediments of the Xingu River in large quantities, as a result of mining, urbanization and deforestation processes (Ribeiro et al., 2017), while studies conducted in the sub Andean region of Peru show that slash and burn deforestation for agricultural and pasture expansion contribute to the enrichment of these metals in sediments (Lindell et al., 2010).

2.2. *Trace element concentrations in relation to size*

Trace element concentrations in fish muscle were not correlated with fish size (p-value > 0.05), although this correlation has been observed in other studies performed at the local scale (Albuquerque et al., 2020; Beltran-Pedreros et al., 2011). In our study, fish of the same species were caught in different locations subject to different contamination sources and exposure levels. In addition, the lack of the exposure level correlation with fish length may be due to changes in dietary habits throughout the fish lifespan, fish physiology and the hydrological dynamics in the different sites (Beltran-Pedreros et al., 2011; Oliveira et al., 2006).

2.3. *Trace element concentrations in relation to feeding habits*

The relationship between the trace element concentration and the fish feeding habits was found to be statistically significant for Cd, Cu, Fe, Hg, Mn, Ni, Pb and Zn (Fig. 2). Carnivorous/piscivorous and herbivorous fish presented lower levels of Cu, Fe and Mn, as compared to detritivorous and omnivorous species. The greater concentration of these metals in detritivorous fish may be associated with a greater presence of these elements in sediments. In the case of omnivorous fish, a significant component of their diet are shrimps, which in turn feed on organic matter debris deposited in sediments, resulting in an increased exposure to these elements (Michael and Ferreira, 1983). For Zn, a different fish accumulation pattern was observed, with detritivorous and omnivorous fish presenting lower fish concentrations as compared to herbivorous and carnivorous individuals. We do not have a clear explanation for this, although it is evident that the main exposure pathway to this trace element may have been different for different species groups. According to Lino et al. (2018), Zn concentrations of fish from the Tapajós River showed no difference between carnivorous and non-carnivorous fish, and the levels found by these authors were similar to those found in this study.

Hg and Pb showed bioaccumulation and biomagnification potential. Regarding Hg, the lowest average concentrations were observed in herbivorous and detritivorous fish (0.08 mg/kg), followed by omnivorous fish (0.13 mg/kg), while the highest concentration levels were recorded in carnivorous/piscivorous fish (0.41 mg/kg). These concentrations are very similar to the average values reported by Beltran-Pedreros et al. (2011) for fish collected from a floodplain lake in the Solimões River (Lago Grande de Manacapuru), which were 0.05 mg/kg for omnivorous and 0.42 mg/kg for piscivorous fish, with biomagnification factors of 0.33 and 0.47 for these two fish species guilds. Variations in Hg concentrations between fish belonging to the same feeding group have been attributed to variability in the age of the fish collected and to water level, being higher (up to two-fold) during the low water period (Dorea et al., 2006; Belger and Forsberg, 2006). Pb showed a similar trend as to Hg, with the lowest concentrations being found in herbivorous fish (0.011 mg/kg) followed by omnivorous and detritivorous fish (0.014 mg/kg), and the highest concentrations being found in carnivorous/piscivorous fish (0.026 mg/kg). In the case of Cd, the concentration of carnivorous/piscivorous fish (0.029 mg/kg) was significantly higher as compared to the omnivorous (0.024 mg/kg), or the detritivorous and herbivorous (0.026 mg/kg) fish groups, however the magnitude of such difference was very low, indicating very limited bioaccumulation potential for this metal.

2.4. *Trace element concentrations in relation to fishing sites*

The Pearson correlation analysis indicated significant correlations between Cr and Ni ($R^2 = 0.92$; $p = 0.007$) and Fe and Cu ($R^2 = 0.88$; $p = 0.016$) in the different locations, indicating that these trace elements can have the same source. Overall, little variability was observed in the mean trace element concentrations measured in the different sites (3 to 6-fold, approximately), altough some outliers were identified for Cd, Hg and Pb (Fig. 3), which may be related to local contamination sources. The highest Cd concentrations were measured in the carnivorous/piscivorous *Hoplias malabaricus*, in the Madeira River (Site 9) and the Tucuruí Dam (Site 20). The Madeira River holds an increasing artisanal mining activity, and showed the highest concentration of Hg in the *Pseudoplatystoma fasciatum* species. The Tucuruí Dam was built in 1984 in Tocantins-Araguaia River basin, downstream of the Serra dos Carajás, one of the main gold mining sites in Brazil (de Araújo et al., 2022; Salomão et al., 2020; Valter et al., 2020). Similar levels of Cd to those reported in the Madeira River and the Tucuruí Dam have been found in the carnivorous fish investigated by Barros et al. (2010), in the Gelado River (Tocantins River watershed), who attributed these high exposure levels to the contamination from the tailings of the upstream ore exploration in the Serra dos Carajás. It should be mentioned, however, that the area near the Tucuruí Dam (Tocantins-Araguaia River watershed) has also been impacted by changes in land use, prin-

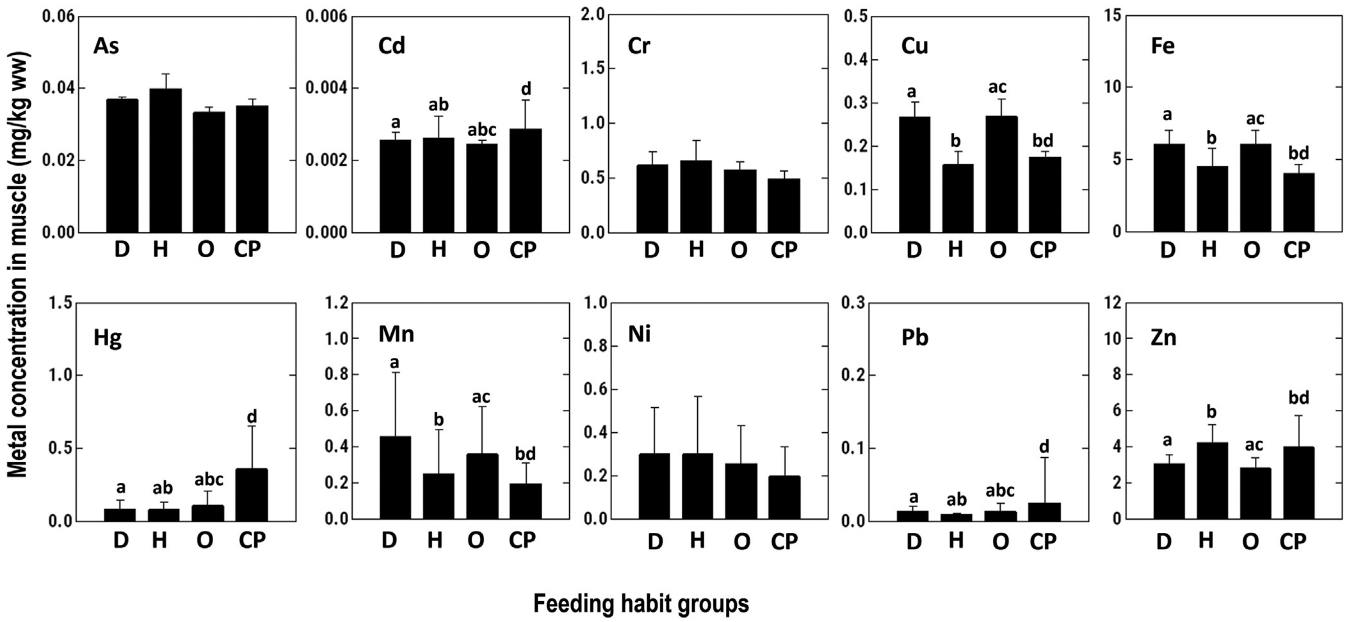


Fig. 2 – Trace element concentrations (mean and standard deviation) in fish with different feeding habits. D: detritivorous (*Procilodus nigricans, Semaprochilodus taeniurus*); H: herviborous (*Myleus* sp.); O: omnivorous (*Astronotus ocellatus, Colossoma macropomum, Osteoglossum bicirrhosum*); CP: carnivorous/piscivorous (*Arapaimas gigas, Cichla monoculus, Hoplias malabaricus, Pdeusoplatystoma fasciatum, Pygocentrum nattereri*). Different letters indicate statistically significant differences between feeding groups (Mann-Whitney contrast test). ww: wet weight.

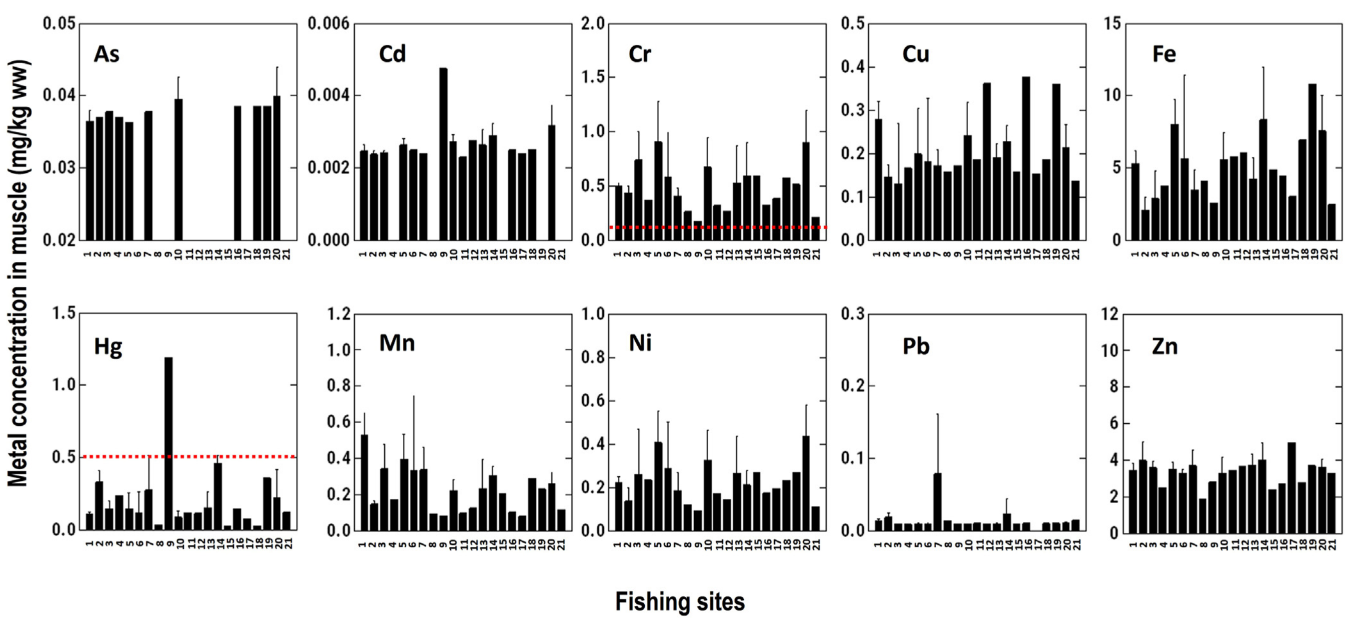


Fig. 3 – Trace element concentrations (mean and standard deviation) in different fishing sites. The dashed red line indicates the maximum allowable limit for fish in Brazil (Brasil, 2013). The name and location corresponding to the different fishing site numbers are shown in Fig. 1.

cipally forest transformation into cattle ranching and agricultural production (Castello and Macedo, 2016).

The highest levels of Pb were found in the carnivorous/piscivorous fish *Arapaimas gigas,* in the Tapauá River, which is in the Purús River basin (Site 7). *Arapaimas gigas* inhabits deep floodplain lakes, which accumulate sediments brought by the up-stream riverine areas during the flooding season. In 2014, it was reported that 15 floating facilities were found in the Tapauá River basin with mining exploration equipment (Aparicio, 2014). Consequently, it is likely that mining activities are taking place in the area and are the main source of Pb contamination.

Table 4 – Estimated daily intake (EDI – mg/kg/day) for each trace element after fish consumption in the Amazon and respective Reference Dose (RfD; USEPA 2000).

Fish species	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn
Arapaimas gigas	2.82E-04	NC	0.0037	0.0012	0.0321	0.0046	0.0017	0.0023	1.22E-03	2.82E-04
Astronotus ocellatus	2.70E-04	2.05E-05	0.0053	0.0027	0.0667	0.0012	0.0034	0.0025	1.43E-04	2.70E-04
Cichla monoculus	NC	2.17E-05	0.0040	0.0013	0.0322	0.0037	0.0013	0.0020	8.35E-05	3.08E-04
Colossoma macropomum	2.94E-04	NC	0.0037	0.0014	0.0404	0.0004	0.0021	0.0017	8.12E-05	2.94E-04
Hoplias malabaricus	2.76E-04	2.93E-05	0.0049	0.0015	0.0528	0.0038	0.0019	0.0020	1.30E-04	2.76E-04
Myleus sp.	3.31E-04	2.17E-05	0.0054	0.0013	0.0404	0.0007	0.0021	0.0026	8.75E-05	3.31E-04
Osteoglossum bicirrhosum	2.94E-04	2.01E-05	0.0047	0.0019	0.0438	0.0012	0.0032	0.0022	8.81E-05	2.94E-04
Prochilodus nigricans	3.11E-04	2.19E-05	0.0061	0.0019	0.0517	0.0007	0.0019	0.0029	8.84E-05	3.11E-04
Pseudoplatystoma fasciatum	NC	2.49E-05	0.0024	0.0018	0.0342	0.0026	0.0011	0.0011	9.15E-05	2.37E-04
Pygocentrum nattereri	3.31E-04	2.20E-05	0.0049	0.0014	0.0479	0.0030	0.0019	0.0023	1.14E-04	3.31E-04
Semaprochilodus taeniurus	2.94E-04	2.04E-05	0.0043	0.0025	0.0494	0.0007	0.0053	0.0021	1.40E-04	2.94E-04
RfD (mg/(kg·day))	**0.0003**	**0.001**	**0.003**	**0.04**	**0.7**	**0.00016**	**0.14**	**0.005**	**0.004**	**0.3**

NC: not calculated (concentrations were below the limit of detection).

Table 5 – Target hazard quotients (THQ) for trace elements based on fish consumption by the Amazonian population and calculated hazard index (HI).

Fish species	As	Cd	Cr	Cu	Fe	Hg	Mn	Ni	Pb	Zn	HI
Arapaimas gigas	0.94	NC	1.24	0.03	0.05	29.01	0.01	0.47	0.31	0.12	32.17
Astronotus ocellatus	0.90	0.07	1.75	0.07	0.10	7.67	0.02	0.50	0.04	0.06	11.18
Cichla monoculus	NC	0.07	1.34	0.03	0.05	23.20	0.01	0.40	0.02	0.08	25.20
Colossoma macropomum	0.98	NC	1.23	0.04	0.06	2.36	0.02	0.34	0.02	0.05	5.09
Hoplias malabaricus	0.92	0.10	1.65	0.04	0.08	23.97	0.01	0.41	0.03	0.08	27.29
Myleus sp.	1.10	0.07	1.82	0.03	0.06	4.28	0.01	0.52	0.02	0.09	8.01
Osteoglossum bicirrhosum	0.98	0.07	1.55	0.05	0.06	7.81	0.02	0.45	0.02	0.07	11.08
Prochilodus nigricans	1.04	0.07	2.03	0.05	0.07	4.21	0.01	0.59	0.02	0.07	8.15
Pseudoplatystoma fasciatum	NC	0.08	0.80	0.04	0.05	16.18	0.01	0.21	0.02	0.06	17.45
Pygocentrum nattereri	1.10	0.07	1.64	0.03	0.07	18.80	0.01	0.46	0.03	0.08	22.30
Semaprochilodus taeniurus	0.98	0.07	1.44	0.06	0.07	4.32	0.04	0.42	0.04	0.07	7.51

NC: not calculated (concentrations were below the limit of detection).

2.5. *Human health risk assessment*

Trace element levels in fish were compared to the maximum limit established for human consumption by the Brazilian Health Surveillance Agency – ANVISA (Brasil, 2013) (Fig. 3; Table 4). Cr levels in the fish flesh exceeded such limit in 83% of the samples, and such exceedance occurred for all species. Levels of Cr above the maximum allowable limit in fish from the Amazon region had been reported in natural lakes (Nascimento et al., 2022) and aquatic systems contaminated by tannery and mining tailings (de Sousa et al., 2016; Lima et al., 2015). Of the non-essential metals, Hg was found to exceed the maximum acceptable limit in 17% of samples. Several studies at different locations in the Amazon have also detected Hg exposure levels above such level (Albuquerque et al., 2020; Azevedo-Silva et al., 2016; Azevedo et al., 2020; Barbosa et al., 2003; Beltran-Pedreros et al., 2011; Dorea et al., 2006; Ferreira da Silva and de Oliveira Lima, 2020). For the rest of elements, mean concentrations in fish muscle were lower than the maximum allowable limit for human consumption set by the Brazilian authorities, although such limits did not consider the fish consumption patterns in the Amazon region, which notably exceed the national average.

The estimated daily intake (EDI) was calculated based on the average element concentration found in the muscles of the different fish species and considering the average fish consumption by the Amazon population. Of the 10 trace elements analyzed, only EDI values for Hg, As and Cr exceeded the RfD (Table 4). The EDI values of Hg were above the RfD for all species. For Cr, all species exceeded the RfD, except the carnivorous *Pseudoplatystoma fasciatum*, while for As the only species that exceeded the RfD were the herbivorous *Myleus* sp., the detritivorous *Prochilodus nigricans,* and the carnivorous/piscivorous *Pygocentrum natereri*.

The results of the chronic toxicological risk assessment are shown in Table 5. The higher the THQ value, the greater the risk for human health. High risks were calculated for Hg, followed by Cr and As. The chronic health risk associated with consuming fish contaminated with Hg was found to range from moderate (1 < THQ < 10) to high (TWQ > 10) for all species, including herbivorous fish. The THQ for Cr was found to be moderate for all species, except for *Pseudoplatystoma fasciatum*, while for As the THQ indicated a moderate risk only for 3 out of the 11 evaluated species (Table 5).

Cr may be present in the environment as Cr(III) or Cr(IV), the latter being toxic to a wide range of living organisms, and having the potential to cause eye and skin irritation, respira-

Table 6 – Estimation of carcinogenicity risk (CR) in adults due to ingestion of fish*.

Fish	As	Cd	Cr	Pb	HI
Arapaimas gigas	4.93E-04	NC	2.17E-03	8.34E-06	2.26E-03
Astronotus ocellatus	NC	4.30E-08	3.07E-03	7.58E-07	3.22E-03
Cichla monoculus	NC	4.57E-08	2.34E-03	6.74E-07	2.20E-03
Colossoma macropomum	NC	NC	2.15E-03	7.52E-07	2.35E-03
Hoplias malabaricus	4.83E-04	6.16E-08	2.89E-03	9.17E-07	3.12E-03
Myleus sp.	5.79E-04	4.57E-08	3.18E-03	1.08E-06	4.68E-03
Osteoglossum bicirrhosum	5.15E-04	4.22E-08	2.72E-03	1.26E-06	1.68E-03
Prochilodus nigricans	5.43E-04	4.60E-08	3.55E-03	8.27E-07	3.86E-03
Pseudoplatystoma fasciatum	NC	5.22E-08	1.39E-03	8.27E-07	1.57E-03
Pygocentrum natereri	NC	4.63E-08	2.86E-03	1.42E-06	3.35E-03
Semaprochilodus taeniurus	5.15E-04	4.29E-08	2.52E-03	8.64E-07	3.34E-03

NC: not calculated (concentrations were below the limit of detection).
* The CR is calculated separately for As, Cd, Cr, and Pb in each fish species, but the Hazard Index (HI), calculated as the sum of CRs per fish species, is also provided.

tory problems, kidney and liver damage, and cancer, among other effects, in humans (Achmad et al., 2017). Cr (III) is insoluble in water and, therefore, tends to be associated with sediment particles. If present in the environment, detritivorous fish are expected to have a higher Cr content than species with other dietary habits. On the other hand, Cr(VI) is easily soluble and can be leached from soils, showing great mobility under tropical environments (Moreira et al., 2018). There was no difference in Cr concentration in fish from the different trophic levels, suggesting that Cr may be present in aquatic environments primarily as Cr(VI).

Most studies in the region address the health risk from exposure to a single trace element, primarily Hg. However, it is important to bear in mind that chronic health risks increase due to co-exposure to different compounds. Thus, during the evaluation of co-exposure to all trace elements, chronic risks were observed for all species, with HI values larger than 5 (Table 5). The species with the lowest overall chronic risk was the omnivore *Colossoma macropomum*, one of the most popular fishes in the Amazon. On the other hand, the highest chronic risk value was determined for the piscivorous *Arapaima gigas* (Fig. 4), which has the highest commercial value in the region and is being exported to other regions of the country.

Fig. 4 shows that there is a variable risk associated to the feeding habits and the position of the fish species in the food chain, with herbivorous fish showing the lowest toxicological risk, followed by detritivorous, omnivorous, and carnivorous/piscivorous species, respectively. In the case of omnivorous fish, *Colossoma macropomum* was found to be the species with the lowest calculated risk as compared to *Astronotus ocellatus* and *Osteoglossum bichirrhosum*. This can be explained by the feeding preference of the first for fruits of the flooded forest, being considered an omnivore/frugivore, while the others are regarded as facultative predators that also feed on mollusks, crustaceans and aquatic insects (Beltran-Pedreros et al., 2011; Oliveira et al., 2006).

High carcinogenic risks were calculated for Cr in all fish species (Table 6). Although no studies exist in Brazil on the relationship between exposure to Cr VI and the incidence of stomach cancer, studies in China and Greece have shown an

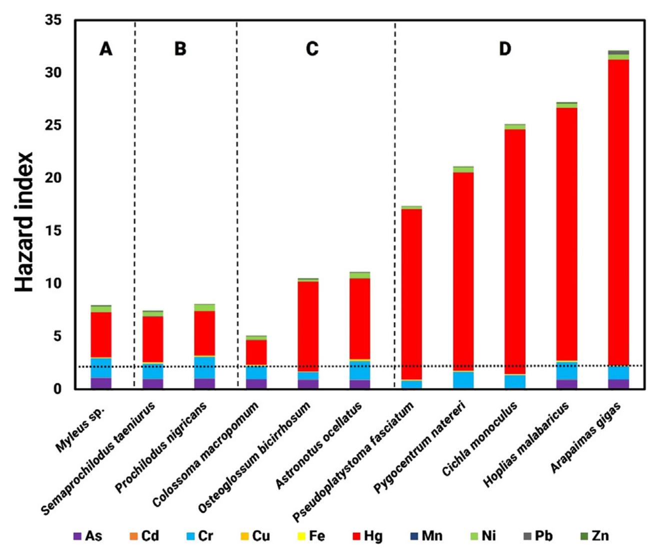


Fig. 4 – Calculated hazard index (HI) for each fish species and relative contribution of the different elements. The dotted line indicates an HI of 1. HIs above this line indicate an exceedance of the risk threshold. A: herbivorous; B: detritivorours; C: omnivorous; D: carnivorous/piscivorous.

increase in the incidence of this type of cancer in areas with Cr-contaminated water (Beaumont et al., 2008; Linos et al., 2011). Studies of cancer incidence in Brazil show that the highest incidence of stomach cancer in men and women occurs in the Amazonian region (INCA, 2019), however further epidemiological studies are needed to investigate its potential causal relationship with fish consumption.

As for As, potential carcinogenic risks were associated with the consumption of the herviborous *Myleus* sp., the omnivorous *Osteoglossum bichirrhosum*, the detritivorous *Prochilodus nigricans* and *Semaprocilodus taeniurus*, and the piscivorous *Arapaimas gigas* and *Hoplias malabaricus* (Table 6). Except for *Hoplias malabaricus*, all other fish are highly appreciated in the region, and particularly *Myleus* sp. and *Semaprochilodus nigricans* are very popular and highly consumed due to their taste and low price. While the highest toxicological risks were associated with consumption of carnivorous fish containing high levels of Hg, the carcinogenic risks were associated to all kind of fish, with herbivorous and detritivorous species also playing an important role. The HIs indicated high carcinogenic risks for all species, mainly due to the high Cr exposure levels. These results suggest that fish consumption in the region should be limited.

3. Conclusions

The levels of essential trace elements and metals have been investigated in 11 commercial fish species from the Amazon region. Measured concentrations in fish muscles were in the range of the values determined in other studies, except for Cr and Ni, which were found to be higher. The measured concentrations of Cr and Hg were found to exceed the Brazilian maximum limits for fish consumption in a significant number of samples. Human health risks were calculated on the basis of the mean fish consumption rate in the Amazon region. Based on this fish consumption value, chronic toxicological risks may be expected, particularly due to the high Hg exposure levels. The fish contamination with Hg was found to be positively correlated to their position in the food chain. Carcinogenic risks may also be expected due to the exposure to Cr (in all species) and As (in different species, including herbivorous and detritivorous fish). The results of this study show that the consumption of wild caught fish in the Amazon region should be limited due to the high levels of metalloids and metals found in the fish flesh, and provides relevant information to perform refined risk assessments, by for example estimating human health hazards related to different fish consumption patterns. According to the results of this study, continued monitoring of trace elements and metals in fish and on the health of the Amazonian population is recommended, particularly for riverine and indigenous communities. Such action is becoming increasingly necessary given the advance of mining activities and associated deforestation in the Amazonian region, which are the most likely causes of the observed fish contamination.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jes.2023.12.029.

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