https://dx.doi.org/10.21577/0103-5053.20230072

J. Braz. Chem. Soc., Vol. 34, No. 12, 1796-1809, 2023

Article

Assessment of the Health Risk of Indigenous People by the Consumption of Fish with Hg and As in Villages Located Close to Mining

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The Amazon suffers from the occurrence of mining along its hydrographic routes, which releases toxic substances into the aquatic environment. The objective of this study was to evaluate the risk of contamination by As and Hg that indigenous peoples are exposed to through the consumption of fish in an area impacted by mining activity in the basin of the Curuá and Baú Rivers, Altamira-PA. In total, 55 fish specimens and 30 surface water samples were collected. The decomposition of the fish samples was done by wet method with the use of nitric acid, hydrogen peroxide, and microwaves. The quantification of total mercury (THg) and total arsenic (TAs) was carried out by inductively coupled plasma optical emission spectrometry with vapor generation (VG-ICP OES). The methodology proposed by the U.S. Environmental Protection Agency was used. The results showed that TAs presents safe levels; however, THg presents a sample with levels up to (4.6 μg g^{-1}), exceeding the permitted levels (1 μg g^{-1}). The risk assessment shows that only the consumption of fish, linked to Hg, represents the existence of deleterious risks to indigenous peoples (hazard quotient (HQ)) > 1). Bioaccumulation factor (BAF) in fish represents a risk to indigenous people, because of the Hg's presence. The study identified that illegal mining activities are responsible for Hg pollution in the Baú and Curuá Rivers. The fish species analyzed can be used as bioindicators of Hg in the rivers, which is relevant for making environmental and public health policy decisions.

Keywords: mining, toxic elements, indigenous, risk factor, ecosystem contamination

Introduction

The artisanal mining activity (AGM) is a practice that history impacts the Amazon basin; it began in the 16th century and its great intensification took place in the 20th century, thanks to the scientific and technological advances offered at the time.[1] This practice usually occurs clandestinely and without environmental concern, and consequently exposes the population and the environment to risks of contamination by the release of toxic elements during this process.[2]

Illegal mining occupations, mainly in search of gold, are encroaching on protected areas[3] and affecting the well-being of indigenous peoples.[4] Currently, there are 332 officially recognized indigenous territories in the Brazilian Amazon. However, it is estimated that 45% of them contain illegal mining activities, of which gold

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Editor handled this article: Maria Cristina Canela (Associate)

mining is very important. The invasion of these lands causes serious environmental effects. For example, the increase in deforestation and intoxication by indigenous people.[5,6] Contaminants include Hg and As, which are highly toxic and non-essential elements.[7]

Intoxication occurs through inhalation, ingestion of water, and consumption of marine species affected by the phenomenon of bioaccumulation, such as fish.[8,9] Continuous exposure to As contaminated food will bring about diseases such as respiratory, and neurological disorders, internal cancers (lung, bladder, liver, and kidney), and skin cancer.[10] Prolonged Hg intoxication can result in cardiovascular, pulmonary and kidney damage, as well as neurological abnormalities that cause sensory disturbances, visual field constriction, deafness, ataxia, and dysarthria.[11]

Considering the risks associated with metal intoxication in the human body, the National Health Surveillance Agency (ANVISA) is responsible for establishing maximum allowed limits for contaminants in carnivorous fish through resolution RDC No. 722, dated July 1, 2022.[12] The accepted values for As and Hg are 1 μg g^{-1} by ANVISA. For the river water, the safety limit is governed by Resolution No. 357, dated March 17, 2005, by the National Council for the Environment,[13] with maximum permitted values for As of 10 μg L^{-1} and for Hg of 0.2 μg L^{-1}.

In the Amazon, fish is the main source of nutrition for traditional peoples (indigenous, riverine, and quilombo communities) in aquatic ecosystems.[14] The increasing content of toxic elements found in fish in the aquatic environment is due to the processes of bioaccumulation and biomagnification in the food chain.[15] Contaminated water affects fish and as a result, it becomes one of the most important sources of contamination for humans. In this way, fish acts as a biomarker in the assessment of risk in humans.[16,17]

The gold mining has caused negative impacts on Amazonian peoples, as several studies have shown. It was found elevated levels of mercury in about a third of the inhabitants of 14 riverside communities of the Xingu River, due to gold mining activity.[17] In addition, it was discovered that 75.6% of participants in urban and riverside areas of the lower Tapajós basin had mercury concentrations above the safe limit established by the World Health Organization.[18] A review highlights that consumption of contaminated fish is the main form of mercury exposure for the Amazonian population, and that the increase in contamination is related to the increase in gold mining in the region.[19]

In addition to mercury contamination, gold mining can cause arsenic contamination; it was evidenced levels above the allowed limit in sediments, water, and soil in gold mining areas in the Amazon, representing a future risk of intoxication for the region's residents.[20] Studies[21,22] in groundwater sources in the Amazon also showed high levels of arsenic. The authors suggest continuous monitoring of groundwater quality, especially in areas with a high presence of gold mining, to protect the health of the local population and ensure the sustainability of the use of these resources.

Because of their intricate interactions with various physical and biogeochemical factors that determine their transit, transformations and fates, As and particularly Hg contamination in fish in the Amazon region is difficult to predict and monitor in aquatic ecosystems.[23] The presence of Hg in Amazonian rivers is very high and the people who live there are suffering intoxication in a silent way.[24,25]

Thus, to understand the potential risk of toxic elements for consumers in a region, it is necessary to quantify the concentrations of elements in the tissues of the most consumed species, to evaluate the risk factor for the prolonged consumption of these contaminated biological samples, in addition to the determination of the bioaccumulative effect present in these species.[25-27]

In this context, this work was developed to evaluate the risk factor and quantify the concentrations of As and Hg in fish species, present in an indigenous conservation area in the municipality of Altamira-PA, Brazil.

Experimental

Study area and sampling sites

The area of this study is inserted in an indigenous reserve of environmental protection, where it houses communities of Indians from 3 villages, namely Baú, Kamurê, and Kamuá. They are located in the municipality of Altamira-PA and belong to the Kayapó tribe. These villages are surrounded by the Curuá and Baú Rivers, the first being in direct contact with illegal mining.

The Curuá River has an area of about 80 km^2 and is located at latitude 7.267044º and longitude −54.878073º. Its source is in Serra do Cachimbo, in the south of Pará, and after about 160 km in length, it ends at the mouth of the Iriri River, Pará. The Baú River is located at latitude −7.892821º and longitude −54.453716º. From these rivers, the indigenous people draw their main source of food, fish. These water bodies are being impacted by the presence of illegal mining. Figure 1 shows the description of the area.

It is important to point out that the authorization to enter the indigenous area was granted through official letter 258/2018/PRM/ATM/GAB1, from the Federal Public Ministry, Attorney General's Office in Altamira.

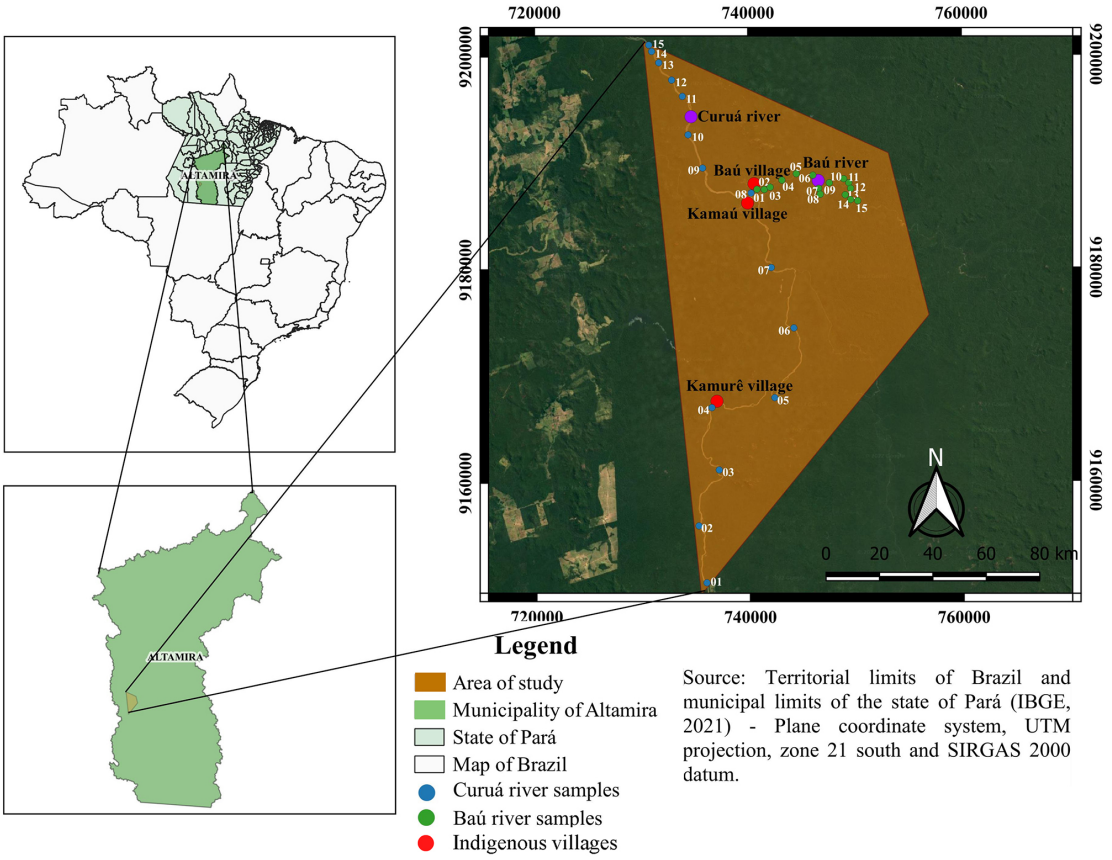

Figure 1. Map of the study area location in the city of Altamira-PA surrounded by the Curuá and Baú Rivers.

Collection and treatment of samples

Fish

Fish sampling was carried out along the Curuá and Baú Rivers, due to the greater proximity to the gold mining. For fish, 22 specimens were collected in the Curuá River and 33 specimens in the Baú River. The species collected were 13 *Ageneiosus brevifilis* ("*Mandubé*"), 17 *Plagioscion squamosissimus* ("*Pescada-Branca*"), and 25 *Pygocentrus nattereri* ("*Piranha-Vermelha*") totaling 55 fish specimens, ranging in length from 7.5 to 58 cm and weight from 35 to 2240 g. These species were selected based on their consumption by indigenous people.

The samples were captured with the help of the indigenous people and later identified, weighed and measurements of width and length were carried out. With the aid of a stainless steel scalpel, the material was filleted, removing the upper white portion of the muscle equivalent to 100 g. These muscles were stored, properly classified, identified in zip lock bags, and placed in styrofoam with ice for later freezing. The next step was to transport the material from Altamira-PA to Belém-PA to the Laboratory of Analytical and Environmental Chemistry (LAQUANAM) at UFPA. Table 1 describes the biometrics of the selected samples.

Samples were decomposed by wet digestion using microwaves. In it, the samples were digested in an acidic solution and taken to the digestion system of the Speedwave four DAP-60+ microwave (Berghof, Eningen unter Achalm, Germany).

The procedure consisted of weighing 500 mg of the sample in the digestion vessel, followed by the addition of 8 mL of 65% nitric acid (Merck, Darmstadt, Germany) and 2 mL of 35% hydrogen peroxide (Merck, Darmstadt, Germany). Then, the mixture was stirred carefully with the aid of a glass rod. After 10 min, the container was closed and heated in a microwave oven. The decomposition procedure followed the methodology described by Berghof.[28]

Water

River water collection stations were defined based on field studies. Fifteen samples of surface water were collected in the Curuá and Baú Rivers, totaling 30 samples. The collection stations were georeferenced through the global positioning system, the GPS (Global Positioning System). The collections were carried out with the help of Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (IBAMA) speedboats, which accompanied the team on their field trips. Water

Table 1. Biometry of the fish specimens chosen for this study

Curuá River				Baú River			
Sample	Length / cm	Sample	Length / cm	Sample	Length / cm	Sample	Length / cm
PSC01	53.0	PNC17	16.0	PSB01	41.0	ABB18	43.0
PSC02	44.0	PNC18	15.2	PSB02	44.0	$ABB_{\bar{x}}$	44.3
PSC03	41.0	PNC19	14.9	PSB03	41.0	PNB06	21.0
PSC11	45.6	PNC20	13.0	PSB04	38.0	PNB07	20.0
PSC22	42.2	PNC21	12.0	PSB05	48.0	PNB08	20.0
$PSC_{\bar{x}}$	45.2	$PNC_{\bar{x}}$	18.6	PSB26	50.9	PNB09	24.0
ABC05	45.0			PSB27	46.9	PNB10	24.0
ABC06	38.0			PSB29	50.3	PNB11	20.0
ABC07	47.0			PSB30	51.2	PNB19	24.0
ABC08	58.0			PSB31	46.2	PNB20	24.0
ABC09	30.0			PSB32	46.9	PNB21	22.0
ABC10	25.8			PSB33	44.8	PNB22	26.8
$ABC_{\bar{x}}$	40.6			$PSB_{\bar{x}}$	45.86	PNB23	21.3
PNC04	32.0			ABB12	48.2	PNB24	28.0
PNC12	21.1			ABB13	40.1	PNB25	7.5
PNC13	23.4			ABB14	50.0	PNB28	20.4
PNC14	22.1			ABB15	46.5	$PNB_{\bar{x}}$	21.6
PNC15	19.2			ABB16	39.0		
PNC16	15.2			ABB17	43.0		

PS: *P. squamosissimus* ("*Pescada-Branca*"); AB: *A. brevifilis* ("*Mandubé*"); PN: *P. nattereri* ("*Piranha-Vermelha*"); C: Rio Curuá; B: Rio Baú. $\bar{x}$: average length value.

collections were performed using a 5-liter vertical hale bottle, complying with the criteria recommended by the Standard Methods.[29] For packaging the water samples, polyethylene bottles with a capacity of 1 liter were used, previously decontaminated with HNO_3 solution (10%).

In the laboratory, the water samples were filtered through GFF membranes (Millipore 0.45 µm*)* using a vacuum filtration system. After filtration, acidification took place with Suprapur 65% concentrated nitric acid (Merck, Darmstadt, Germany) until pH < 2.

Previously, the quantification of As required the use of a pre-reduction technique for sample preparation. In this technique, 1% potassium iodide solution (≥ 99.5%, Sigma-Aldrich, Missouri, United States) and ascorbic acid (ACS grade, Merck, Darmstadt, Germany) in 10% hydrochloric acid (trace metal grade 32-36%, Fisher Chemical, Massachusetts, United States) were added to the samples for 5 h at room temperature. For the determination of Hg, the samples were acidified with 10% hydrochloric acid (Fisher Chemical, Massachusetts, United States).

Metal analysis and analytical quality

The quantification of total arsenic (TAs) and total mercury (THg) in the samples was performed by ICP-OES technique (optical emission spectrometry with inductively coupled plasma). The analytical performance required for these two elements has been enhanced by the use of a dedicated continuous flow hydride generation sample introduction system. The equipment used for quantification with the generation of hydrides was the iCAP 7000 Series ICP-OES (Thermo Fisher Scientific, Massachusetts, United States). The pre-reduction and quantification procedures followed the methodology described by Bartsch.[30]

The analytical curves of the elements were constructed with a certified standard solution (Merck ICP Multi-element, Darmstadt, Germany). The limit of quantification (LOQ) was calculated as the concentration equal to 10 times the standard deviation of 15 blank measurements divided by the slope of the analytical curve for each element. For the accuracy and precision studies, the material certified 1642b for Hg and 1643d for As in natural water from the National Institute of Standards and Technology (NIST) and DORM-2 from the National Research Council (Ottawa, Canada), for elements in fish tissue were used. The recoveries ranged from 96.8 to 111% for water and 91.3 to 109% for fish, showing that the method adopted is suitable for analyzing the elements.

Statistical treatment

The results obtained from the analyzes of the concentrations of TAs and THg in fish and river water underwent a series of statistical treatments. Data analysis included the calculation of position (average) and dispersion (standard deviation) parameters, as well as Box Plots, analysis of variance (ANOVA), Pearson's

correlation, and principal component analysis (PCA), as a function of dimension 1 (Dim1) and dimension 2 (Dim2). The statistical treatments were performed using the R programming language (version 4.0.3), RStudio[31] (version 1.4.1717, developed by Posit PBC), and OriginPro 2022[32] (SR1 9.9.0.225, developed by Originlab) on the 64-bit platform for Windows platform. The concentrations of quantified metals were compared with the standards prescribed by the legislation of Reference ANVISA RDC No. 722-Ministry of Health of July 01, 2022,[12] and CONAMA Resolution No. 357, of March 17, 2005.[13]

Risk assessment for fish consumption

The methodology used in the risk assessment involved the calculation of the hazard quotient (HQ) and the hazard index (HI) proposed by the U.S. Environmental Protection Agency (USEPA) (2018).[33] The HQ and HI are used to assess the deleterious risk that people are subject to prolonged exposure to a chemical substance(s). The assessment of the risk factor to human health for the consumption of fish with Hg and As contents by the indigenous peoples was determined according to the reference doses (RfD). For the elements, the reference values were used according to the values available in the USEPA (2000).[34] The reference doses for Hg and As are: Hg 0.1 RfD / μg kg^{-1} day^{-1} and As 0.3 RfD / μg kg^{-1} day^{-1}, where RfD is the reference dose.

Indigenous people consume fish daily, and may be exposed to these elements. Thus, the determination of muscle tissue intake (It) was the first step to assessing possible risks associated with prolonged consumption of these species. It was calculated using equation 1.

$$It = C \times \frac{IR \times EF \times ED}{BW \times T} \quad (1)$$

where It is the ingestion of contaminated muscle tissue (μg kg^{-1} day^{-1}); C is the concentration of the element in muscle tissue (μg g^{-1}); IR is the rate of food intake (g day^{-1}); EF is the exposure frequency (days $year^{-1}$); ED is the average duration of exposure (year); BW is the subject's average body weight during exposure (kg); T is the average exposure period, in days.

In determining fish consumption, the variables were selected based on the characteristics of the Brazilian population.[35,36] Thus, an average weight of 70 kg for adults and 15 kg for children; daily fish intake (35 g *per* person *per* day) for adults and (7 g *per* person *per* day) for children; and the average exposure period will be counted from the time of collection.

With the determination of It, it was possible to assess the risk quotients in the population as a function of the HQ and HI ratios, described in equations 2 and 3.

$$HQ = \frac{It}{RfD} \quad (2)$$

$$HI = \sum \frac{It}{RfD} \quad (3)$$

where It is the ingestion of contaminated muscle tissue (μg kg^{-1} day^{-1}); RfD is the reference dose (μg kg^{-1} day^{-1}).

The determined ratios (HQ and HI) inform whether there are deleterious risks to human beings during their exposure to one or more chemical substances. However, without the risk of presenting cancer problems or genetic mutation.[37] Values of HQ and HI > 1 indicate that there are risks of deleterious effects to humans, HQ and HI ≤ 1 indicate that there are no risks.

Bioaccumulation factor in fish

The bioaccumulation factor (BAF) is a mathematical criterion that allowed the evaluation of the occurrence of this process in fish. This factor was determined according to equation 4. The BAF was determined by dividing the concentration of the element in muscle tissue by the average concentration of the element in water.[34]

$$BAF = \frac{\text{Element concentration in the body } (\mu g\ kg^{-1})}{\text{Element concentration in the water } (\mu g\ L^{-1})} \quad (4)$$

Maximum BAF values in freshwater fish tissue were compared with that is recommended,[38] for As (400 L kg^{-1}) and Hg (1000 L kg^{-1}).

Results and Discussion

Fish

The results of the quantification of the elements in the muscle tissue of the fish species collected in the Baú and Curuá Rivers are described in Table 2.

TAs concentrations varied from < LOQ-0.307 and < LOQ-0.116 μg g^{-1} between species in Baú and Curuá Rivers, respectively. The THg presented variations of 0.014-0.996 and 0.107-4.552 μg g^{-1}, in Baú and Curuá Rivers, respectively. Among the species, higher TAs concentrations in muscle were observed in *P. squamosissimus* > *P. nattereri* > *A. brevifilis* (Baú River) and *P. squamosissimus* > *P. nattereri* > *A. brevifilis* (Curuá River). For THg, *P. squamosissimus* >

Table 2. Descriptive statistics on the concentration of chemical elements in fish

	THg / (μg g^{-1})				TAs / (μg g^{-1})			
Baú River	Mean	Sd	Max	Min	Mean	Sd	Max	Min
Plagioscion squamosissimus	0.356	0.240	0.860	0.113	0.074	0.085	0.307	< LOQ
Ageneiosus brevifilis	0.241	0.147	0.385	0.014	0.059	0.060	0.146	< LOQ
Pygocentrus nattereri	0.191	0.251	0.996	0.028	0.066	0.071	0.182	< LOQ
Curuá River	Mean	Sd	Max	Min	Mean	Sd	Max	Min
Plagioscion squamosissimus	0.434	0.141	0.667	0.323	0.038	0.047	0.116	< LOQ
Ageneiosus brevifilis	1.013	1.748	4.552	0.107	0.025	0.023	0.057	< LOQ
Pygocentrus nattereri	0.240	0.114	0.462	0.117	0.027	0.037	0.108	< LOQ

Sd: standard deviation; Max: maximum; Min: minimum. Reference ANVISA RDC No. 722-Ministry of Health of July 01, 2022: 1 μg g^{-1} for As and Hg; LOQ: limit of quantification (ng g^{-1}), 0.400 for Hg and 2.833 for As.[12]

A. brevifilis > *P. nattereri* (Baú River) and *P. squamosissimus* > *P. nattereri* > *A. brevifilis* (Curuá River).

For all samples analyzed, TAs concentrations were within the safe limits established by the RDC No. 722/2022 standard. It is of great importance to emphasize that the presence of high levels of As during gold processing is directly linked to the presence of arsenopyrite in some specific areas. The leaching of this element, which was previously retained in rocks, causes it to be transported to water bodies, significantly contaminating them.[21,39] The values found for THg showed dangerous levels; the species *A. brevifilis* from the Curuá River presented an average of 1.013 μg g^{-1}, thus an average higher than the 1 μg g^{-1} established as the permitted limit of RDC No. 722. The high levels of mercury found in the species *A. brevifilis* may be attributed to its migration and residence time in more contaminated areas or to the ingestion of food and prey with high levels of mercury.[40]

The species *P. nattereri* and *P. squamosissimus*, despite their averages being within the allowed limit, in some samples, concentrations of 0.996 and 0.860 μg g^{-1} were obtained, respectively, which are values close to the maximum limit established by legislation.

When comparing the levels of THg within the same species, it is observed that the species *A. brevifilis*, *P. nattereri*, and *P. squamosissimus* from the Curuá River presented levels 4.20, 1.22, and 1.26 times higher than the average values for this element in the Baú River, respectively. These results show that illegal gold mining activity is directly affecting the Curuá River and negatively impacting these species. Regarding the presence of TAs, it is observed that the species *A. brevifilis*, *P. nattereri*, and *P. squamosissimus* captured in the Curuá River presented concentrations 2.36, 1.95, and 2.44 times lower than the average concentrations observed in the Baú River, respectively. However, the concentrations found are considered very low and do not represent a significant risk of environmental contamination.

The ANOVA test for the mean concentrations of THg and TAs at $p = 0.05$ did not show a significant difference between the species. The conditions for the null hypothesis (H0) to be accepted were for As $F_{calculated}$ (0.138) < $F_{critical}$ (3.316) with p-value (0.871) > p-value (0.05) and Hg $F_{calculated}$ (1.693) < $F_{critical}$ (3.316) with p-value (0.201) > p-value (0.05) for fish from the Baú River and As $F_{calculated}$ (0.202) < $F_{critical}$ (3.522) with p-value (0.819) > p-value (0.05) and Hg $F_{calculated}$ (1.439) < $F_{critical}$ (3.522) with p-value (0.262) > p-value (0.05) for fish from the Curuá River.

The statistically similar concentrations for TAs and THg observed in the three species, both in the Baú and Curuá Rivers, can be attributed to all of them occupying comparable trophic levels.

The findings of the present work indicate that the main sources of exposure to As in the fish under study are probably natural from the Amazonian geochemistry and that even the extreme results of the species *P. squamosissimus* in the rivers, end up being at safe levels. In contrast, the Hg present in the species is due to illegal gold mining; therefore, the extreme results for the species *P. nattereri* (Baú River) and *A. brevifilis* (Curuá River) are attributed to these fishes due to their presence in areas with high levels of Hg (Figure 2).

Only the PSC samples showed a positive Pearson correlation coefficient for Hg (r = 0.930) in the linear relationships shown in Figure 3. For As, the PSC and ABB samples had the best correlations (r = 0.842 and r = 0.737, respectively). These positive correlations can be explained by the presence of lower sample demand, which results in fewer points in the correlation.

According to the results, only PSC species showed a positive correlation with Hg (r = 0.930), as illustrated in the linear relationships in Figure 3. This means that the increase in mercury concentration is associated with the increase in the species' length variable. Regarding As, PSC

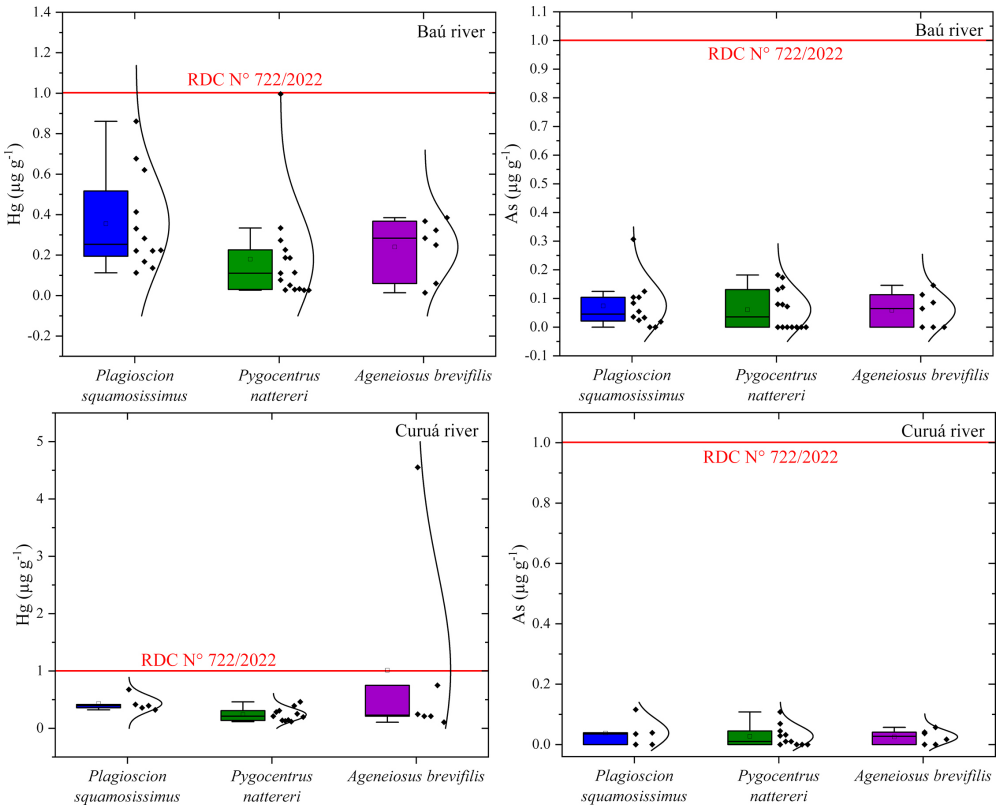

Figure 2. Box plots of TAs and THg concentration values (µg g^{-1}).

and ABB species had the best linear correlations (r = 0.842 and r = 0.737), respectively. However, it is important to note that these positive correlations may be explained by the presence of a smaller sample size, resulting in fewer points in the correlation.

It was evidenced that size is not a factor that contributes to the higher levels of As and Hg in fish. This result explains that although the species *P. nattereri* is up to 2.5 times smaller than *P. squamosissimus* and *Ageneiosus brevifilis*, it has Hg levels similar to them. Another factor that can contribute to higher concentrations of Hg and As for small species is their diet, leaving them more exposed to these pollutants.[41]

From the PCA result (Figure 4), from the perceptual map, it can be noted that the first individual factor (Dim 1) explains 58.1% of the data variability, while the second (Dim 2) explains 41.9% in the Baú River and Dim1 equal to 62.7% and Dim2 equal to 37.3% in the Curuá River. It can be considered that the two-dimensional perceptual maps presented are adequate to evaluate the relationships between the variables since they explain a large part of the variability of the data.

As a result, the elements Hg and As are not positively correlated with each other, which means that fish samples with high concentrations of Hg do not necessarily tend to also have a high concentration of As. This implies that any principal component will not cause a correlated response in terms of the other component.

Tissue concentrations of Hg and As have been studied in a wide selection of fish species in the Amazon region and in the world in recent decades (Table 3), as expected the accumulation of these metals occurs at high levels in carnivorous fish species. The presence of As in these studies was at acceptable levels by Brazilian legislation (1 µg g^{-1}). For Hg in species frequently found in the Tapajós, Madeira, Branco, Owasco, and Ebro Rivers, the values were above the maximum levels allowed in Brazil (1 µg g^{-1}). These high levels show that the Hg problem extends beyond the rivers of the Amazon region; as a result, these rivers and their species need to be closely monitored.

As concentrations in the species in this study were below or equal to the levels reported in the literature, as indicated in Table 3. Compared to the levels quantified in Brazil, the results for Hg found in the species *P. squamosissimus*, *A. brevifilis*, *P. nattereri* (Baú River) and *P. squamosissimus*, *P. nattereri* (Curuá River) were lower than those of 90% of the authors. However, the species *A. brevifilis* (Curuá River) had an average of more than 40% of these authors. The lower concentrations of Hg found in this study compared to the findings of Brazilian authors may be due to the fact that Tapajós and Madeira Rivers, for example, suffer from the activity of gold mining for a longer period.

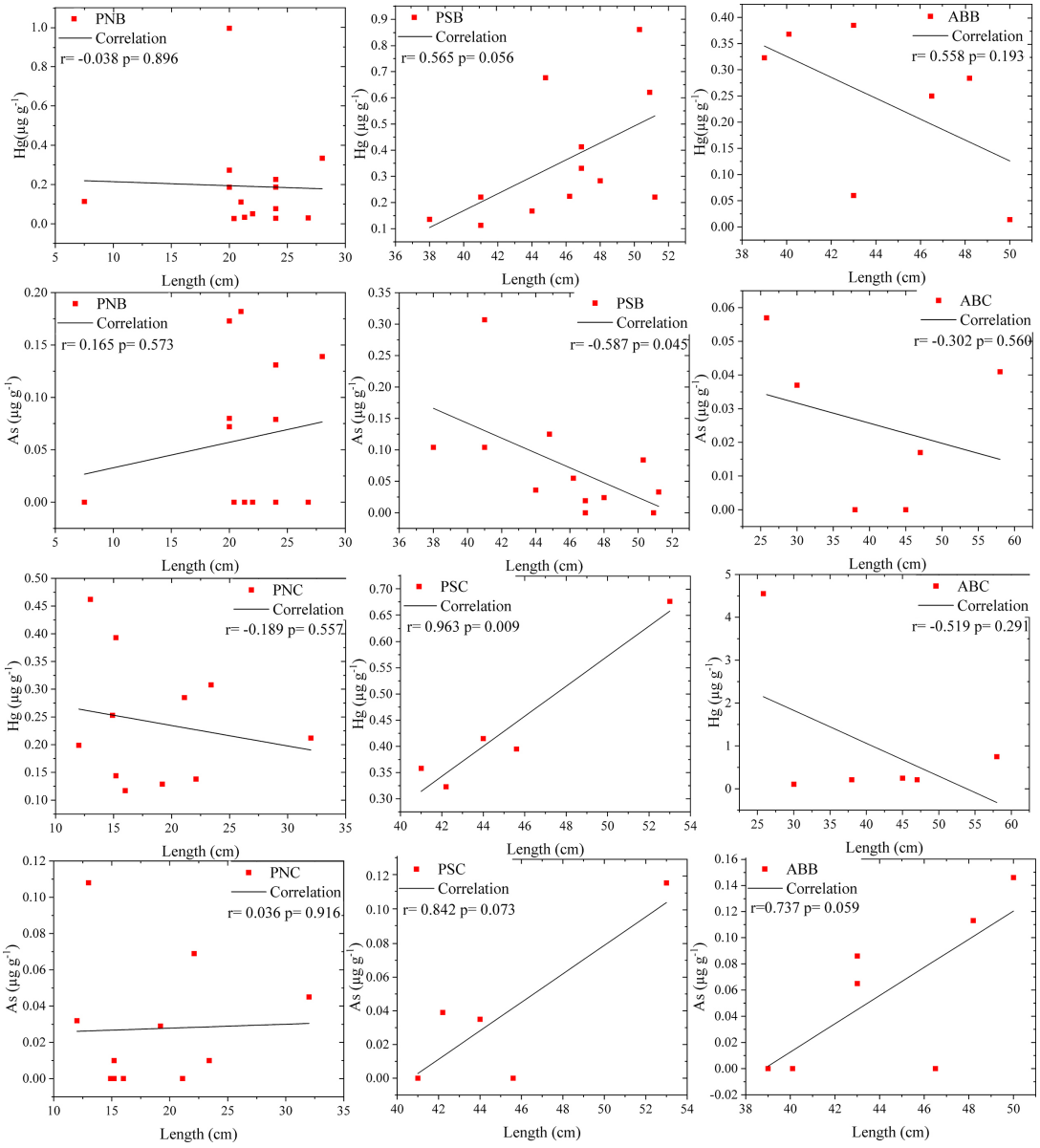

Figure 3. Pearson's linear correlation between TAs and THg concentrations and length values recorded in fish samples. PS: *P. squamosissimus*; AB: *A. brevifilis*; PN: *P. nattereri*; C: Curuá River; B: Baú River.

In comparison with the average concentrations of Hg found by authors from other countries, the species from the Baú River obtained levels above 45% of these authors. In turn, the species from the Curuá River obtained levels above 54% of them, with emphasis on *A. brevifilis*, which showed a concentration above 81% of these studies. These comparisons highlight the serious problem that the Amazon region currently faces due to the high levels of Hg in the region.

Water

The results for the concentration of elements in the water in the Baú and Curuá Rivers are presented in Table 4. Both element averages complied with the legislation adopted in Brazil.

The gold mining activity did not influence the exposure of As in the rivers under study, so far, which suggests that its presence is natural. However, in the Baú River, there was a sample for Hg with values above the allowed (0.220 µg L^{-1}). The presence of Hg in natural waters is of concern due to its high toxicity and ability to accumulate in biota, which can cause it to reach higher levels in the food chain, like humans.[57]

It is worth mentioning that values quantified in surface water tend to have lower levels compared to deep water.[58] This placement is evidenced in the literature,[59] in which the concentrations for deep water were up to 6.5 times

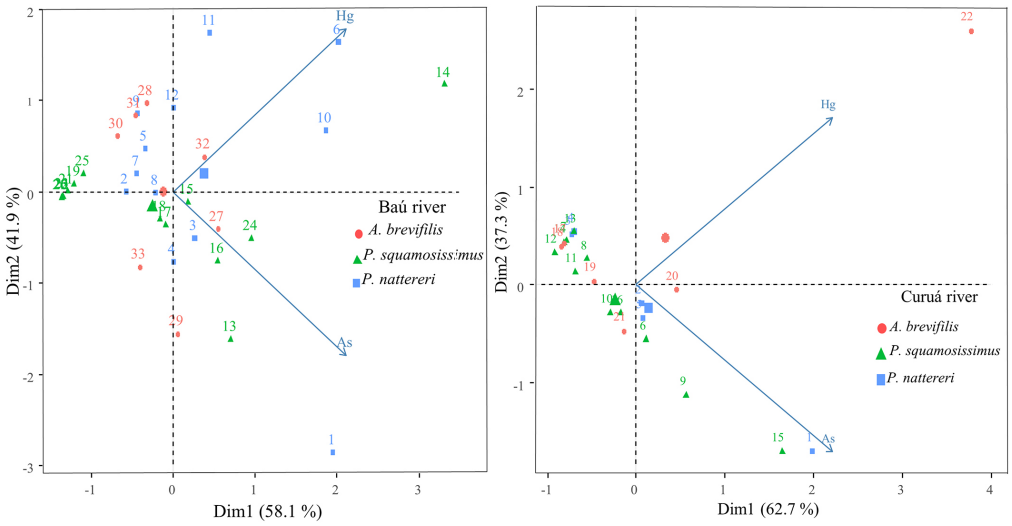

Figure 4. PC1 (Dim1) and PC2 (Dim2) principal component charts and factor loads.

Table 3. Mean concentrations of Hg and As in fish

Country (State)	Species	Hg / (μg g^{-1})	As / (μg g^{-1})	River/Sea	Reference
Brazil (PA)	*Plagioscion squamosissimus*	0.356	0.074	Baú	current study
Brazil (PA)	*Ageneiosus brevifilis*	0.241	0.059	Baú	current study
Brazil (PA)	*Pygocentrus nattereri*	0.193	0.066	Baú	current study
Brazil (PA)	*Plagioscion squamosissimus*	0.434	0.038	Curuá	current study
Brazil (PA)	*Ageneiosus brevifilis*	1.013	0.025	Curuá	current study
Brazil (PA)	*Pygocentrus nattereri*	0.240	0.027	Curuá	current study
Brazil (SP)	*Pygocentrus nattereri*	–	0.730	Piracicaba	42
Brazil (PA)	*Plagioscion squamosissimus*	0.098	–	Tapajós	43
Brazil (RO)	*Brachyplatystoma filamentosum*	1.970	–	Madeira	44
Brazil (PA)	*Ageneiosus brevifilis*	0.650	–	Bacajá	45
Brazil (PA)	*Plagioscion squamosissimus*	0.540	–	Bacajá	45
Brazil (AC)	*Plagioscion squamosissimus*	0.617	–	Purus	46
Brazil (PA)	*Plagioscion squamosissimus*	1.550	–	Tapajós	47
Brazil (AM)	*Cichla ocellaris*	4.549	–	–	48
Brazil (PA)	*Cichla ocellaris*	3.250	0.600	–	49
Brazil (AM)	*Hypostomus affinis*	0.620	0.160	–	49
Brazil (RO)	*Pygocentrus nattereri*	1.751	–	Branco	50
Colombia	*Hoplerythrinus unitaeniatus*	0.120	–	Amazon	51
Colombia	*Caquetaia kraussii*	0.490	–	Amazon	51
United States	*Lepomis microlophus*	0.061	0.300	Colorado	52
United States	*Sander vitreus*	1.529	–	Owasco	52
Spain	*Cyprinidae*	0.013-3.470	0.030-0.800	Ebro	53
China	*Carassius carassius*	0.016	–	Chagan	41
Serbia	*Alburnus alburnos*	0.010	0.010	Sava	54
Serbia	*Alburnoides bipunctatus*	0.100	0.150	Sava	54
Serbia	*Squalius cephalus*	0.467	0.122	Sava	54
Slovakia	*Salmo trutta*	0.363	–	Malachov	55
Iran	*Cyprinus carpio*	0.580	0.800	Caspian	56

Brazilian states: PA: Pará; AM: Amazonas; RO: Rondônia; AC: Acre; SP: São Paulo.

Table 4. Descriptive statistics on the concentration of chemical elements in river water

	Hg / (μg L^{-1})				As / (μg L^{-1})			
	Mean	Sd	Max	Min	Mean	Sd	Max	Min
Baú River	0.114	0.051	0.220	0.001	0.367	0.521	1.560	< LOQ
Curuá River	0.062	0.050	0.159	< LOQ	0.405	0.442	1.290	< LOQ

Sd: standard deviation; Max: maximum; Min: minimum. Reference CONAMA 357/2005: 10 μg L^{-1} for As and 0.2 μg L^{-1} for Hg; LOQ: limit of quantification (ng L^{-1}), 0.377 for Hg and 1.384 for As.[13]

higher (THg) compared to those found on the surface and 5.8 times higher for methyl-Hg in the same ratio. This can be explained by the physical-chemical properties of Hg, which have a greater tendency to deposit in deep waters and, consequently, be affected by bacteria.[60] This evidence is verified in the literature, which mentions the presence of methyl-Hg at depths greater than 1000 meters.[61] Thus, the presence of mercury in the rivers under study may have higher levels.

Currently, although there are values on Hg limits for different exposure channels, these reference values should not be interpreted as a clear threshold that separates safe from unsafe.[62] That is, the safe limit would be the null presence of Hg and anything outside of that can be considered unsafe for human health in the face of exposure to Hg. For example, doses well below the current reference levels considered safe in environmental sources caused neurotoxic problems in children.[63]

Toxicological risk assessment for fish consumption

Table 5 shows the indexes used in the risk assessment (HQ and HI) found.

It was possible to verify that, in relation to As, the HQ presented higher values for Hg, which was expected since the quantified Hg concentrations were higher and more dangerous than those of As. When analyzing the HQ values for Hg, it is noted that only the HI_{PN}, for an adult, in the Baú River has a value below 1. In this same river, the HQ value for children is less than 1 of HQ > 1 determined, indicating that the continued consumption of these fish represents a significant risk of diseases related to Hg for the indigenous people.

The fish from the Curuá River obtained the highest HQ values, especially *A. brevifilis*, which has a ratio of 5.1 for an adult male and 4.7 for children. The highlight in the Baú River was given to *P. squamosissimus* with values of 1.8 for an adult person and 1.7 for children. This fact indicates that these species are those that, when consumed for a long period, will cause more quick problems of intoxication by Hg.

The HQ values for children are on average 7.4%, lower than for adults. However, in the prolonged consumption of these species, children are more likely to have Hg intoxication, as they are more immunologically vulnerable than adults.[64]

According to HI, all species under study have HI > 1, which would indicate that the consumption of these fish would cause harmful health effects, due to the simultaneous exposure to both elements (Hg and As). However, the determined values of HI > 1 are attributed to the high concentrations of Hg quantified in the muscular tissue of these fish. Therefore, the deleterious effects that this population will acquire will be solely caused by the concentration of Hg in the fish.

Bioaccumulation factor (BAF)

Table 6 shows the values of BAF found in the species.

The assessment of the bioaccumulation factor plays an important role in verifying the intoxication that a certain population is suffering, and the safety of aquatic

Table 5. HQ and HI for children and adults

Risk factor	Curuá River				Baú River			
	Adult		Child		Adult		Child	
	Hg	As	Hg	As	Hg	As	Hg	As
HQ_{PS}	**2.2**	0.1	**2.0**	0.1	**1.8**	0.1	**1.7**	0.4
HQ_{PN}	**1.2**	0.1	**1.1**	0.1	0.9	0.1	0.9	0.1
HQ_{AB}	**5.1**	0.1	**4.7**	0.1	**1.2**	0.1	**1.1**	0.0
HI_{PS}	**2.2**		**2.1**		**1.9**		**2.1**	
HI_{PN}	**1.3**		**1.2**		**1.1**		0.9	
HI_{AB}	**5.1**		**4.8**		**1.3**		**1.1**	

HQ: hazard quotient; HI: hazard index; AB: *A. breviphilis*; PS: *P. squamosissimus*; PN: *P. nattereri*. Values > 1 for both reasons mean the possibility of harm to health; Values < 1 indicate zero health risk. In bold, values that do not conform to the reference adopted by USEPA.[37]

Table 6. Bioaccumulation factor in fish

Bioaccumulation factor	Curuá River		Baú River	
	As / (L kg^{-1})	Hg / (L kg^{-1})	As / (L kg^{-1})	Hg / (L kg^{-1})
BAF_{PS}	94	**7000**	202	**3123**
BAF_{PN}	67	**3871**	180	**1675**
BAF_{AB}	57	**16883**	161	**2114**

AB: *A. brevifilis*; PS: *P. squamosissimus*; PN: *P. nattereri*. In bold, values that do not conform to the reference adopted by National Council on Radiation Protection and Measurements (NCRP).[38]

ecosystems and all the biota that live in them is a factor of great importance, as they play a central role in providing water and food for humans, especially those living close to these ecosystems.[65]

The BAF showed that Hg presents significant values in relation to As in all biological species. All fish BAF values for As were < 400 L kg^{-1}. This suggests low bioaccumulation for this element in the fish studied. However, the values for Hg were > 1000 L kg^{-1}, indicating that this element has high levels of bioaccumulation in these species. These values have raised concerns since Hg can harm human consumers of these fish who may acquire high levels of this element in their bodies as a result of its toxicity.

In the Curuá River, the BAF of Hg was higher in *A. brevifilis* > *P. squamosissimus* > *P. nattereri*. Although fish body size is a factor in the bioaccumulation process[66] and there is a tendency for high concentrations to be found in larger fish, the *A. brevifilis* species has a smaller average length (40.6 cm) than the *P. squamosissimus* species (45.2 cm), as observed in Table 1.[38] Thus, the *A. brevifilis* species is absorbing more mercury in its body, probably due to its diet and location in areas with higher levels of mercury. In turn, the BAF values for the *P. nattereri* species can be attributed to its average length (18.6 cm), which is 2.2 and 2.4 times smaller compared to the *A. brevifilis* and *P. squamosissimus* species, respectively. Thus, resulting in less absorption of mercury in its muscle tissue.

In the Baú River, the increasing order of BAF was *P. squamosissimus* > *A. brevifilis* > *P. nattereri*. Unlike the species in the Curuá River, the higher BAF values were attributed to the larger average size of the species, which were 45.8, 44.3, and 21.6 cm, respectively, as observed in Table 1.[38] In both rivers, the species are directly affected by the mercury present in the environment, and thus the higher their level in the food chain, the more accumulation of this element they can have in their body.[67] Therefore, the high values for the species' bioaccumulation factor are explained because they are carnivorous species and occupy high trophic levels in the food chain.

The high bioaccumulation of Hg in carnivorous fish in areas contaminated by this element was expected due to their food diversity, in addition to other factors such as mobility, foraging location, and migratory characteristics.[68] Toxic elements can penetrate the tissues of fish by natural absorption and/or as a function of their food in the food chain.[69]

The BAF values found in this study indicate that these fish are absorbing the existing Hg in the Curuá and Baú Rivers, generated from gold mining. Thus, there is a high probability that these levels of Hg are acquired by indigenous peoples since these fish are the most consumed by these people.

The continuous operation of gold mining suggests that the existing local biota will still accumulate high concentrations of Hg, which together with the phenomenon of biomagnification will affect in an accelerated way the indigenous peoples who depend on the food originated from the rivers in this study.

Conclusions

The presence of TAs in the fish evaluated in this study showed safe levels, suggesting that these levels are natural to the region's geochemistry. Regarding THg, only one sample showed a concentration above the safety limit established by ANVISA's RDC No. 722/2022 reference. However, due to the physical-chemical properties of Hg, it is expected to have higher concentrations in deeper waters.

The study revealed that only Hg has bioaccumulative characteristics above the reference adopted in the fish tissues, due to the generation of the element during the gold mining process. Therefore, consuming these fish can lead to increased levels of Hg in the indigenous people's bodies, representing a significant health risk, especially for children. Finally, this determination reflects local contamination by Hg, particularly in the aquatic ecosystem.

The HQ for fish consumption shows that As has zero risk of causing health effects to residents of the indigenous communities studied. However, in relation to the HQ of Hg, in fish, it indicates that the prolonged consumption of these species presents a high risk for the indigenous people to develop health problems, especially children. As for simultaneous exposure to As and Hg,

as determined by the HI, it indicates significant risks in fish consumption only, based only on quantified Hg levels. Therefore, the occurrence of deleterious effects in indigenous communities will result from the presence of Hg in the fish body.

The study suggests that illegal gold mining activities are identified as the source of Hg contamination in the analyzed fish species, thus, they can be used as bioindicators of this Hg in rivers. In this context, fish can be important for making environmental policy decisions, as they are bioindicators of pollution.

Acknowledgments

We are grateful for the support of the Coordination for the Improvement of Higher Education Personnel (CAPES - Brasil), Federal Public Ministry of Altamira-PA, especially Dr Cristiane Costa Carneiro, Secretary of Health and Environment of the State of Pará, the Brazilian Institute for the Environment and Renewable Natural Resources, Special Indigenous Sanitary District of Altamira, Federal University of Pará and Indigenous people for their help in collecting the samples.

Author Contributions

L.V.D.S. was responsible for funding acquisition, resources, data curation, investigation, formal analysis, data processing, writing original draft, writing-review and editing; S.F.P.P. for conceptualization, funding acquisition, project administration, resources, sample collection, data processing, writing original draft, writing-review and editing; C.C.C. for data curation, investigation, writing original draft, writing-review and editing; T.M.S. for software, investigation, data curation, writing the original draft; R.M.R. for investigation, formal analysis, data processing, writing original draft; H.C.C. for software, investigation, data curation, writing the original draft; C.S.S. for data curation, investigation, writing original draft, writing-review and editing; A.M.F.S. for software, investigation, data curation, writing the original draft; M.L.S.S. for investigation, resources, writing-review and editing.

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Submitted: January 14, 2023
Published online: May 11, 2023