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RISK ANALYSIS DUE TO INGESTION OF TRACAJÁ WITH ARSENIC IN A KAYAPÓ INDIGENOUS VILLAGE

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ABSTRACT

The objective of this work was to evaluate the presence of As in the tracajá tissue consumed by indigenous people and added to the environment by the gold mining activity. The study area was the Kayapó indigenous region, on the Baú river, in the municipality of Altamira-PA, Brazilian Amazon. HNO_3 and microwave were used for sample digestion. As was quantified by Inductively Coupled Plasma Optical Emission Spectrometry with Hydride Generation. For the risk assessment, the ingestion of muscle tissue was used, and then methodologies were used to calculate the hazard quotient (HQ) and hazard index (HI). The HQ values for children were slightly lower compared to adults, but in prolonged consumption of the species, children are more likely to have As intoxication. The hazard index (HI) presented valuesbelow 1 in the samples, which indicates that the consumption of tracajá would not cause harmful health effects in an individual by the simultaneous exposure to As. The results of this study showed that the concentrations of As found in the tracajás presented rates below the maximum concentration allowed (1 $\mu g\ g^{-1}$ of As) by ANVISA. As much as the HQ and HI values for As are below the established reference, care must be taken when consuming foods with As, as it is a food that is widely consumed by the indigenous people, and its weekly consumption rate may take in the future to some risks to the health of indigenous people, especially children.

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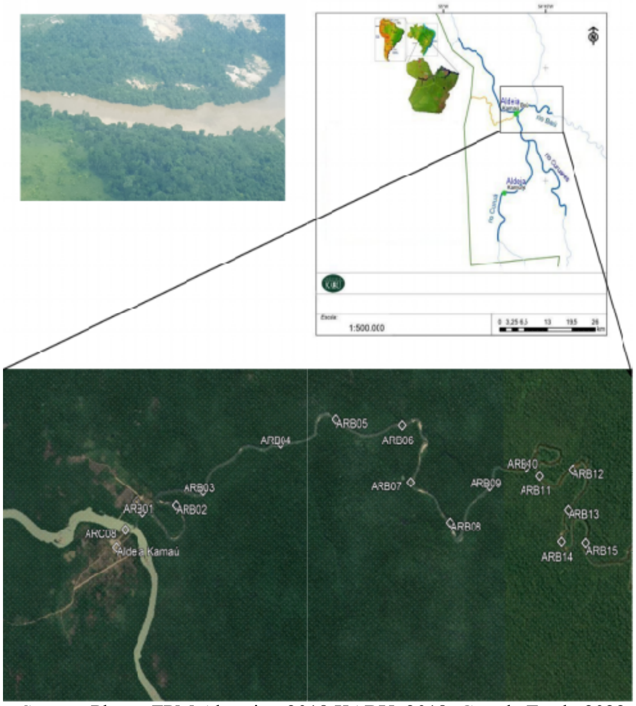
INTRODUCTION

Toxic elements such as As are added naturally to the human environment and through human activities such as mining. Because As is considered an Au tracker, it receives greater environmental attention in gold exploration areas (NETO *et al*., 2020). The main environmental threats caused by gold mining include deforestation, acid mine drainage, and air and water contamination by toxic elements such as As, reported by Veiga *et al*. (2006) and DeNicola & Stapleton (2002). Environmental contamination by As is a major global problem and has become a challenge for scientists around the world, and this occurs mainly through the ingestion of contaminated water (BARRA *et al*., 2000; MATSCHULLAT *et al*., 2000). As pollution of groundwater is widespread and there are several regions where As contamination is significant, according to Ravenscroft *et al.* (2009), it will only increase as the global demand for natural resources is increasingly driving the extraction of local resources and the use of land according to DeFries *et al*. (2010). According to the World Health Organization (2001), about 200 million people worldwide are chronically exposed to As present in natural waters at levels above the proposed safety standard of 10 µg/L. Non-industrial informal mining has increased dramatically in developing countries, with these increases having serious environmental and health consequences for these countries, Swenson *et al*. (2011) and Larmer (2009). As contamination in Brazil, regardless of the source of contamination (natural or anthropogenic), has been discussed in the last two decades. Authors such as Borba *et al*. (2003), Santos *et al*. (2003), Matschullat *et al*. (2007), Keim (2011), Costa *et al*. (2015), and Silva *et al*. (2018), warned of the imminent risk of contamination after quantifying high levels of As in water in their studies. In the 1950s, substantial artisanal and small-scale gold mining activities began in the Amazon basin (LOBO *et al*., 2016). This mining activity has caused alarming social impacts, thousands of miners have taken over indigenous territories throughout the Amazon, making the region with the largest number of illegal mining in the world according to Couto *et al*. (2021). The population that resides around the mines is directly and indirectly affected, the mines cause destruction, contamination of ecosystems and populations, and other negative impacts. In Brazil, in Minas Gerais Ono *et al*. (2012) reported the high presence of As in tailings in gold mining areas. The As released by mineral extraction can reach rivers and contaminate the local biota. Contaminated water affects aquatic biota and as a result, it becomes one of the most important sources of contamination for populations that consume these foods. Continuous ingestion of food contaminated by As can bring serious diseases to humans. The effects of excess As in living beings involve damage to various organs and systems such as the cardiovascular system, vascular disorders followed by gangrene and black foot disease, blood system, reproductive system, increased frequency of spontaneous abortions, skin tumors, hyperpigmentation, hyperkeratosis, liver, liver dysfunction, nervous system, and respiratory system, in addition to respiratory, neurological disorders, internal cancers (lung, bladder, liver, and kidney) and skin cancer (SMEESTER & FRY, 2018; MOCHIZUKI, 2019; SINHA & PRASAD, 2020; PALMA-LARA *et al*., 2020; OLIVEIRA *et al*., 2021). In the aquatic biota, in addition to fish, other species such as tracajás are impacted by the accumulation of toxic elements in their body. It was found that their reproductive success is being hampered by the presence of toxic elements in the mother's body (BELL *et al*., 2004; RAFFERTY *et al*., 2011; PERRAULT *et al*., 2012). As with fish, tracajás are contaminated through the ingestion of contaminated food and water (PERRAULT *et al*., 2013). Tracajá is part of the diet of riverine and indigenous peoples of the Amazon, it is also very targeted by the illegal trade in the region (BRAGA & MUDURUKU, 2020). When adult, a tracajá whose scientific name is Podocnemis unifilis, reaches 45 cm in length, weighing about 8 kilos (KLOSOVSKI, 2003). An easy target, the tracajá is hunted during spawning, when it is on dry land when its movements are slower. Although they choose the ravines of rivers and lakes to reproduce, this tactic does not always work (BRAGA & REBÊLO, 2015). In this way, the tracajá can act as a biomarker in the assessment of risk to humans as well as fish (MEHANNA *et al*., 2016; GU *et al*., 2017; BORHAN *et al*., 2017; VAROL; SÜNBÜL, 2017; SHEIKHZADEH; HAMIDIAN, 2021). The contamination of this important food for the native populations of the Amazon can bring an impact on the health of these populations and the analysis of the risk that this ingestion of food contaminated by As is fundamental for the conservation of the health of these populations. Due to the concern for the food safety of the population, as a result of single or simultaneous exposure to several chemicals, government agencies have given considerable attention to this problem, and thus, methodologies and guidelines have been developed to calculate the cumulative risk (BOPP *et al*., 2018; EFSA *et al*., 2019). The methodologies most used in risk assessment are the hazard quotient (HQ) and the hazard index (HI) (USEPA, 2018). The HQ and HI are used to assess the deleterious risk that people are subject to prolonged exposure to a chemical substance. The villages Kamurê, Kamuá, and Baú are located in the municipality of Altamira-PA. These indigenous people and the ecosystem are being impacted by the presence of the Esperança IV mine. The objective of this work was to evaluate the risk of ingestion of tracajá with As contents by indigenous people of the Kayapó ethnicity, captured in the Baú river basin, municipality of Altamira-PA, Brazilian Amazon.

METHODOLOGY

COLLECTION PLACE: The area of this study is part of an indigenous reserve of environmental protection that houses indigenous communities from three villages, Baú, Kamurê, and Kamuá. They are located in the municipality of Altamira-PA, in the Brazilian Amazon, and belong to the Kayapó tribe (Figure 1). These villages are surrounded by the Curuá and Baú rivers, the first being in direct contact with illegal mining. The Baú River is located at latitude -7.892821º and longitude -54.453716º. The Baú village is directly in contact with this river. Rivers and local biota are being impacted by mining activity as shown in Figure 2.

COLLECTION PROCEDURES: The collection of all tracajá samples took place along the Baú River with the help of the Kayapó indigenous people and the employees of the KABU Institute, the Special Indigenous Sanitary District (DSEI) of Tapajós, the National Indian Foundation (FUNAI) of Brasília and the Federal Public Ministry(FPM), who was responsible for storing and conserving this material. Sample collection followed the protocols recommended by the National Council of Animal Experimentation Control (CONCEA), Standard Methods (APHA *et al*., 2017), and other procedures. The tracajás were captured in different places of the Baú river, having as reference the water collection stations. The markings refer to the water collection stations taken as a basis for capturing the tracajás. It is worth mentioning that the tracajás were selected given their consumption in the Baú village. Tracajá samples were identified, weighed, and measured in width and length. Ten tracajás were captured with a means width of 24.5 cm, length of 26.31 cm, and weight of 1,735 g. These 7 tracajás were male and 3 were female. In the field, with the aid of a stainless steel scalpel, filleting of the material was carried out, and a portion of the tissue equivalent to 100 g was removed. These muscles were stored, properly classified, identified in zip lock bags, and immediately placed in styrofoam with ice for later freezing and transport from Altamira-PA to Belém-PA to the Laboratory of Analytical and Environmental Chemistry (LAQUANAM) at UFPA.



Source: Photo: FPM Altamira, 2018.KABU, 2018, Google Earth, 2022

Figure 1. Study area location map

TREATMENT AND DIGESTION OF TRACAJÁ SAMPLES: All materials used were previously decontaminated by immersion in 10% HNO_3 (v v^{-1}) for 4 hours, then washed 5 times with ultrapure water and dried in a laminar flow hood before use. Analytical grade reagents and ultrapure water from the Milli-Q purification system were used for the treatment of samples, and preparation of standards and solutions. Supra pure HNO_3 acid and microwave opening were used to open the tracajá samples. Tracajá samples were submitted to a wet digestion procedure adapted from the procedures recommended by Aldoghachi *et al*. (2016) and Olmedo *et al*. (2013) Berghof (2013).

The samples were previously dried and crushed and sent for digestion. The procedure consisted of weighing 500 mg of the dry sample in the digestion vessel, followed by the addition of 8 mL of nitric acid (65%) and 2 mL of hydrogen peroxide (35%). Then, the mixture was stirred carefully with the aid of a glass rod. After 10 minutes the container was closed and heated and taken to the Speedwave four DAP-60+ microwave digestion system (Berghof, Germany). After the programming was completed, the pumps were cooled for 10 minutes at 40 °C to ensure that there was no loss of arsenic, which is volatile.

Fonte: FPM - Altamira, 2018.

Figura 2. Atividade garimpeira no rio Curuá- Altamira-PA

ANALYSIS OF AS IN TRACAJÁ: To quantify the concentrations of As present in the tracajás, the technique of Inductively Coupled Plasma Optical Emission Spectrometry with Hydride Generation (HG ICP-OES) was used. The ICP-OES technique uses an inductive plasma source in the equipment and is suitable for quantifying polluting and toxic metals according to Smichowski, *et al*. (2005) the generation of hydrides transforms the species of As (III) present in the sample into arsine (AsH_3) (SKOOG *et al*., 1998). The analytical performance required for As 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 HGICP-OES (Thermo Fisher Scientific, Waltham, Massachusetts, USA).
The sample preparation and quantification procedures followed the methodology described by Bartsch (2016).

ANALYTICAL QUALITY: The analytical quality of the tracajá matrix is shown in Table 1. As it was not possible to acquire standard samples of tracajá for the accuracy and repeatability studies, due to its inexistence, the technique of standard addition was used, where an amount of the analyte was added to one of the samples, and all procedures were repeated on the sample without addition, the procedure was repeated 10 times and the achieved recovery and repeatability were calculated.

STATISTICAL ANALYSIS: The results obtained from the analysis for As concentrations underwent a series of statistical treatments. Data analysis included the calculation of position (average) and dispersion (standard deviation) parameters, as well as BoxPlots and Linear Correlation Test. Statistical treatments were performed using Excel (2016 office version) and Statistica (version 10.0.228.8) programs. on the 64-bit platform for Windows.

RISK ANALYSIS DUE TO TRACAJÁ INGESTION: The assessment of the risk to human health from the consumption of tracajás with As levels by the indigenous people were determined according to the reference doses (RfD). The reference dose (RfD) for As is 0.3 µg kg^{-1} day^{-1} according to USEPA, 2020. 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 through equation 1.

$$It = Cx\,\frac{IR \times EF \times ED}{BW} \times \frac{1}{AT} \qquad (1)$$

Where:

It = It is the intake of muscle tissue (µg kg^{-1} day^{-1});
C = It is the concentration of the element in muscle tissue (µg g^{-1});
RI = It is the rate of food intake (g day^{-1});
EF = It is the exposure frequency (days $year^{-1}$);
ED = It is the average duration of exposure (year);
BW = Is the subject's average body weight during exposure (kg);
AT = It is the average time of exposure, in days.
In determining the It is consumption of tracajás, the variables in equation (1) were selected based on the characteristics of the Brazilian population (IBGE, 2009; SILVA; SANTOS, 2016).

Thus:

- The average weight of 70 kg for men and 15 kg for children;
- Daily fish intake (35g/person/day) for adults and (7g/person/day) for children;
- And the average exposure period was accounted for as reported at the time of collection;

With the determination of It, it was possible to assess the risk in the population as a

(Equation 2) and HI (Equation 3) ratios.

$$HQ = \frac{If}{RfD} \qquad (2)$$

$$HI = \sum\left(\frac{If}{RfD}\right) \qquad (3)$$

Where:

If = It is the ingestion of contaminated muscle tissue (µg kg^{-1} day^{-1});
RfD = It is the reference dose (µg kg^{-1} day^{-1}). The ratio determined in equation 2 informs whether there are deleterious risks to humans during their exposure to a chemical substance. However, without the risk of having cancer problems or genetic mutation. Values of HQ>1, presents risk and H≤1, does not present risk (USEPA, 1989; ANDRADE *et al*., 2018).

RESULTS AND DISCUSSION

CONCENTRATION OF AS IN TRACAJÁ: Table 2 shows the As concentrations analyzed in the tracajás captured in the Baú River and their characteristics. As the Brazilian legislation does not have a reference for Tracajá, the reference of non-carnivorous fish was used in Reference ANVISA RDC Nº 487, of March 26, 2021, from the Ministry of Health. All the tracajá samples evaluated showed As levels within the safety limit, which is 1 µg g^{-1}. Low levels of toxic metals are expected in herbivorous species, such as tracajás, compared, for example, with predatory species, according to a study by Souza-Araújo *et al*. (2015), the first hypotheses on this evidence were linked to its herbivorous habit, feeding mainly on plants.

STATISTICAL ANALYSIS: The female species presented a mean As of 0.040±0.064 µg g^{-1} with a range of <LOD to 0.114 µg g^{-1}. This mean represents 2.35 times greater than the mean found for males with a mean of 0.017±0.035 µg g^{-1} and a range of <LOD to 0.095 µg g^{-1}. The variability of results was greater in females, with an anomalous result in the male group (Figure 3). Regarding the width, it was observed that there was no discrepancy in the concentration of As in the small species (<average) that presented an average of 0.023±0.041 µg g^{-1} ranging from <LOD to 0.095 µg g^{-1} about the large ones (> mean) whose mean was 0.024±0.050 µg g^{-1} ranging

Table 1. Analytical quality for the tracajá matrix

Element	λ (nm)	LOD ($\mu g\ g^{-1}$)	a	b	R^2	CV (%)	Recovery (%)
As	193.759	0.00098	0.086	0.293	0.959	3.8	91

λ: Wave-length; LOD: Detection limit; a: Angular coefficient; b: Linear coefficient; R^2: Determination coefficient; CV: Coefficient of variation.

Table 2. As concentration in Tracajás ($\mu g\ g^{-1}$)

Sample	As	Width (cm)	Length (cm)	Mass (g)
Mean	0.024	24.5	26.3	1735
Standard deviation	0.043	3.6	4.5	821
Minimum	<LOD	19.0	19.0	675
Maximum	0.144	31.5	34.8	3515

<LOD: Below Detection Limit (0.00098 $\mu g\ g^{-1}$); M: Male; F: Female

Table 3. Correlation Coefficients Concentration of As versus variables

Coefficient	Width	Length	Mass	Coefficient	Width	Length	Mass
Pearson	0.220	0.249	0.356	Pearson's (Fisher)			
Spearman	0.287	0.287	0.288	Alpha	0.050	0.050	0.050
Kendall	0.255	0.255	0.258	Correlation	0.220	0.249	0.356
Pearson's (t test)				z	0.547	0.623	0.911
Alpha	0.050	0.050	0.050	p-value	0.584	0.533	0.362
Correlation	0.220	0.249	0.356	Below	-0.520	-0.497	-0.404
t	0.596	0.680	1.007	Above	0.771	0.784	0.825
p-value	0.570	0.518	0.348				
Below	-0.652	-0.617	-0.480				
Above	1.092	1.115	1.191				

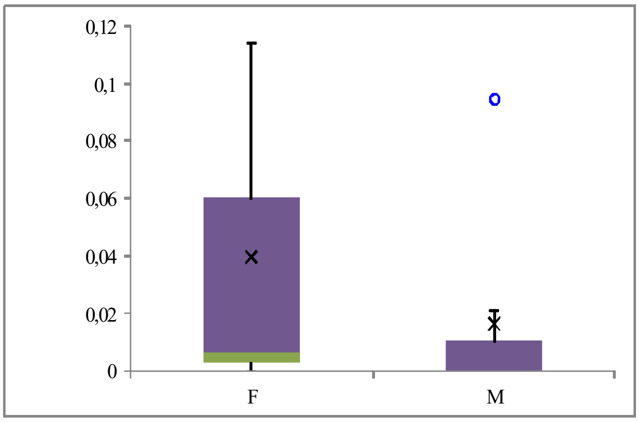


Figure 3. Results of As in tracajá by gender ($\mu g\ g^{-1}$)

from <LOD to 0.114 $\mu g\ g^{-1}$. The results showed greater variability in the species that presented a width below the average than those above the average with an anomalous result in each of the groups (Figure 4).

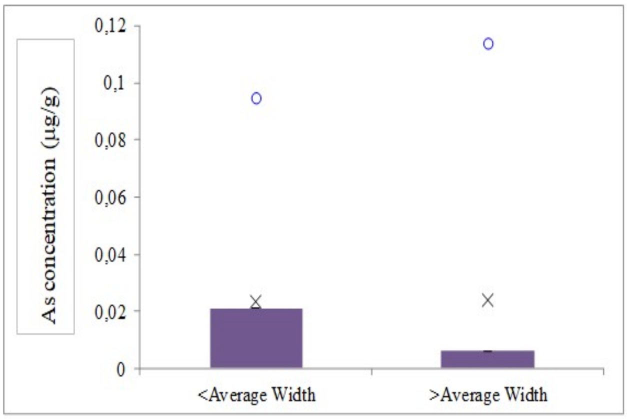


Figure 4. Results of As in tracajá versus width ($\mu g\ g^{-1}$)

The same behavior was observed about the length, which did not change the levels of As found. In samples whose length was below average, the average concentration of As was 0.023±0.041 $\mu g\ g^{-1}$, ranging from <LOD to 0.095 $\mu g\ g^{-1}$, whereas in species with a length above the average, the concentration of As was 0.023± 0.041 $\mu g\ g^{-1}$ ranging from <LOD to 0.114 $\mu g\ g^{-1}$. The same behavior of the width was also observed for the variability of the results about the length. The greatest variability was found in species below the average length with an anomalous result in each group (Figure 5).

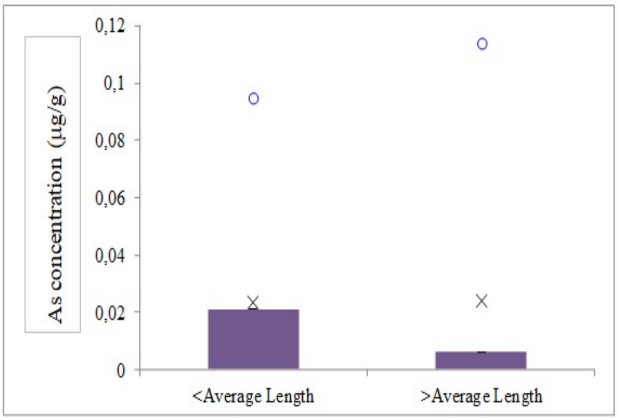


Figure 5. Results of As in tracajá versus length ($\mu g\ g^{-1}$)

A different behavior was observed about the mass of the tracajá specimens captured, where the specimens with the highest mass (average of 0.030±0.056 $\mu g\ g^{-1}$, ranging from <LOD to 0.114 $\mu g\ g^{-1}$) had an average content of As 1.58 times greater than those with below-average mass (mean of 0.019±0.038 $\mu g\ g^{-1}$, ranging from <LOD to 0.095 $\mu g\ g^{-1}$). The greatest variability was found in species above the mass average with an anomalous result in each group (Figure 6). No significant correlation (p-value <0.050) was found between the concentration of As and the variable's width, length, and mass, showing that these variables did not interfere with the results found (Table 3).

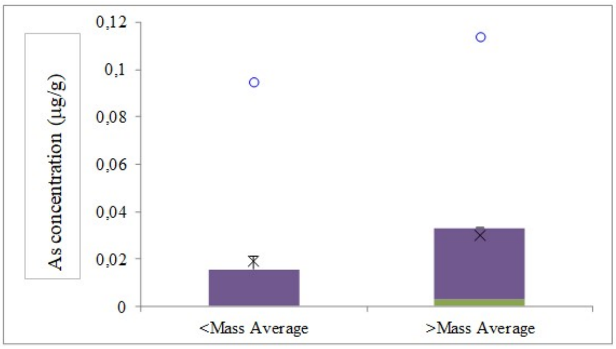


Figure 6. Results of As in tracajá versus mass (µg g^{-1})

RISK ASSESSMENT BY TRACAJÁ CONSUMPTION

The quantification of concentrations of toxic elements in proteins consumed in areas with imminent environmental contamination is the first step to assessing the risks that this population faces (NIENCHESKI *et al*., 2001; SILVA & SANTOS, 2016). Since the risk quotient (HQ) and hazard index (HI) are reasons that assess potential exposure to one or more substances, Table 4 determines these reasons to verify whether the consumption of tracajás will have adverse health effects for the indigenous. As a reference, values > 1 for both reasons mean the possibility of damage to health, whereas values < 1 indicate zero risk to health.

The tracajá species presented HQ values below 1, which suggests that its consumption by indigenous communities will not bring any deleterious risk to them. This value can be explained by the fact that tracajás are essentially herbivores and thus occupy lower trophic levels in the food chain, when compared to carnivorous fish, for example. The HQ values found for children are a little lower compared to adults in tracajás. However, in the prolonged consumption of these species, children are more likely to have As intoxication, as they are more vulnerable due to their developing immune system and metabolism (SOLDIN *et al*., 2003). The hazard index (HI) has values below 1 in all species evaluated, indicating that the consumption of tracajá would not cause harmful effects on health in an individual, due to the simultaneous exposure to As.

CONCLUSION

The results of this study showed that the concentrations of As found in the tracajás of the indigenous community present rates below the limits allowed by ANVISA Resolution No. 487/2021 (1 µg g^{-1} of As). As much as the values of the Hazard Quotient and the Hazard Index for As in tracajá caught in the Baú River are below the established reference, it is necessary to be careful when consuming foods that present As contents, as it is food that is largely consumed by the indigenous people, and its weekly consumption rate may lead to some risks to the health of indigenous people in the future, especially children.

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