UNIVERSIDADE FEDERAL DO OESTE DO PARÁ
PRÓ-REITORIA DE PESQUISA, PÓS-GRADUAÇÃO E INOVAÇÃO TECNOLÓGICA
PROGRAMA DE PÓS-GRADUAÇÃO SOCIEDADE NATUREZA E DESENVOLVIMENTO

OSVALDO GATO NUNES NETO

ENVIRONMENTAL BIOMONITORING OF TOXIC AND ESSENTIAL METALS IN AMAZONIAN ECOSYSTEMS USING ANIMAL TISSUES = BIOMONITORAMENTO AMBIENTAL DE METAIS TÓXICOS E ESSENCIAIS EM ECOSSISTEMAS AMAZÔNICOS UTILIZANDO TECIDOS ANIMAIS

SANTARÉM
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Thesis presented to the Graduate Program Society, Nature and Development of the Federal University of Western Pará as a requirement to obtain the title of Doctor in Environmental Sciences, Area of Concentration "Society, Nature and Development".
Advisor: Ph.D Antonio Humberto Hamad Minervino

SANTAREM – PA

2024

Dados Internacionais de Catalogação-na-Publicação (CIP)
Sistema Integrado de Bibliotecas – SIBI/UFOPA

N972e Nunes Neto, Osvaldo Gato
Environmental biomonitoring of toxic and essential metals in amazonian ecosystems using animal tissues = Biomonitoramento ambiental de metais tóxicos e essenciais em ecossistemas amazônicos utilizando tecidos animais./ Osvaldo Gato Nunes Neto . – Santarém, 2024.
104 p. : il.
Inclui bibliografias.

Orientador: Antonio Humberto Hamad Minervino.
Tese (Doutorado) – Universidade Federal do Oeste do Pará, Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação Tecnológica, Programa de Pós-Graduação Sociedade, Natureza e Desenvolvimento, .

1. Mercúrio. 2. Peixe. 3. DMA-80. 4. ICP-MS. 5. Amazônia. 6. Contaminação ambiental. I. Minervino, Antonio Humberto Hamad, *orient.*. II. Título.

CDD: 23 ed. 615.92566309811

Bibliotecária - Documentalista: Cátia Alvarez – CRB/2 843

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Date of presentation: 29/02/2024

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To my parents, my son Caio and Paula

THANKS

I thank Jesus Christ for every day I have lived so far. Although there have been unforeseen events, errors, delays, pandemics and uncertainties, the conclusion of this thesis no longer means just an academic stage, but a concrete period of a journey. In this journey, there were central people, such as Professor Antonio Minervino, the one who guided and was an authority and arbiter in all the decisions of this research. Thank you very much, Professor. I would like to thank the entire LARSANA-UFOPA team, especially Dr. Fábio Edir and PhD student Salatiel Dias, for their help in data collection, organization, revision and writing.

All gratitude to my parents, Francisco and Eunice, and all the family members who will rejoice at the end of this journey. And finally, the Federal University of Western Pará, for all the academic support.

Finally, I would like to thank the Amazon Foundation for the Support of Studies and Research (FAPESPA) for the support provided to the realization of this thesis.

"If the days are good, make good use of them, if they are bad, consider: God made both the one and the other" Ecclesiastes 7:14

RESUMO

A bacia do rio Tapajós é uma das regiões de destaque para a contaminação por mercúrio proveniente de atividades de mineração. Vale ressaltar que o elemento mercúrio não é o único metal pesado com potencial malefício à saúde das populações amazônicas. Arsênio, cádmio e chumbo são metais tóxicos conhecidos por estarem presentes na região, seja por ação antrópica ou natural. Além das florestas tóxicas, é preciso alertar para os riscos do consumo excessivo até mesmo de metais considerados essenciais. Esta tese apresenta quatro capítulos que tratam do monitoramento de metais tóxicos e essenciais na mesorregião do Baixo Amazonas. No capítulo I, foi realizada uma revisão dos quatro elementos tóxicos abordados na tese, a saber, mercúrio, arsênio, chumbo e cádmio. No capítulo II, determinamos a sensibilidade e a especificidade da detecção de Hg realizada com DMA-80 e se esses resultados são confiáveis em comparação com aqueles obtidos com o Espectrômetro de Massa com Plasma Indutivamente Acoplado (ICP-MS), que é o padrão-ouro. A análise de Bland-Altman mostrou que o DMA-80 apresentou um viés positivo de 6,5% em relação à ICP-MS, o que é mais evidente em amostras com altas concentrações de Hg. O DMA-80 foi eficiente em determinar se os níveis de Hg excediam os níveis máximos permitidos exigidos pela União Europeia, EUA e Brasil, com especificidade e sensibilidade acima de 95%. No capítulo III, considerou-se a avaliação da concentração de metais tóxicos, como mercúrio (Hg), chumbo (Pb), cádmio (Cd) e arsênio (As), em amostras de tecido muscular de bovinos pertencentes a seis municípios da região do Baixo Amazonas. Neste estudo, os resultados do cálculo da ingestão diária de metais tóxicos (EDI), que representa ingestão diária de metais tóxicos por meio do consumo diário de carne bovina (71 g por pessoa), em geral, indicaram que as concentrações de metais estavam ligeiramente abaixo dos valores de referência (RfDo). Os resultados da análise do quociente de risco alvo (HQ) e dos dados de exposição a múltiplos contaminantes (TTHQ) revelaram que os valores dos quatro elementos tóxicos não foram associados aos riscos por exposição isolada (THQ<1). A cidade de Monte Alegre apresentou as maiores concentrações de THQ e TTHQ para os metais As e Pb. Entretanto, em Itaituba e Oriximiná, as concentrações de elementos tóxicos resultaram nos maiores valores de THQ de Hg e Cd, levando a um TTHQ de 0,06 a 0,02, respectivamente. Os resultados da presente pesquisa indicam que carne bovina proveniente de animais criados na região é segura para consumo. No Capítulo IV, foram determinadas as concentrações de elementos essenciais como cobalto (Co), cromo (Cr), cobre (), ferro (Fe), manganês (Mn), molibdênio (Mo), níquel (Ni), selênio (Se) e zinco (Zn) em tecido hepático de bovinos consumidos em municípios da região oeste do Pará. O EDI de Se e Zn manteve-se acima dos valores quando avaliados os tecidos hepáticos de Oriximiná. O HQ referente à ingestão de tecido hepático revelou risco quanto aos efeitos adversos em todos os municípios avaliados. O quociente de risco alvo total (TTHQ) revelou que todos os municípios estavam expostos aos riscos do consumo excessivo desses minerais.

Palavras-chave: Mercúrio. Peixe. DMA-80. ICP-MS. Amazonia. Contaminação ambiental.

ABSTRACT

The Tapajós River basin is one of the prominent regions for mercury contamination from mining activities. It is worth mentioning that the element mercury is not the only heavy metal with potential harm to the health of Amazonian populations. Arsenic, cadmium, and lead are toxic metals known to be present in the region, either by anthropogenic or natural action. In addition to toxic forests, the risks of excessive consumption of even metals considered essential must be warned. This thesis presents four chapters that deal with the monitoring of toxic and essential metals in the Lower Amazon mesoregion. In chapter I, a review was carried out on the four toxic elements addressed in the thesis, namely Mercury, Arsenic, Lead and Cadmium. In chapter II, we determined the sensitivity and specificity of Hg detection performed with DMA-80 and whether these results are reliable compared to those obtained with the Inductively Coupled Plasma Mass Spectrometer (ICP-MS), which is the gold standard. The Bland-Altman analysis showed that DMA-80 showed a positive bias of 6.5% in relation to ICP-MS, which is more evident in samples with high Hg concentrations. DMA-80 was efficient in determining whether Hg levels exceeded the maximum allowable levels required by the European Union, USA and Brazil, with specificity and sensitivity above 95%. In chapter III, we considered the evaluation of the concentration of toxic metals, such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As), in muscle tissue samples from cattle belonging to six municipalities in the Lower Amazon region. In this study, the results of the calculation of daily intake of toxic metals (EDI), which represents the daily intake of toxic metals through the daily consumption of beef (71 g per person), in general, indicated that the concentrations of metals were slightly below the reference values (RfDo).The results of the analysis of the target risk quotient (THQ) and the multiple contaminant exposure data (TTHQ) revealed that the values of the four toxic elements were not associated with risks by isolated exposure (THQ<1). The city of Monte Alegre presented the highest concentrations of THQ and TTHQ for the metals As and Pb. However, in Itaituba and Oriximiná, the concentrations of toxic elements resulted in the highest THQ values of Hg and Cd, leading to a TTHQ of 0.06 to 0.02, respectively. The results of the present research indicate that beef from animals raised in the region is safe for consumption. In Chapter IV, we determined the concentrations of essential elements such as cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) in liver tissue of cattle consumed in municipalities of the western region of Pará. We also assessed the risks arising from the daily consumption of these elements. The daily intake of bovine liver tissue calculations were higher than those established for the copper element in all municipalities evaluated. The EDI of Se and Zn remained above the values when the hepatic tissues from Oriximiná were evaluated The total target risk quotient (TTHQ) revealed that all municipalities, were exposed to the risks of excessive consumption of these minerals, indicating that the degree of accumulation of essential elements in cattle in the Amazon region presents a significant risk for the consumption of the local population.

Key words: Mercury. Fish; DMA-80. ICP-MS. Amazon. Environmental contamination.

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LIST OF ACRONYMS

ALA-D.	δ-aminolevulinic acid dehydrase
ANVISA.	National Health Surveillance Agency
CAESA.	State Water Company
COBRAC.	Brazilian Lead Company
DL.	Lethal dose
DMA.	Dimethylmercury
DNA.	Deoxyribonucleic acid
FAO.	Food and Agriculture Organization
JECFA.	Food Additives Experts
LEPIMOL.	Molecular Epidemiology Laboratory
MAPA.	Ministry of Agriculture, Livestock and Food Supply
MetilHg.	Methylmercury
MMA.	Monomethylmercury
MPF.	Federal Prosecutor's Office
MSMA.	Methane Arsenate Acid Monosodium
NASA.	North American Space Agency
NR.	Regulatory Standard
OMS.	World Health Organization
PIB.	Gross Domestic Product
PNCRC.	National Plan for the Control of Contaminating Residues
SIE.	State Inspection Service
SIM.	Municipal Inspection Service
SNC.	Central Nervous System
T.e.	Boiling point
T.f.	Melting Point
UFPA.	Federal University of Pará

UFRJ.	Federal University of Rio de Janeiro
UNEP.	United Nations Environment Programme
USDA.	United States Department of Agriculture
UV.	Ultraviolet

SYMBOL LIST

Hg	Mercury
Cd	Cadmium
As	Arsenic
Pb	Lead
Z	Atomic number
M	Molar mass
g/cm^3	Gram per Cubic Centimeter
Zn	Zinc
HgS	Cinnabar
Hg_2^{2+}	Mercurous
Hg^{2+}	Mercúrico
$HgNO_3$	Mercury Nitrate
HgI_2	Mercury Iodide
HgO	Mercury Oxide
°C	Degrees Celcius
ng	nanogram
CH_3HgCl	Methylmercury chloridate
mg/L	milligram per liter
mg/cm^3	milligram per cubic centimeter
CH_3	methyl
pH	Hydrogen potential
Au	Gold
Hg^0	Elemental Mercury
µg/Kg	microgram per kilogram
Kg	kilogram
Km	Kilometer

Ca^{2+}	Calcium Ion
$\mu g/m^3$	microgram per cubic meter
ATP	adenosine triphosphate
Na^+	Sodium Ion
Mn	Manganese
PbS	Lead sulphide
$PbSO_4$	Lead sulfate
$PbCO_3$	Lead carbonate

SUMARY

1. INTRODUCTION

The Amazon region is currently established, based on an abundant collection of scientific productions, as an ecosystem potentially contaminated by the element mercury. The Tapajós River basin, in the state of Pará, is one of the most prominent regions in terms of mercury contamination (Albuquerque et al., 2020). With the mining activity present, the use of mercury by man during the mining and extraction processes of gold ore, results in a high and significant environmental contamination and cases of human poisoning, already existing in the state (Khoury et al., 2013). However, it is important to note that the contamination of this element is not restricted to aquatic environments, since the natural cycle of mercury allows this heavy metal to reach the atmosphere as well as the soils of the regions (Albuquerque et al., 2021). It is estimated that fires in the Amazon are responsible for emitting tons of mercury per year (Lacerda et al., 2007).

It is worth noting that the element mercury is not the only heavy metal with potential harm to the health of populations in the Amazon (Maciel, 2019). Arsenic, cadmium and lead are toxic metals known to be present in the region, whether due to human or natural action (Albuquerque et al., 2020). Studies have been carried out regarding the levels of arsenic in rivers of the Amazon, contamination by Pb in the Brazilian Amazon, especially in the state of Pará, for example, has been discussed in the soil, water, sediment compartment, in traditional hunting and fishing practices and in food (Santana et al., 2017). It is estimated that the main sources of cadmium release into the air and emissions of anthropogenic origin may exceed those of natural origin.

Obviously, when natural compartments are contaminated, there is a possibility of increased levels of these elements in animal organisms. In this context, the Ministry of Agriculture, Livestock and Supply, through its Secretariat of Agricultural Defense and its Coordination of Residues and Contaminants, outlines risk management actions to mitigate chemical hazards in food by verifying the presence of residues of chemical substances potentially harmful to the health of the consumer, such as residues of veterinary products, pesticides or the like, environmental contaminants and inorganic contaminants (Borges, 2013), among which the element mercury stands out. The presence of toxic metals in fabrics and animal products can result from both natural occurrence and industrial contamination processes (Filho, 2011).

It is important to highlight notable mining-related incidents in the region, including the direct discharge of effluents consisting of bauxite tailings rich in iron (Fe), aluminum (Al) and silica (Si) into the Potato Lake in Oriximiná between 1979 and 1989 (Albuquerque, 2021) and the historical mercury contamination in the Tapajós River basin associated with artisanal gold mining. In addition, natural processes such as the resuspension of soil particles by winds, volcanic emanations, forest fires, and soil and surface water emanations can contribute to natural emissions of heavy metals into the ecosystem (Siqueira & Aprile, 2012). It is important to emphasize that the presence of heavy metals in the environment is not strictly a consequence of human action. As demonstrated by Fadini & Jardim (2001), the natural concentrations of Hg in the Rio Negro basin considerably exceed those referring to mercury concentrations resulting from human actions.

Despite the existence of numerous reports in the national literature of the quantification of heavy metals mercury in food samples, especially mercury, there are few studies that monitor the presence of these toxic metals in products such as beef, collected randomly so that the conclusion of the study can better represent the reality of the meat product consumed in Brazilian municipalities (Filho, 2011). Thus, the present study aims to measure the concentration of heavy metals such as Mercury (Hg), Cadmium (Cd), Arsenic (As) and Lead in the liver and muscle tissues of cattle herds belonging to municipalities in the Western region of Pará, in addition to verifying the risk to which the population of these municipalities may be exposed.

It is important to emphasize that not only toxic metals have deleterious effects on human health. Essential metals, when in concentrations above the established limits, can also be mentioned as risk factors for the body. Taking this into account, an additional objective of the present thesis was the evaluation of the concentration of essential metals to the organism in hepatic and muscular tissues of cattle, with the objective of verifying if there is any probable influence of mining activities on such values and if the concentrations found may pose risks to human health from their consumption.

It is important to emphasize that the results of the analyses of the present research are not necessarily intended to be used for the adoption of regulatory actions, but that, however, they are used for guidance regarding the real risk of a certain unknown substance or the best way to manage the risk.

In addition to evaluating potential contaminants and residues, the present study also intends to suggest the bovine species as a monitor of pollutants. Cattle as environmental biomarkers can have a number of advantages over data provided by chemical or physical sensors, being able to accumulate biologically available forms for pollutants that are always present in the environment, thus allowing continuous monitoring of pollutants (Hashemi, 2018).

1. CHAPTER 1

Mercury, Lead, Cadmium, and Arsenic: A Brief Review

With a cattle herd of 222 million head in 2018, Brazil came to occupy the first position in the world ranking of the herd, and held the second world leadership in commercial beef production, behind the United States (FAO, 2018). In that same year, 44.23 million head of meat were slaughtered and 10.9 million tons of meat were produced under sanitary inspection, of which 20.1% was exported and 79.6% was destined for the domestic market, responsible for a per capita consumption of 34.4 kg/year (FAO, 2021).

Currently, Brazil occupies the second position both in the herd and in the production of beef (ABIEC, 2023). Beef exports already represent 3% of Brazilian exports, with the major markets represented by China, the United States, Africa, the Middle East, Japan and South Korea. It is also responsible for 8.7% of the Gross Domestic Product (GDP) or 30% of the GDP of Agribusiness, with a value equivalent to 597.22 billion reais, which has increased by 45% in the last 5 years (ABIEC, 2023).

The record numbers recorded are the result of continuous improvement in all stages of the production process, which allows Brazil to comply with the most demanding international rules, with quality and competitive meat (ABIEC, 2021). Thus, this record demonstrates the recognition of the Brazilian product both in the domestic and international markets, which allowed the United States Department of Agriculture (USDA, 2018) to classify Brazil in 2027 as the first exporter of beef, with India being the second, followed by Australia and the United States (MAPA, 2019).

With the significant increase in Brazil's participation in the world meat market, the control over the safety of these products has become strict (Filho, 2011). Currently, Brazil has the National Plan for the Control of Residues and Contaminants – PNCRC/Animal, the risk management tool adopted by MAPA that aims to promote chemical safety of food of animal origin produced in Brazil. The main legal basis of the program is SDA Normative Instruction No. 42, of December 20, 1999.

The strict quality control of beef when subjected to the Federal Inspection System is evident. However, there are two other seals in relation to the rules for the commercialization of meat: the Municipal Inspection Service (SIM), which allows the sale of meat only in the territory of the municipality where the slaughterhouse is located, and the State Inspection Service (SIE), which allows the trade only within the limits of the state. In these two areas of inspections, there is a lack of data related to the control of contaminants of meat products, of relevant publications in the area of food safety, including several documents and panels that would assess the risks to the human health of consumers of these products, even though there are regulations that require these analyses. Therefore, it is important to carry out studies in Brazilian municipalities that gather data on the occurrence of the main contaminants in meat products, so that their frequency and geographic distribution can be better known, and thus be able to subsidize, with reliable scientific information, public policies for the control and monitoring of contaminants.

2.1 TOXIC METALS

The term toxic metal refers to a class of elements that do not have beneficial or essential characteristics for living organisms, causing harmful effects on normal metabolic functions even if concentrations are apparently negligible. These elements are often called, in vague terms, heavy metals, that is, they have a high specific weight (Munhoz, 2010). Since the first metals described as toxic and bioaccumulative were mercury, lead, and cadmium, all three of which had a high specific weight, all other elements of the same nature came to be inserted into the imprecise terminology of heavy metals (Rocha, 2009). The appearance of heavy metals in tissues and products of animal origin can be a consequence of both the natural occurrence of these elements in the soil and the processes of anthropogenic contamination. The following toxic weeds will be highlighted in this research: Mercury (Hg), Arsenic (As), Lead (Pb), and Cadmus (Cd).

2.2 MERCURY

The chemical element mercury is symbolized by Hg, a reduction of the Greek word "Hydrargyrus" ("Hydrargyrum" in Latin), which means liquid silver, as this element was known in antiquity (Damas et al., 2014). Mercury is an element that has an alchemical symbol (figure 1) and is the only element that remains as the alchemical name to this day (Almeida, 2005). The name, Mercury, is an inheritance of the Egyptian civilization that, having knowledge of the stars such as Sun, Moon, Venus, Mars, Jupiter, Saturn and Mercury, began to associate them with the elements already known as Gold, Silver, Copper, Iron, Lead and Mercury. The latter, due to its peculiares volatility, came to be associated with the rapid movement of the planet Mercury, observed by Egyptian scientists.

The first scientific account of Mercury was written by Aristotle (384-322 B.C.) who described it as "liquid silver." It is currently recognized as highly toxic to human health, posing a special threat to the development of the child in the womb and early life (OMS, 2021). However, the ignorance of these properties of mercury by previous societies allowed them to have unrestricted contact with the element.

Tratamiento de la SÍFILIS en TODAS sus MANIFESTACIONES

ENESOL

Salicilarsinato de Mercurio (38, 46 % de Hg
14, 4 % de As)

VENTAJAS :

DÉBIL TOXICIDAD, 70 veces menor que Hg I^2.

INDOLENCIA de la INYECCIÓN, indicada por todos los autores.

VALOR ESPECÍFICO DOBLE : 1° Como **hidrargírico** simple de eliminación fácil; 2° Como **arsenical** (acción electiva del arsénico sobre el espiroqueta). El elemento arsenical, por otra parte, obra como antitóxico con respecto al mercurio, que puede ser administrado en dosis más fuertes, sin accidente.

El **ENESOL** hace desaparecer rápidamente la reacción de Wassermann y asegura una esterilización persistente.

FARMACOLOGÍA y DOSIS :

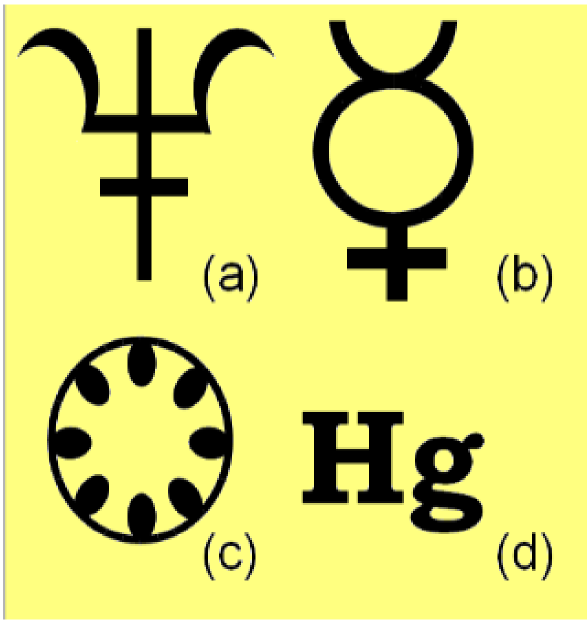
Ampollas de 2 c.c. y de 5 c.c. de una solución dosificada á 3 centigr. por c.c.

Inyecciones intramusculares de 2 c.c. por día (dosis media), ó de 4 á 6 c.c., cada **dos** ó tres días é **inyecciones intravenosas** de 2 á 10 cc. (dosis **masivas** ó de saturación) según el sujeto, la urgencia y la gravedad del caso, cada 2 ó 3 días.

LABORATORIOS CLIN, 20, Rue des Fossés-Saint-Jacques, PARIS 1501

Figure 1: Mercury salicylacinate used in the treatment of syphilis (Cabacas, 2018)

For example, the medicinal use of mercury compounds occurred between the fifteenth century and the beginning of the twentieth century, especially in the treatment of syphilis (Figure 1), by means of ointments, oral administration, or by heating the patient in an oven, which released elemental mercury vapors (Damas et al., 2014). Other civilizations have overestimated mercury's medicinal powers. In 133 B.C., in China, an alchemist offered a mercury compound to the Han emperor Wu-i, promising that consumption could provide him with eternal life. The Hindus believed that, because of the similarity to the color of the blood, Mercury had resurrection power.



Figur2 2. Representations of mercury at different times: (a) and (b) alchemical symbols; (c) symbol created by John Dalton at the beginning of the century. XIX; (d) modern representation, proposed by Jacob Berzelius (Damas, et al., 2014).

Currently, the properties of Mercury are very well established. In 1869 Dmitry Mendeleev created the Periodic System of Chemical Elements. After 154 years, the tool that makes it possible to predict the properties of matter on Earth, or anywhere in the Universe, is available. In this table, it is located in the sixth period and family IIB, arranged with an atomic number: Z = 80 and Molar mass: M = 200.59 g.mol-. Its Melting Point: Tf = -38.83 °C, Boiling Point: Te= 356.73 °C and Density: 13.534 g.cm3

Hg is the only metallic element that is in a liquid state at room temperature. The most acceptable explanation for this singularity is related to the 6s orbital in Hg that is completely filled and relativistically contracted, which disfavors metal-metal bonds and results in high ionization energy (1007 kJ.mol-1) compared to elements of the same family in the periodic table such as Zn and Cd, which have 906.1 and 876.5 kJ.mol-1, respectively), moreover, there is a weak interaction in the 6p orbital implying a weak Hg–Hg bond. (Norrby, 1991).

Mercury is found in the environment in association with other elements. The most common is sulphur, which makes up cinnabar (HgS), which is characteristic of a red or black colour, the largest reserves of which are found in Almadén, Spain, and Italy (Micaroni et al., 2000). In the Almaden mines, there are records of mercury exploitation for at least 2,000 years, while in China, the use of cinnabar as a pigment in red ink points to a period of 3,000 years ago (Miranda et al., 2007). Other exemplifiable natural sources of mercury are volcanic eruptions, natural evaporation and mercury mines, which are responsible for mercury emissions in the order of 2700-6000 tons/year (Micaroni et al., 2000).

Mercury species are classified into three distinct groups; elemental mercury, inorganic species and organic species, and can also exist in three oxidation states: zero, elemental mercury, (I), mercury mercury (Hg_2^{2+}) and (II), mercury mercury (Hg^{2+}) (Almeida, 2005). Inorganic mercury compounds are used in the dye industry (ex. HgS, $HgNO_3$, HgI_2) and in the production of dry batteries (HgO). The high catalytic capacity of various compounds, such as oxides, chlorides, or sulfides, in organic reactions for the synthesis of synthetic polymers, alcohols, ketones, and synthetic acids, can also be added to the properties of mercury.

Organic Hg species are those that combine carbon atoms in their chemical structures. There is potentially a high number of organic mercury compounds, however, the most common of them in the environment is methylmercury (metilHg). The metilHg it is the most toxic species of mercury and the interest in its study is mainly due to its ability to be bioaccumulated through adsorption on superficial bodies, in the ingestion of food, especially fish, as well anthropogenic entry into the environment, up to one million times along the aquatic food chain (BisinotI & Jardim, 2004). Table 1 highlights the main physicochemical characteristics of methylmercury.

Table 1: Physicochemical characteristics of the metilHg (BisinotI & Jardim, 2004)

Properties	**Valores**
Molecular Formula	CH_3 HgCl
Melting point	170 ºC
Stability	Stable
Toxicity	Lethal dose for rats (DL-50) 1,95 mg kg^{-1}
Blood levels	Blood methylHg level of 200– 500 ng mL^{-1}
Solubility at 25 ºC	> 6 mg/L
Saturation Concentration	94 mg/m^3

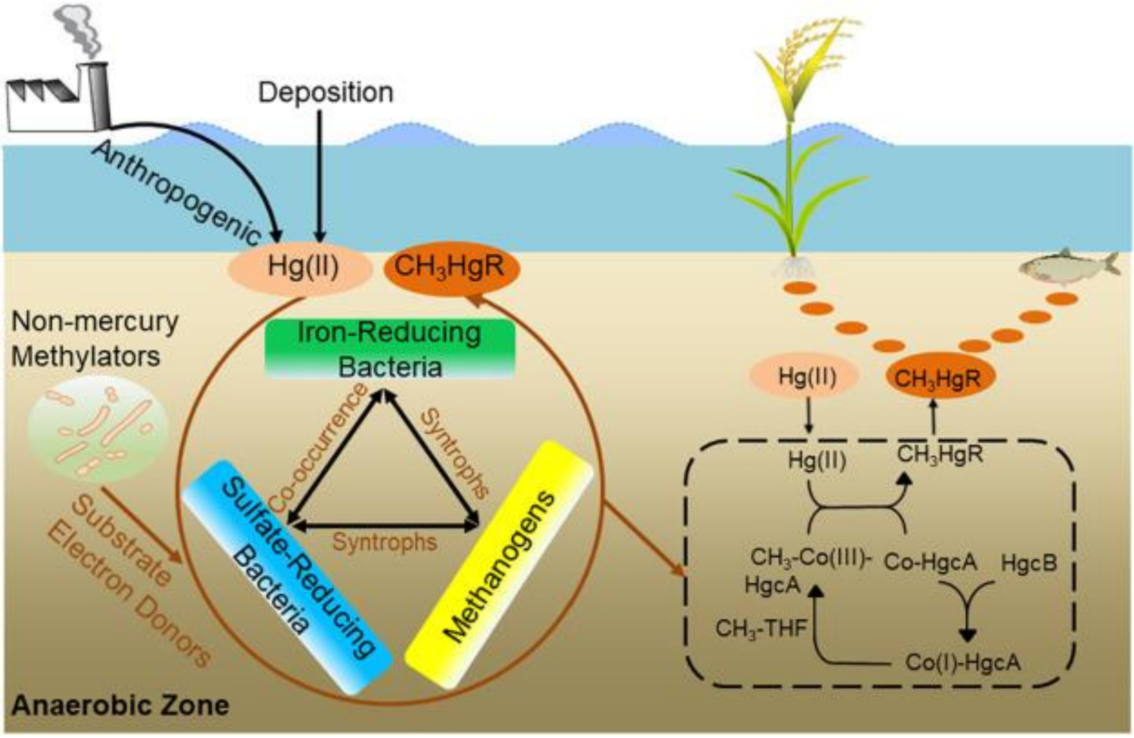
The MethylHg is synthesized from a process called methylation, characterized by the addition of a methyl group (CH_3) to inorganic mercury. Methylation began to be taken into consideration after the research of Jensen & Jernelov (1969) who perceived high levels of methylHg in aquatic organisms without the entry of organomercurial compounds into the aquatic ecosystem studied. The addition of the methyl group can be characterized into biotic and abiotic. Biotic methylation is mediated by the coenzyme methylcobalamin, synthesized by methanogenic bacteria and fungi, such as Neurospora crassa (Landner, 1971).

The main abiotic methylation is phytochemical, which occurs from the addition of methyl mediated by the transalkylation reaction, i.e., the addition of methyl in the presence of UV rays. The process is highlighted in Figure 1. Importantly, in addition to microbial activity and bioavailable mercury, other factors influence mercury methylation. Among these factors can be mentioned the physical and chemical parameters of the water such as pH, temperature, salinity, conductivity, oxidation-reduction potential, dissolved oxygen concentration, turbidity, presence of sulfide ions and dissolved organic carbon. (Kasper et al., 2007)

Once established, methylmercury becomes part of the food chain through strong binding with aquatic biota proteins, reaching its maximum level in fish tissues at the top of the food chain due to bioaccumulation (Micaroni et al., 2000) The same author states that the main reasons that impact the concentration of methylmercury in fish are: diet/tropic level of the species, age, microbial activity, mercury concentration in the upper layer of the local sediment, dissolved organic carbon content, salinity, pH and redox potential.

The classification of mercury emissions in the biosphere is usually separated into natural and anthropogenic sources (MIRANDA et al., 2007). However, according to the UNEP report in 2003 it adopted four categories: 1. Emission from natural sources; 2. Unplanned emission, characterized as those from anthropogenic sources and the product of the exploitation of materials in a natural state in which mercury is as an impurity. Examples: the burning of fossil fuels, mainly coal, and to a lesser extent gas and oil, as well as the synthesis of cement and the extraction, treatment and recycling of minerals, such as zinc; 3. Planned emission - anthropogenic sources in which the emission of Hg is a consequence of its balanced use in various industrial contexts. Emission occurs during the process of synthesis, leakage, discarding or burning of unused products; 4. This group includes the release of mercury through remobilization from soils, sediments, water bodies, landfills, and tailings piles, characterized as reservoirs.

FIGURA 3: Mercury Methylation



Source: Parks et al., (2013)

A biogeochemical cycle is established in which methylmercury and Hg^{2+}, dimethylmercury and Hg^0 compounds undergo conversions in atmospheric, aquatic and terrestrial systems (Almeida, 2005). A low fraction of the Hg0 that reaches the atmosphere is transformed into water-soluble varieties (possibly Hg^{2+}) (Brito et al., 2021). These varieties can be accumulated in the soil and released back into the atmosphere in Hg^0 species. The atmosphere retains Hg^0 for long periods of time and, consequently, this compound can be transported over great distances (Brito et al., 2021).

The irresponsible use of mercury, whether in industrial or artisanal activities, are the most significant examples of anthropic interference in natural cycles, and a series of fatalities are described, with mercury as a central element in their triggers (Transande, 2016). The first environmental catastrophe of notorious impact and that made explicit the risks of mercury occurred in 1953 in Minamata Bay, Japan. A hydroelectric company that manufactured fertilizer products used mercury sulfate as a catalyst for the synthesis of acetic acid and mercury chloride as a catalyst in the production of vinyl chloride, with methylmercury being a byproduct of these reactions (Silva et al., 2017). The company had been active in the synthesis of these products since 1930. During this long period, methylHg was released into the effluent and infected the marine biota and nearby waters, reaching the population through the ingestion of fish (Semionov, 2018).

"Minamata Disease" was officially diagnosed in 1956 when four patients were admitted to a town on the western coast of the island of Kyusho. The patients had severe seizures, a bout of psychosis, and a coma. Since 1953, idiopathic symptoms have been described near Minamata Bay: people have experienced numbness in the fingers, lips and tongue; Fish and shellfish deaths have been observed. By 1960, at least 111 patients were diagnosed with the disease and mortality was about 20% and survivors were permanently disabled (Micaroni et al., 2000).

In Brazil, Hg is used in alluvial gold mining, mainly in the Amazon region (Kasper et al., 2007). In this activity, the metal is released into the environment in high quantities to form amalgam with gold. It is assumed that about 128 tons of mercury were released per year in the early 1980s, a period of maximum gold exploration in the region (Almeida, 2005). There is a vast and considerable amount of studies that demonstrate high concentrations of mercury in fish as well as in the riverine population of the Amazon (Hacon et al., 2008).

It is interesting to note that this high concentration of mercury is described both in populations that are neighboring gold exploration areas, as well as in regions where there are no records of this type of exploratory activity (Crespo-Lopes et al., 2021). An example is the study carried out by Aula et al., (1995) who evaluated the concentrations of methylHg in fish in the region and revealed high concentrations of this compound in fish, thus affecting the local population, even though it does not coexist with sources of the metal in neighboring áreas. Fadini & Jardim (2001) also observed that the natural concentrations of Hg in the Rio Negro basin, where there are no exploratory activities, considerably exceed those referring to mercury concentrations resulting from human actions.

The Tapajós River basin, in the state of Pará, is one of the prominent regions in mercury contamination. In this region, the exploitation of mineral resources is quite evident. With the mining activity present, the use of mercury by man during the mining and extraction processes of gold ore, results in a high and significant environmental contamination and cases of human poisoning, already existing in the state (Oliveira, 2014). In a study conducted by Khouri et al., (2013), the mean concentrations of total Hg in communities in the Tapajós region (mercury-contaminated areas) were 8.66 ± 9.24µg/g. Other studies in the region have already found 150 µg/g, measured in hair roots, and the first clinical signs of contamination symptoms can appear from 50 µg/g (Santana et al., 2017)

Research developed by LEpiMol (Laboratory of Molecular Epidemiology) of UFOPA (Federal University of Western Pará), in partnership with Fiocruz (Oswaldo Cruz Foundation) and WWF-Brazil, showed a high concentration rate of mercury (Hg) in the blood of residents of urban and riverine areas of the Lower Tapajós basin, in the state of Pará. The metal, which is used in mining areas to separate and extract gold, was found in all 462 participants in the research. Of this total, 75.6% showed exposure above the safety limit of 10 µg/L established by the WHO (Meneses et al., 2022.).

In addition to the series of several other studies that point to the biomagnification of mercury in the Tapajós River basin (Silva et al., 2006.; Brabo et al., 1999.; Gonsalves & Gonsalves, 2004.; Lima de Sá, 2006) it is important to highlight the presence of mercury bioaccumulation in other locations in the Brazilian Amazon, such as that found by Lacerda et al., (2004) in a study carried out on fish from the Madeira River, as well as the research carried out in the Trombetas River, located in an area of bauxite exploitation (Albuquerque et al., 2020.; Albuquerque et al., 2021)

For a long time, all the mercury found in fish in the Amazon basin was attributed entirely to mining activities. Currently, it is certified that this activity is not the only source of mercurial emissions in the Amazon in particular (Santos et al., 2003). High levels of mercury are found in fish from places hundreds of kilometers away from gold exploration and for this reason, many researchers have studied other sources of mercury release and movement, such as weathering, soil erosion, evaporation of Hg from water, soils and plants, deposition of mercury from silver and gold mines in the last century in neighboring countries. industrial activities, burning forests and fossil fuels, and other diffuse sources (Santos et al., 2003)

With regard to mercury emission resulting from fires in the Amazon, an estimate of Hg emission between 0.78 and 1.76 kg/km2 of burned forest was presented by Lacerda (1995) and Veiga et al. (1994). In 1995, some close to 30,000 km2 of forests were burned in the Amazon, which allows us to stipulate that about 23 to 52 t of Hg may have been emitted into the atmosphere. Artaxo et al. (2000), analyzing smoke particles in the Amazon, estimated that 30% of the mercury in the air comes from vegetation, about 60% from mines and the rest from dispersed sources (Veiga et al., 2002). Currently, it is estimated that fires in the Amazon are responsible for emitting 13% of the atmospheric mercury in Brazil, which is equivalent to 8.7 tons per year (Lacerda et al., 2007).

FIGURE 4 - Image of a satellite of the American space agency (NASA) with the fires detected from August 15 to 22, 2019. Source: https://earthobservatory.nasa.gov/images/145498/uptick-in-amazonfire-activity-in-2019 (Crespo Lopes et al., 2021)

Taking into account the neurotoxic potential of methylHg, in 2003 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) established a tolerable weekly intake of 1.6 µg/kg body weight in terms of dietary exposure to methylmercury in order to protect a developing foetus from any neurotoxic effects. In 2006, JECFA clarified that life stages, with the exception of the embryonic and fetal phases, may be less sensitive to the adverse effects of methylmercury (WHO, 2021).

In adults, ingestion of up to about twice the tolerable weekly dose would not pose any risk of neurotoxicity. However, the available data did not allow strong conclusions to be drawn for children (aged up to 17 years), as they may be more sensitive than adults. In this sense, the tolerated intake established in 2003 also applies to children. In 2010, JECFA established a provisional tolerable weekly intake of inorganic mercury of 4 µg/kg body weight, applicable to dietary exposure to total mercury in foods other than fish and shellfish (Who, 2021).

Values proposed in the WHO guideline

- Values proposed in the WHO guidance Water: 6 µg/litre for inorganic mercury.
- Air: 1 µg/m^3 (annual average).
- The WHO has calculated a tolerable concentration of 0.2 µg/m3 for long-term inhalation exposure to elemental mercury vapours, and a tolerable daily intake of total mercury of 2 µg/kg body weight.

Fonte: OMS, 2021

In the northern region of Brazil, mercury levels are closely related to fish intake. It is noticeable that even within the limits designated by Brazilian legislation, a population that has a high consumption of fish has a high concentration of Hg in the body. Amazonas, for example, is the Brazilian state with the highest consumption of fish per year. The per capita intake is approximately 155g of fish daily. Each person consumes approximately 55 kg of fish per year in this state, while the Brazilian average is 7 kg (Jesus, 2007).

It is important to highlight the status of mercury contamination in the Tapajós River basin, in the state of Pará. In April 2023, the Federal Public Prosecutor's Office (MPF) recommended to the Ministry of Health's Health Surveillance Secretariat to initiate an administrative procedure and recommend the declaration of a public health emergency of national importance, due to mercury contamination in the Tapajós River basin, western Pará, especially in relation to the Munduruku indigenous people.

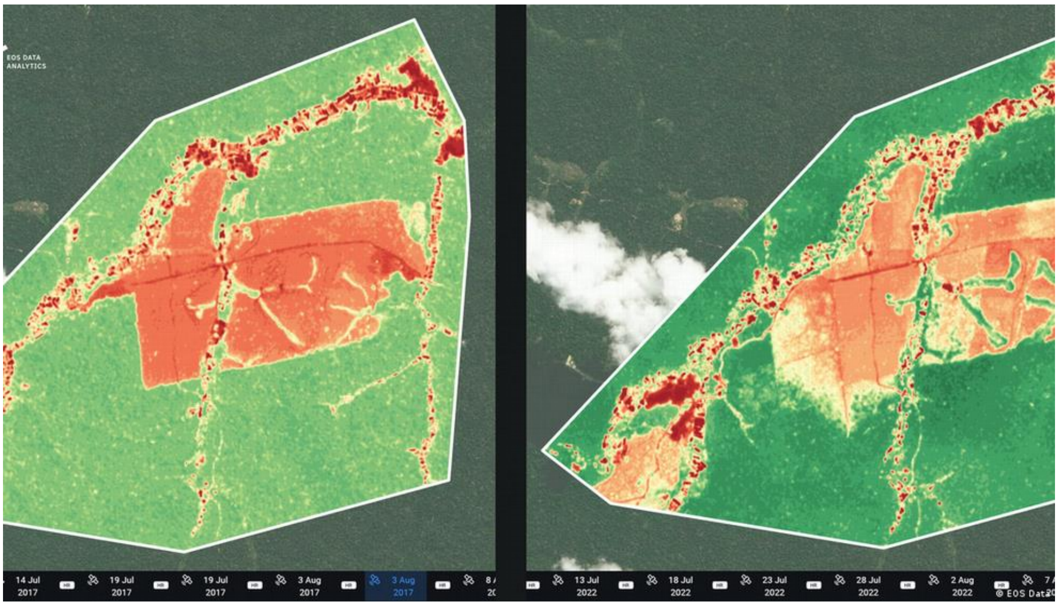


FIGURE 5 - Satellite images of the Munduruku Indigenous Land show a mining area in 2017 (left) and in 2022, with more degraded vegetation — Photo: Reproduction/EOS Data

The MPF's investigation is supported by two studies carried out by the Oswaldo Cruz Foundation (Fiocruz), the Federal University of Rio de Janeiro (UFRJ) and Imperial College London, which measured rates of mercury contamination incident on the Munduruku people. In these studies, it was found that the analysis of mercury levels for the 197 participants who provided hair samples reveals a prevalence of contamination of 57.9% of the population evaluated.

The nervous system is identified as the one that suffers the most impairment when exposed to mercury (Milioni et al., 2017). The toxicity of organomercury elements is a consequence of their higher absorption and lower excretion (Sousa & Santana, 2013). When mercury leaves the bloodstream and diffuses into the nervous tissue, it binds to proteins and loses its solubility, which makes it impossible for it to leave the CNS when associated with the intrinsic characteristics of the blood-brain barrier (Milioni, 2015). The main actions of Hg in the CNS are related to the reduction of Ca2+ concentrations, blockade of voltage-gated Ca2+ and Na+ channels, blockade of mitochondrial enzymes and reduction of ATP synthesis (Zachi, 2007).

The symptoms resulting from exposure to methylHg are neurogenic and are characterized by visual alterations such as scotomas (blurred vision) and decreased visual field, ataxia (uncoordinated gait), characteristic symptoms of Hunter-Russell syndrome. Paraesthesia (numb skin), neurestenia (neuraugia), deafness, dysarthria (difficulty articulating words), mental deterioration, muscle tremor, motility disturbance and, in situations of severe exposure, paralysis and death, are also effects resulting from exposure to methylHg. It has been found that regions of the brain such as the cortex, especially the portion responsible for vision, are the most sensitive to the effects of mercury (Kasper et al., 2007).

The effects of mercury are not restricted to the CNS, but reach multisystem status. Very well established as other conditions resulting from exposure to Mercury, more precisely to methylHg, are the genotoxic effects, which detect mutations, both at the chromosomal level and at the genetic level, which may be related to the emergence of neoplasms and other hereditary diseases, teratogens, capable of causing fetal malformation, mercury can also influence hormonal status by acting on the hypothalamic-pituitary system as well as triggering reproductive problems and immunosuppression (Cardoso et al., 2002).

As mentioned, there is a vast bibliography and studies on mercury contamination in the Amazonian environment that attest to this element in rivers, soils, air, as well as its bioaccumulation along the trophic chain, allowing an intimate relationship with local populations that traditionally have fish as one of their main sources of food. However, there are no studies that refer to the scenario of the concentration of this element in another product of animal origin potentially consumed in the region: beef.

Although the topic related to mercury contamination in fish is widely addressed in the scientific literature, in cattle, this study is still limited. In addition, there are also frequent studies that relate the effects of these chemical elements on human health or on some specific environmental compartment, such as soil, sediments, vegetation or water. Obviously, when these compartments are contaminated, there is a possibility of increased levels of these elements in animal organisms (Sousa et al., 2009).

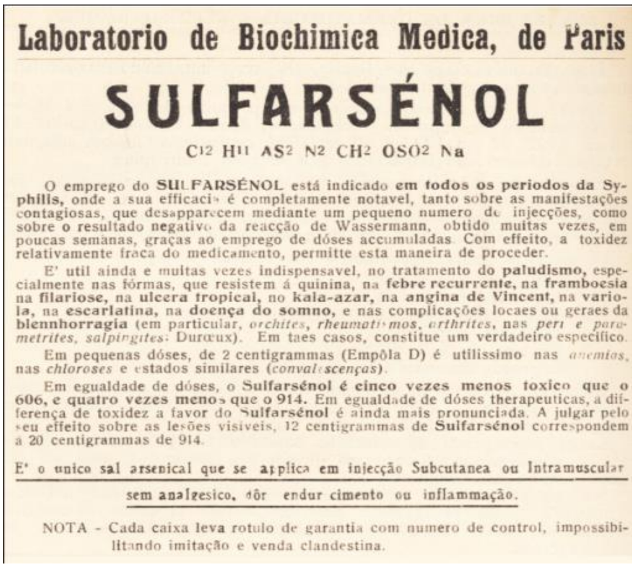
Mercury poisoning in livestock occurs almost exclusively as a result of accidental supply of grains, pelleted feed, or concentrates treated as mercury-based antifungal agents (Jordana et al., 2011). Accidental ingestion, acute or chronic, of mercury in cattle can result in numerous conditions that, on certain occasions, may not be clinically noticeable. Because they are not very selective animals, cattle can become contaminated by chewing objects containing this chemical element or by ingesting contaminated water or food (Sousa et al., 2009)

Thus, it is important to carry out in the Amazonian municipalities that already have certification of mercurial contamination, studies that gather data on the occurrence of this element in meat products, so that its frequency and geographic distribution can be better known, and thus can subsidize, with reliable scientific information, public policies for the control and monitoring of contaminants.

2.3 ARSENIC (As)

Arsenic (As) is a chemical element with atomic mass 75, toxic and widely distributed in the biosphere, and can be perceived in the atmosphere, water, soils, sediments and organisms (Sousa et al., 2014). Arsenic (As) is a metalloid of solid, crystalline, grayish nature, with the chemical valences , 0, 3^+ (arsenite) and 5^+ (arsenate), whose chemical form predominates in water and depends essentially on the pH and potential of the medium (Andrade & Rocha, 2019). Arsenic is part of the select group of substances that have a sensational scientific history, since its therapeutic use dates back to 400 B.C. and there are writings of its use by Hippocrates, Aristotle, Dioscorides and Pliny the Elder (Gontijo e Bitencourt, 2005).

Arsenical constituents were then used, with or without scientific basis, in the resolution of dermatoses such as psoriasis, pemphigus, eczema, dermatitis herpetiformis, acne, lichen planus, leishmaniasis, prurigo and syphilis (Figure 5). Further justifying its status as a medicine that cures numerous diseases, it was still widely prescribed as tonics and fortifiers, attenuating conditions such as "stomach problems", "nervousness" and "fits", or even in the treatment of malaria, epilepsy and asthma, (Gontijo and Bitencourt, 2005). Outrora classificado como um famigerado elemento de cura, atualmente o arsênio está em primeiro lugar no *Ranking da Priority List of Hazardous Substances* da *Agency for Toxic Substances and Dissease Register* (ATSDR), órgão federal do departamento de saúde dos Estados Unidos (Gonsalves, 2015).



Laboratorio de Biochimica Medica, de Paris

SULFARSÉNOL

$C^{12} H^{11} AS^{2} N^{2} CH^{2} OSO^{2} Na$

O emprego do **SULFARSÉNOL** está indicado **em todos os periodos da Syphilis,** onde a sua efficacia é completamente notavel, tanto sobre as manifestações contagiosas, que desapparecem mediante um pequeno numero de injecções, como sobre **o resultado negativo** da reacção de Wassermann, obtido muitas vezes, em poucas semanas, graças ao emprego de dóses accumuladas. Com effeito, a toxidez relativamente fraca do medicamento, permitte esta maneira de proceder.

E' util ainda e muitas vezes indispensavel, no tratamento do **paludismo,** especialmente nas fórmas, que resistem á quinina, na **febre recurrente,** na **framboesia** na **filariose,** na **ulcera tropical,** no **kala-azar,** na **angina de Vincent,** na **variola,** na **escarlatina,** na **doença do somno,** e nas complicações locaes ou geraes da **blennhorragia** (em particular, *orchites*, *rheumatismos*, *arthrites*, nas *peri e para-metrites, salpingites*: Durœux). Em taes casos, constitue um verdadeiro especifico.

Em pequenas dóses, de 2 centigrammas (Empôla D) é utilissimo nas *anemias*, nas *chloroses* e estados similares (*convalescenças*).

Em egualdade de dóses, o **Sulfarsénol é cinco vezes menos toxico que o 606, e quatro vezes menos que o 914.** Em egualdade de dóses therapeuticas, a differença de toxidez a favor do **Sulfarsénol** é ainda mais pronunciada. A julgar pelo seu effeito sobre as lesões visiveis, 12 centigrammas de **Sulfarsénol** correspondem a 20 centigrammas de 914.

E' o unico sal arsenical que se applica em injecção Subcutanea ou Intramuscular

sem analgesico, dôr endurecimento ou inflammação.

NOTA - Cada caixa leva rotulo de garantia com numero de control, impossibilitando imitação e venda clandestina.

Figura 6: Arsenic as a panacea (Gontijo e Bitencourt, 2005)

Arsenic is found in ecosystems in various chemical forms, as a result of its participation in biological complexes, chemical processes, and some industrial applications, such as the manufacture of certain glasses, semiconductor materials, and photoconductors, among others (Barra et al, 1999). Its distribution is considerable in the earth's crust and consists of more than 200 minerals, and can be described in the natural form of sulfide, which can be mentioned as Copper, Lead, Nickel, Cobalt and Iron (Andrade and Rocha, 2019). Industrially, it is used in the manufacture of non-ferrous alloys, for the production of semiconductors, including those for light emission, lasers, integrated circuits and solar cells, bleaches, whiteners and air bubble dispersants in the production of glass bottles and other glassware (Andrade and Rocha, 2019).

The existence of As in waters and soils is a consequence of the presence of this element in oxidized weathered surfaces, in mineralized rocks and enriched with sulfide ore bodies found in outcrops and in the walls of deactivated mines, presenting as examples, mainly, arsenopyrite, pyrite and pyrrhotite, (Gonçalves et al., 2010). The action of weathering processes on ore bodies promotes the oxidation of the constituent sulfides. The oxidation process acts more intensely in the surface zone, and with less force in veins, fissures and fractures of deep aquifers (Gonçalves et al., 2010). From this oxidation, the release of As, both from the altered surface mantle and from the mineralized rocks occurs (Gonsalves & Lena, 2013).

Once Arsenic is found in the environment, it can change the status of the area in question to contaminated environment. In order for this classification to be established, it is necessary to take as a basis the occurrence of substances in concentrations that may cause risks of relevant effects on human health, called CETESB Intervention Values (2001) (Gonsalves & Lena, 2013).

In Brazil, there are three regions with the status of contamination by Arsenio, where the studies currently carried out have converged, in which the high occurrence of As can be verified due to anthropogenic activities such as the extraction of base metals, which leads to a severe contamination of river systems and soils (Pataca et al., 2005). The highlighted regions are: the Iron Quadrangle region in Minas Gerais – in which arsenic is released into the environment through activities related to Au mining; the Ribeira Valley region, in Santa Catarina and São Paulo, highly contaminated due to mining, processing and refining of "lead-zinc-silver" ores; and the Amazon region, in which As is naturally found in rocks and soils, including the area of the municipality of Santana, in the State of Amapá, where As is associated with manganese ores (Mn) exploited in the last 50 years (Rodrigues & Malafaia, 2010). In the Port of Santana, on the premises of the former ICOMI, there is a source of risk of environmental contamination represented by the deposition area and the old tailings basin (Santos et al., 2003).

After decades of economic activity resulting from the extraction of manganese by the Brazilian Society of Industry and Commerce of Iron and Manganese Ore (Icomi), the former district of Santana today sustains the consequences of an ore exploitation marked by the lack of concern for collective or environmental health. One year after the end of the company's activities, Jaakko Pöyry Engenharia Ltda (JPE) carried out surveys at Icomi's facilities and identified foci of contamination of the water table by heavy metals and arsenic (Santos et al., 2003).

In 2001, research carried out by the Federal University of Pará (UFPA) with hair fragments from residents of Vila Elesbão found that, out of every 100 inhabitants, 98 had an arsenic level higher than that tolerated by the World Health Organization (WHO). In 2002, a survey carried out by the Evandro Chagas Institute revealed the presence of arsenic concentrations under high conditions in the industrial area of Icomi, which triggered or increased levels of Arsenio in the water, bottom sediments and suspended particulate matter in the Elesbão 1 and Elesbão 2 streams in areas closer to the tailings dam.

A population-based study in the community of Elesbão aimed to diagnose health conditions and assess the exposure to As of about 70% of the residents. This community is located in the port of Santana, next to the ICOMI tailings accumulation area, on the banks of the Amazon River, and is under the influence of the tides. In this region, basic sanitation is deficient and the entire community is built on stilts, and pedestrians are moved on stilts. The water for the community's consumption is collected directly from the Amazon River, without any treatment, or received from the State Water Company (CAESA), undergoing a simplified decantation process. Garbage is discharged into the Amazon River and into the streams and banks, as well as waste (Santos et al., 2003).

Seyler and Boaventura (2001) calculated the levels of arsenic in rivers of the Amazon. The highest levels of arsenic are found in Andean rivers, such as the Solimões and the Madeira. In Óbidos, the Amazon River has values higher than 0.8 mg/m3 of arsenic, indicating that about 0.5 tons of the element are taken to the sea daily, that is, more than 200 tons annually. It is admitted that the maximum acceptable concentration of arsenic in drinking water in the Brasileiras is 10 mg/m3, according to Ordinance 518/2004 of the Ministry of Health. Figure 5 shows the total arsenic transported by the rivers of the Amazon Basin, in tons per day.

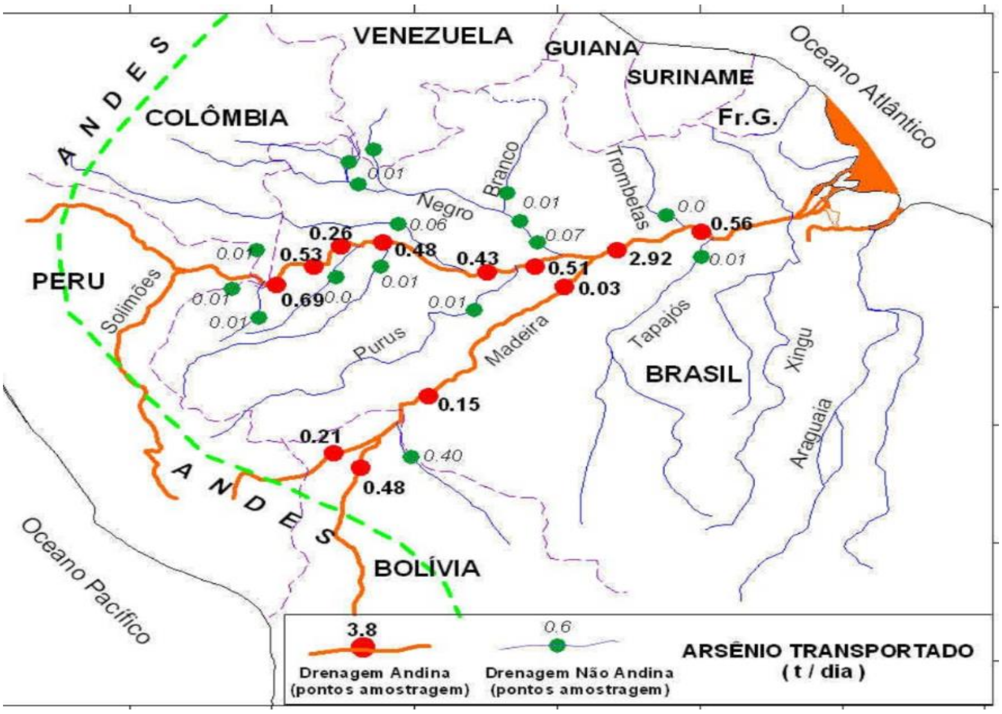


Figura 7: Total arsenic transported by the rivers of the Amazon Basin, in tons per day (Scarpelli, 2021).

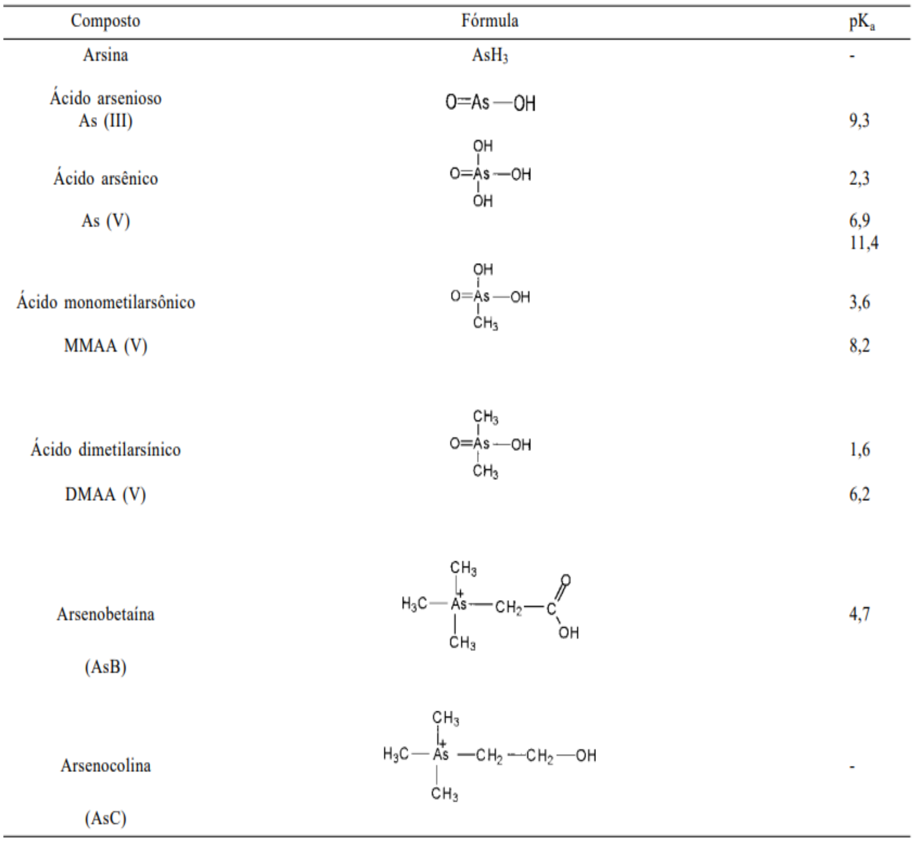
The Lower Amazon region (western Pará, northern Brazil) is greatly affected by mining (particularly artisanal gold mines) and other intensive industrial and agricultural activities with potentially strong impacts on aquatic ecosystems. Although such impacts include contamination with various toxic elements. In a study carried out by Albuquerque et al., 2020, 351 specimens of fish widely consumed in the region, such as arapaima and acari, were evaluated, and values between 2–238 µg/kg of Arsenio were found on average in the species evaluated.

Currently, it is the ingestion of arsenic through water that has been classified as a major public health issue (Sousa et al., 2022). Arsenic found in water bodies through natural deposits or by human activities, mainly mining, has consequently caused an increase in human exposure to this element, increasing the likelihood of the appearance of cancers and numerous pathological effects, such as: cutaneous, gastrointestinal, vascular diseases and diabetes (Sousa et al., 2022).

The World Health Organization (WHO, 2001), supported by toxicological evidence related to exposure to Arsenic, was motivated to lower the limit of the permissible concentration of Arsenic in drinking water from 0.05 mg/L to 0.01 mg/L. In 2003, Brazil, through CONAMA resolution 357, of March 2005, also reduced the threshold for the concentration of as in drinking water (class 1 to 3) to 0.01 mg/L.

As compounds are easily absorbed, both orally and airbornely, and the extent of absorption depends on the solubility of each compound. The toxic effect of arsenic species depends mainly on their chemical form. For example, arsenic in natural waters can occur as As (III) (arsenite), As (V) (arsenate), monomethylarsonic ion (MMA), and dimethylarysinic ion (DMA).

Groundwater contains arsenic as arsenite and arsenate. In sea waters, lagoons, lakes, and where there is a possibility of biomethylation, arsenite and arsenate occur along with MMA and DMA (Barra et al., 2000). Figure 6 demonstrates the main Arsenic Compounds of Interest in studies.



Composto	Fórmula	pK_a
Arsina	AsH_3	-
Ácido arsenioso As (III)	$O{=}As{-}OH$	9,3
Ácido arsênico As (V)	$O{=}As(OH)_3$	2,3 6,9 11,4
Ácido monometilarsônico MMAA (V)	$O{=}As(OH)_2{-}CH_3$	3,6 8,2
Ácido dimetilarsínico DMAA (V)	$O{=}As(CH_3)_2{-}OH$	1,6 6,2
Arsenobetaína (AsB)	$H_3C{-}\overset{+}{As}(CH_3)_2{-}CH_2{-}C(=O){-}OH$	4,7
Arsenocolina (AsC)	$H_3C{-}\overset{+}{As}(CH_3)_2{-}CH_2{-}CH_2{-}OH$	-

FIGURE 8: Major Arsenic Compounds of Interest in Studies (Barra et al., 2000).

In terms of inorganic As (As-i), pulmonary absorption is rated at 30-60%, and approximately 95% of ingested As is absorbed from the digestive tract. Taking into account the dermal pathway, there are few studies that have underestimated the absorption through this pathway (Sousa et al., 2015).

Once in the bloodstream, 95 to 99% of the As is associated with red blood cells, bound mainly to hemoglobin and after about 24 hours, the As is sent to most tissues. Soluble inorganic compounds are rapidly taken to organs or tissues rich in sulfhydryl groups such as the liver, kidneys, spleen and adrenal glands and in giving great affinity by binding to sulfhydryl groups, compounds of As can be detected in high concentrations in hair, nails and skin 2 to 4 weeks after exposure, accumulating in bones, which coincides with decreased concentrations in the liver and kidneys (Gontijo & Bitencourt, 2005).

It is important to note that arsenic is defined as a protoplasmic toxicant that promotes its toxicity through the inactivation of approximately 200 enzymes, in particular, those included in the synthesis of cellular energy and those involved in the synthesis and repair of DNA (Andrade & Rocha, 2019). The toxic intervention of arsenic in the human body is established as an inhibitor of cellular respiration, as it is possible to observe a large accumulation of this compound in mitochondria, thus causing systemic impairment in the functions of protein metabolism arising from the effect of its properties very similar to those of phosphorus (Andrade & Rocha, 2019).

Arsenic's affinity for sulfur, especially in the pentavalent state, which causes arsenic to silently penetrate the body, harms the organism where it causes changes in glycolysis, hindering the whole process by replacing a phosphorus atom of an important metabolite, 1,3-bisphosphoglycerate, causing the energy that would be transferred to ATP to be lost, and, mainly because it binds to the sulfur atoms in the active center of enzymes, making them incapacitated, also inhibiting an enzyme of the citric acid cycle, a stage of cellular respiration, alpha ketoglutarate decarboxylase, and by binding to the hydrogen sulfides of its cofactor, lipid acid, blocking the entire process and even all metabolic pathways acting in the cycle, causing serious damage to the entire organism, and can quickly lead to death by the total blockade of several important reactions of human metabolism (Andrade & Rocha, 2019).

The main adverse effects reported with long-term ingestion of inorganic arsenic in humans are skin lesions, cancer, neurotoxicity, cardiovascular disease, abnormal glucose metabolism, and diabetes (Gontijo & Bitencourt, 2005). Neurotoxicity is described primarily with acute exposure from deliberate poisoning or suicide, or in high concentrations in drinking water. Evidence of cardiovascular disease (Blackfoot's disease, peripheral vascular disease, coronary heart disease, myocardial infarction, and stroke) and diabetes in areas with relatively low levels of exposure to inorganic arsenic remain inconclusive (Filho, 2011).

A new attention has been drawn to herd animals since arsenic-based herbicides in organic form have been used for weeds in post-emergence in cotton, sugarcane and citrus crops, as is the case of MSMA (methane arsenate monosodium acid) (EMBRAPA, 2007). Poisoning by this compound in herd animals can occur when the product is released into native grassland or pastures where animals are kept. For this reason, the concentration of arsenic should also be monitored in the production environment (Batista, 2012).

It is important to note that there is a lack of geochemical reports on the arrangement of the element Arsenio in the Brazilian territory, which inevitably has profound implications for public health. Added to this is the lack of data relating the existence of the element to the prevalence of diseases in the Brazilian context.

Another factor that can help in the elucidation of obscure issues related to the context of As pollution in Brazil refers to the development of efficient analytical methods, appropriate for routine analysis and adequate for monitoring the element in regions with probable contamination by it, maintaining the requirements of the National Health Surveillance Agency (ANVISA) and the specifications of the *Standard Methods for the Examination of Water and Wasterwater* (da *American Public Health Association* – APHA) e da *International Standartization Organization* (ISO) (Rodrigeus & Malafaia, 2010).

In this context, we suggest that the determination of As concentration from bovine animal models emerges as a viable alternative for monitoring this element, and biological responses can be considered more representative than data provided by chemical or physical sensors. In addition, animal models as bioindicators make it possible to estimate the levels of pollutants and, even more importantly: the impact on biological receptors (Sousa et al., 2018).

Certainly, the stimulus to studies related to extension research and, simultaneously, to the peculiarities attributed to As, will make it possible to produce data that will fill several gaps, making it possible to compare studies already published in the literature, which deal mainly with the methodological aspect chosen in data collection, to the type of contact (chronic or acute) through which the population is subjected. the way in which the results obtained are externalized, the interindividual variations within a studied population and the reference values in the human population (Rodrigues & Nantes, 2010).

Many countries have formalized regulations regarding tolerance limits for arsenic in tissues of animal origin, creating demands for reliable test methods. In Brazil, the monitoring of this element in tissues of animal origin (liver, kidney, and muscle) is regulated by the National Residue Control Plan (PNCRB), of the Ministry of Agriculture, Livestock, and Supply (MAPA), which establishes 1000 μg/kg as the maximum limit allowed for this contaminant in bovine liver (MAPA, 2019). Despite being described as a difficult matrix, the liver was the tissue chosen for initial monitoring by the PNCRB due to the methylation of arsenic in this organ during the metabolization and detoxification process (Borges, 2013).

It is noteworthy that the growing need for large-scale food production requires the development of fast and efficient methods for the determination of contaminants. Toxic elements at trace levels are a growing concern for the quality control of commodities and the concentration of these elements has been investigated, because when accumulated they can be transferred to final consumers, which can become a public health problem (Silva et al., 2013)

2.4 LEAD

The origin of the name of the element lead is Anglo-Saxon, from the Latin "Plumbum", from which its periodic symbol, Pb, originates.. Like the first metals, lead was also associated with a planet, Saturn being the chosen star. Always established as wear-resistant, lead pipes with the emblem of the Roman Empire were used as a drainage system for baths.

Lead along with carbon, silicon, germanium and tin form group 14 of the periodic table. It is found in its natural state in mineral deposits, from which 3 to 10% of Pb are extracted. It is a bluish-gray heavy metal, with an atomic number of 82, and a relative atomic mass and density equal to 207.2 atomic mass units (1 um = 1.6605402 x 10-27 kg) and 11340 kg m-3, respectively. Its melting point is 327.4 °C, while its boiling point is 1749 °C (Moreira & Moreira, 2003).

Lead is a natural element, whose use is intertwined with the beginnings of civilization (Bosso & Enzweiler, 2008). Due to the numerous industrial activities that favor its wide distribution, it has become a ubiquitous element. All humans have lead in their bodies, originally as a result of exposure to exogenous sources (Moreira & Moreira, 2003).

Natural deposits of lead occur all over the world, with galena (PbS) being the most abundant ore and from which most of the commercially obtained lead is extracted. Due to its low melting point and ease of work, this metal has been transformed into a huge variety of objects and utensils, such as ammunition, pipes, types for printing, radiological protection, lead sheets, brass and bronze products, among others (Moreira & Moreira., 2003).

In addition, it has been used in various forms, such as pigment in paints, varnish for ceramics, welds, and anti-knock additives in fuels. It is the most widely used non-ferrous metal, and until the beginning of the 90s, 52% was used in storage batteries and as antiknocks, and approximately 48% in cable protection, pigments, alloys, acid-resistant installations, crystals, vitrified ceramics and stabilizers for plastics and rubbers (Moreira & Moreira., 2003).

Lead contamination is caused by the various uses of this metal and its compounds, summarized in Table 2, which also details the types of occurrence of Pb, chemical formula and solubility products of minerals, and bioavailability

TABLE 2: Main uses of lead and its compounds (Teixeira et al., 2014)

Substance	Principais Usos
Metallic Lead	Batteries, welds, ammunition, metal alloys
Lead stearate	Drying of lacquers and varnishes, greases, waxes
Lead monoxide	Batteries, medicines, paints
Lead naphthenate	Wood preservative, insecticide, lubricating oil
Lead naphthenate	Antiknock for gasoline, ethylation reactions
Lead carbonate	Paints
Lead sulfate	Paints
Lead silicate	Glass compositions

Among the various lead compounds, two toxic groups are highlighted: Inorganic and Organic Lead. Inorganic lead poisoning occurs mainly through the respiratory and digestive systems, and this species is distributed primarily in soft tissues and subsequently undergoes deposition in bones, teeth and hair. The largest fraction of circulating inorganic lead is associated with red blood cells. Organic lead has fat-soluble characteristics, being easily absorbed by the integumentary tissue, digestive system and respiratory system (Schifer et al., 2005).

Occurrence	Chemical formula
Anglesite	$PbSO_4$
Cerussite	$PbCO_3$
Galena	PbS
Fluorpyromorphite	$Pb_5 (PO_4)_3 F$
Hydroxypyromorphite	$Pb_3 (PO_3)_3 OH$
Chloropyromorphite	$Pb_3 (PO4)_3 Cl$
Hysdalite	$PbAl_3 (PO_4)(OH)_6 SO_4$
Plumbogumite	$PbAl_3 (PO4)_2 (OH)_5 H_2 O$
Corkita	$PbFe_3 (PO_4)(SO_4)(OH)_6$

TABLE 3: Type of occurrence of Pb, chemical formula and solubility products of minerals and bioavailability (Bosso & Enzweiler, 2008).

Lead poisoning can occur in a chronic or acute form, the latter being difficult to occur, since it is necessary to ingest 10 to 40 g of the metal. Chronic intoxication, called saturnism, can occur from the daily absorption of 1 to 2 mg of lead for a week (Teixeira et al., 2014). Saturnism is the intoxication produced by excess lead in the body, and the term is a reference to the god Saturn, idolized in ancient Rome. The Romans believed that lead, "the oldest metal", was a gift that Saturn gave them and with it they built aqueducts and produced lead acetate, used by the aristocrats of the time to sweeten wine (Martins, 2014).

The normal plasma lead rate is 5 to 40 μg dL-1, being considered toxic above 80 μg dL-1 or by a urinary elimination above 0.08 to 0.10 mg L-1 (Teixeira et al., 2004). The American Conference of Government Industrial Hygienists in the United States states the maximum biological exposure index to be 30 μg/dL (VoltarellI et al., 2022). Poisoning by lead or compounds containing this metal can cause serious damage to health and even cause death. In Brazil, Ordinance No. 685 of 08/27/1998, of the National Health Surveillance Secretariat, establishes maximum tolerance limits for lead in food, while Regulatory Standard No. 7 (NR 7), of Ordinance No. 24, of the Department of Occupational Safety and Health of 12/29/1994, determines the maximum biological index values allowed for lead in humans (Schifer et al., 2005).

The biological effects of lead are the same regardless of the route of entry (inhalation or ingestion), since there is interference in the normal functioning of the cell and in numerous physiological processes, with the nervous system, bone marrow and kidneys being critical sites in lead exposure, while disturbances in the function of the nervous system and deviations in heme synthesis are considered as critical toxic effects (Moreira & Moreira, 2004). The toxicity of lead results mainly from its interference with the functioning of cell membranes and enzymes, as it is able to form stable complexes with ligands containing sulfur, phosphorus, nitrogen, or oxygen (e.g., –SH, –H2, PO3, –NH2, –OH groups), which function as electron donors. For example, as a result of the biochemical interaction of lead with –SH groups of an enzyme, the activity of this enzyme can be affected and thus produce toxic effects (Bosso & Enzweiler, 2008).

As previously mentioned, the hematological system is an important target of lead toxicity, compromising the synthesis of the heme group and consequently causing anemia

(Sousa & Tavares, 2009). The pathophysiology of anemia that accompanies lead poisoning is not fully understood and seems to be the result of several inhibitory effects of lead on the production of erythrocytes (Sousa & Tavares, 2009). It is suggested that lead inhibits the body's ability to produce hemoglobin by affecting several enzymatic reactions, which are critical for heme synthesis. The activity of three enzymes (5-aminolevulinate dehydrotase, coproporphyrinogen oxidase, and ferrochelatase) is inhibited by lead. This weakens heme synthesis and decreases the synthesis of 5-aminolevulinate synthetase, the initial and rate-limiting enzyme of heme biosynthesis (Minozo et al., 2009).

The nervous system is the most sensitive set of organs to lead poisoning, and encephalopathy is one of the most serious toxic shunts induced by lead in children and adults (Moreira & Moreira, 2004). Childhood cognitive impairment, encephalopathy, and seizures can be caused by lead exposure (Lima et al., 2012). The mechanisms of the neurotoxic effects of lead are not well understood, but there are indications of disturbances in carbohydrate metabolism, abnormal nucleotide synthesis, inhibition of cellular respiration, blockade of neuronal SH groups, and changes in neuraminic acid and RNA levels (Moreira & Moreira, 2004).

To assess lead concentrations in the body, its biomarkers can be measured in the bloodstream and urine. In humans, biological markers reflect the amounts of blood lead from a recent exposure, and may also reveal values close to the concentration of the accumulated metal and its effects on the different organ systems of the body (Schifer et al., 2005). In this context, two enzymatic activities stand out as examples of biological markers of lead exposure, ALA-D (δ-aminolevulinic acid dehydrotase) and erythrocyte protoporphyrin (Telisman, 2001).

In Brazil, the most prominent lead contamination occurred in Santo Amaro (BA) and has been the subject of studies for approximately 4 decades, since the evidence found in the Subaé River and in blood samples from workers of the Brazilian Lead Company (Cobrac), and from the urine and hair of fishermen. The persistence of contamination continues to support new research, even 20 years after the shutdown of Cobrac (Andrade & Moraes, 2013).

In the Brazilian Amazon, Pb contamination in the state of Pará, for example, has been discussed in the soil, water, sediment compartment, in traditional hunting and fishing practices, and in food (MacieL, 2019). According to Faria et al., (2004), the geological nature of the middle and lower Tapajós region, between the municipalities of Santarém and Itaituba, consists of recent sediments, with coarse sandstone, gravel, sand and clay along the river. With ages between 135 and 65 million years (Cretaceous/Mesozoic) and 0.01 to 106 million years (Quaternary/Cenozoic), they are part of the Alter do Chão formation (K2ac) with Alluvial Deposits and presence of Galena-shaped Pb.

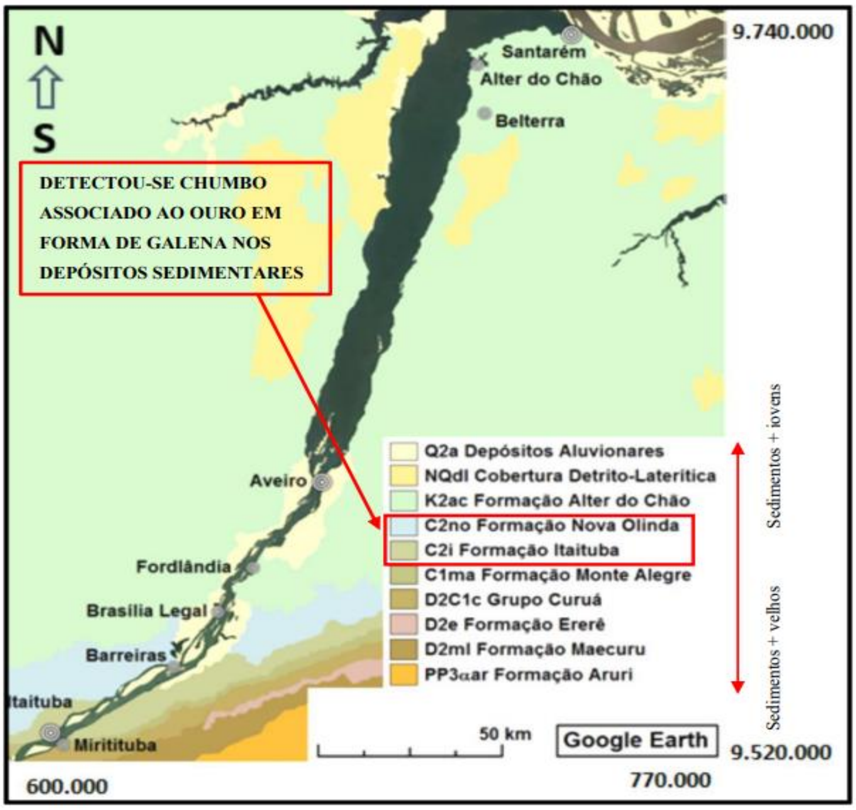


Figure 9: Geological map of the Study Area: Tapajós River Basin (PA), Brazilian Amazon. Source: (Faria et al., 2004)

Rocha (2017) characterized the distribution and availability of total lead in surface waters, humic substances, and particle size fractions of sediments collected from springs of the three different types of waters in the Amazon basin, i.e., white-water rivers, black-water rivers, and clear-water rivers. The researcher determined that the highest mean concentration values were in the river with white water type, then in the river with clear water type and river with black water type, and the following decreasing order of mean Pb concentration in the sediments could be established: white water> clear water> black water.

Regarding domestic animals, cattle are considered one of the most sensitive species (Peres, 2009). Its propagation in this species can occur through the ingestion of forages, water and compromised mineral formulations. From an economic point of view, when lead is transmitted to animals by ingestion of contaminated food, it can cause important organic alterations, such as significant impairment of the reproductive system (Marçal et al., 2004). Cases of lead poisoning are commonly related to the exposure of livestock to waste batteries, paints, lubricants, motor oils, industrial smoke, herbicides, insecticides, and pastures contaminated by industrial waste (Sant'ana et al., 2009).

Alternative sources of raw materials, sought by scientists to ensure animal productivity at lower costs, have allowed the appearance of many mineral compounds, some of which contain components from various sources, including international ones. This fact has given rise to concern among nutritionists, veterinarians and technicians specialized in animal health and production, since it is estimated that some new mineral formulations may be impregnated by toxic elements, such as heavy metals and radioactive substances (Marçal et al., 2004).

Clinical signs of lead poisoning include, as in humans, neurotoxicity, including blindness, muscle spasms, hyperirritability, depression, seizures (especially in young animals), teeth grinding, empty chewing, ataxia, walking in circles or directionlessly, and head pressure (manifestation of cerebral edema) (Filho, 2011). These signs of intoxication can also be confused with those characteristic neurological diseases such as polyencephalomalacia and listeriosis. Other signs such as excessive salivation, anorexia, tympanism and diarrhoea may be included. Miscarriages can also occur as a consequence of lead poisoning. The lethal dose ranges from 400 to 600 mg/kg body weight in calves and 600 to 800 mg/kg body weight in adult cattle (EFSA, 2004).

Nervous alterations derive from edema due to the accumulation of lead in the endothelium of blood vessels, while injuries to the gastrointestinal tract occur due to the corrosive action of lead salts on the mucosa. Some cattle develop normocytic normochromic anemia in chronic envenomations resulting from decreases in the life span of circulating red blood cells and in the production of the heme fraction of hemoglobin. This mechanism results from the increase in protoporphyrin resulting from the suspension of the action of the enzyme hemessetantatase, making it impossible for iron to bind to protoporphyrin (Radostits et al., 2007.; Sant'ana et al., 2009).

Because they are not very selective animals, cattle can become contaminated by chewing objects containing these chemicals or ingesting contaminated water or food (Souza et al., 2009). In addition, lead can be found in considerable concentrations in feeds used as a nutritional supplement for cattle, and this is favored by the low quality of the by-products used for the preparation of these feeds (Marçal et al., 2004)

Contaminants transferred through the food network also play an important role in the action and persistence of lead in the ecosystem. The metal can be slowly accumulated in the tissues of individuals over time, with characteristics of low toxicity until it reaches levels that can be harmful and even lethal to the body (Jesus et al., 2018). The transfer of lead from the tissues of organisms to those of their predators will always lead to concentrations of greater magnitudes in the higher trophic levels, since the metal has an accumulative character (Munhoz, 2010).

In the national literature, there are numerous reports of quantification of lead in food samples, however, there are few studies that monitor the presence of the main toxic metals in a large number of samples collected randomly and with full traceability from the farm to the analytical laboratory, so that the conclusion of the study can better represent the reality of Brazil (Filho, 2011).

The model in which livestock animals are used as biomonitors to recognize environmental pollution has been widely used and its practice has been observed for a long time. Research on lead in bovine blood has proven that this animal species is a good bioindicator of environmental contamination (Marçal et al., 2003; Munhoz, 2010).

It is known that the toxicological manifestation in animals raised in their natural ecosystem will always be earlier than in humans, due to the more restricted relationship they have with nature – this is due to a possible constant exposure when they feed on pastures and water contaminated by the metal (Moreira & Moreira, 2004). Although direct links between ecological effects and human health are sometimes difficult to establish, the use of animal species as sentinels of environmental problems is still the conceptual basis for this connection (Munhoz, 2010).

In Brazil, the monitoring of this element in tissues of animal origin (liver, kidney, and muscle) is regulated by the National Residue Control Plan (PNCRB), of the Ministry of Agriculture, Livestock, and Supply (MAPA), which establishes 500 µg/kg as the maximum limit allowed for this contaminant in bovine liver (MAPA, 2019).

2.5 CADMIUM

The discovery of Cadmium is relatively recent when compared to other heavy metals with a past established throughout the history of the various social organizations of the human species. Described for the first time in 1817 by the German Fredrich Stromeyer, it quickly became a very important metal at an industrial level, namely in the production of batteries, paints and plastics (Rocha, 2009). They are listed in Table 4.

Chemical Symbol	Cd
Atomic number	48
Atomic mass	112,411
Melting point	320,9 ºC
Boiling point	765 ºC
Density (25 ºC)	8,65 g/cm3
Solubility	Insoluble in water; Soluble in organic acids, NH_2NO_3 e H_2SO_4

TABLE 4: Main chemical properties of Cadmium.

Cadmium is often found not in ecosystems as an isolated metal, but rather as compounds involving other elements such as oxygen (cadmium oxide), chlorine (cadmium chloride) or sulfur (cadmium sulfate and sulfite), and in the sulfite forms of zinc, copper, and lead. Its ability to combine with other metallic elements (zinc and copper mainly) favors the occurrence of environmental contamination (EFSA, 2009).

The natural origins of cadmium in the atmosphere are the result of volcanic eruption, erosion of sedimentary and phosphate rocks, and forest fires (Fernandes & Manier, 2014). Industrial sources relate to the activities of mining, preparation, consumption and disposal of products that use cadmium (nickel-cadmium batteries, pigments, stabilizers of PVC products, coating of ferrous and non-ferrous products, cadmium alloys and electronic components) and sources classified as "inadvertent" in which cadmium is a natural participant in the product being processed or consumed such non-ferrous metals. zinc, lead and copper alloys, emissions from iron and steel industries, fossil fuels (coal, oil, gas, peat and wood), cement and phosphate fertilizers (Vieira et al., 2015).

Cd is one of the five most toxic metals present in nature, and is not an essential element for humans or domestic animals. Its presence in biological tissues is the result of exposure due to food, environmental or occupational contamination (Marin & Garcia, 2016). Relatively, large amounts of Cd are found in commercial phosphate-containing fertilizers (Bizarrro et al., 2008).

The main sources of cadmium release into the air and emissions of anthropogenic origin can exceed those of natural origin. Cadmium and cadmium compounds have negligible vapor pressures, but they can exist in the air, as suspended particles, in industrial emissions, in the combustion of fossil fuels, or in soil erosion (Coutinho, 2018). In processes involving extremely high temperatures (e.g. iron and steel), cadmium can volatilize and be emitted as a vapour (EFSA, 2009). The industrial applications of cadmium are wide, and the metal is mainly used in the manufacture of nickel-cadmium (Ni-Cd) batteries, an activity described as responsible for about 83% of the total use of cadmium (Marinha, 2011). Atmospheric Cd is in the form of particles, which can be very small in size (<10μm) if they are produced by combustion processes (Filho, 2011).

The main chemical species found in the air is cadmium oxide, however, some cadmium salts, such as cadmium chloride, can also be found in the atmosphere. These are stable compounds that do not undergo significant chemical transformations (Bonanni et al., 2006). In relation to aquatic systems, it is estimated that rivers carry about 30,000 tons of Cd from weathering and erosion processes to the oceans, In addition, between 4 and 13,000 tons of cadmium are deposited in aquatic environments around the planet by atmospheric deposition of emissions from natural and anthropogenic sources (Nava-Ruiz & Mendez-Armenta, 2011). Other important sources of cadmium in the hydrosphere include domestic wastewater, smelting and refining of non-ferrous metals, and the manufacture of chemicals and metals.

Since cadmium is absorbed by plants, a higher concentration in the soil can result in increased levels in food and feed (Buture & Marçal, 2005). However, the concentration of cadmium in soils is not the main determinant of cadmium in plants. Cadmium is much less mobile in soil than in air and water (Bonanni et al., 2006). The main factors that regulate cadmium mobility in soils are speciation, pH, soluble organic matter content, hydrated metal oxide content, clay type and content, presence of organic and inorganic binders, and competition from other metal ions (Pierangeli et al., 2005).

Higher concentrations of cadmium in soils have also been reported after the application of sewage sludge and barnyard manure, which contain concentrations of this element in variable and sometimes excessive concentrations (Matta, 2011). In addition, soils can be contaminated after the dispersion of mining waste or industrial processes associated with the smelting of metals such as zinc or lead (Nava-Ruiz & Mendez-Armenta, 2011). Since cadmium is retained in the soil, concentrations can increase rapidly if the application of these materials to the soil continues for long periods.

Another form of exposure of cattle to Cd can occur through mineral formulations (Buture & Marçal, 2005). Researchers from various parts of the world have demonstrated the possibility of mineral formulations contaminated by heavy metals reaching the food food chain, reaching cattle and, consequently, humans through the consumption of notably toxic animal products and/or by-products, which represents a potential risk to public health (Marçal et al., 2004).

Buture & Marçal (2005) conducted an investigation in mineral mixtures, quantifying the elements lead and cadmium in different formulations, marketed in the state of Paraná. Considering as a parameter of comparison the references of the National Research Council (1996) and the Association of American Feed Control Official Incorporated (2001), in 30 different brands analyzed, 21 brands presented levels of inorganic lead higher than the maximum acceptable limit of 30 ppm. In all samples, the cadmium level found exceeded the limit of 0.5 ppm, which is the maximum acceptable value. The results demonstrated the urgent need for monitoring with manufacturers and/or dealers, as some mineral mixtures can be eminently dangerous, enabling toxic cumulative effects of lead and inorganic cadmium in cattle (Marçal et al., 2004)

Wildfires also contribute to the dispersion of heavy metals in the environment. Shin et al. (2002) demonstrated results that indicated excessive concentrations of Cd in samples collected in the region of forest fires in South Korea. Samples of burnt wood showed high concentrations of Cd and are considered the main source of which is transported to the coastal regions of the country. In 2019, 40 thousand fires were registered in the Amazon region (INPE, 2019).

Cadmium has a prolonged biological half-life and is therefore considered one of the environmental poisons most likely to accumulate in the body. Due to the high toxicity of cadmium, even at the trace level, global health authorities are concerned with establishing measures to reduce the concentration of these metals in food, since they are considered the main source of ingestion of these contaminants (Ribeiro et al., 2015).

An important aspect of Cd metabolism is the lack of a homeostatic control mechanism, especially in the kidneys and liver, and the strong interactions with other divalent metals, both at the level of absorption in tissues. Once absorbed, Cd first accumulates in the liver and then travels to the kidneys. Thus, the presence of Cd residues in the viscera of animals such as cattle that have had the opportunity to accumulate cadmium from contamination of soil, pastures or other foods during their lives is frequent (Sousa et al, 2009).

Even with the existence of indices that point to increased cadmium concentrations in animals at the apex of the food chain, correlations between animals at different levels of the chain are rare, and accessible studies on bioaccumulation are inconclusive. However, the uptake of cadmium from the soil by food crops can result in high levels of cadmium in beef (especially in the liver and kidneys).

Zanini & Oga (1995), in research with cattle and equine kidneys, found high levels of cadmium and stated that continuous control of metal levels in organs of slaughtered animals is important in the assessment of potential risks. Both for consumers and for animals, since the feed contains organ residues removed in the slaughter of animals that are indirectly consumed by humans (FIlho, 2011).

The absence of adequate urban planning has led to a disorderly industrial growth in some Brazilian cities, with undesirable consequences for farm animals. Rural locations that previously served only as habitats for raising farm animals now share space with factories and industries that pollute the air, soil, plants and water sources, making these animals vulnerable to the toxicity of industrial effluents. Cattle, mainly, and horses have already been used as bioindicators to assess the environmental pollution caused by a metalliferous industry in a rural location in the state of Paraná (Marçal et al., 2003)

CHAPTER 2

Comparative analysis between mercury levels in fish tissues evaluated using direct mercury analyzer and inductively plasma-coupled mass spectrometer

Abstract

Recent ecotoxicological studies have indicated mercury (Hg) contamination in aquatic ecosystems in the Amazon Basin. Although Hg contamination can be associated with small-scale gold mining, the soils of the Amazon region have naturally high Hg concentrations, and can be transported to aquatic ecosystems via deforestation and mining activities. Biomagnification of Hg can pose risks to the local human population; therefore, its concentration in fish tissues must be monitored consistently. Fast and sensitive Hg determination is required for continuously monitoring ecosystems impacted by mineral exploration. The direct mercury analyzer (DMA-80) is widely used for determining total Hg levels in tissue samples; it is fast and cost-effective, without requiring sample preparation. Here, we determined the sensitivity and specificity of Hg detection accomplished using DMA-80, and whether these results are reliable compared to those obtained using Inductively Coupled Plasma Mass Spectrometer (ICP-MS), which is the gold standard. We obtained 106 paired dried samples of muscle tissue from fish species occupying different trophic levels in the Lower Amazon region, and analyzed them using both equipment (DMA-80 and ICP-MS). The results obtained using DMA-80 had an overall Hg mean of 1.90 ± 0.18 µg/kg which was higher ($p<0.05$) than the mean of those obtained using ICP-MS (1.55 ± 0.13 µg/kg). Linear regression analysis comparing the Hg levels obtained using both devices was within the 95% prediction interval, and a high coefficient of correlation showed agreement between the devices (r = 0.979; 0.069 to 0.986, 95% CI). Bland-Altman analysis showed that DMA-80 had a positive bias of 6.5% in relation to ICP-MS, which is more evident in samples with high Hg concentrations. DMA-80 was efficient in determining whether the Hg levels exceeded the maximum allowed levels required by the European Union, USA, and Brazil, showing a specificity and sensitivity of above 95%.

Keywords: mercury; fish; DMA-80; ICP-MS; Amazon; environmental contamination;

1. Introduction

Mercury (Hg) is a metal capable of associating with most living organisms that constitute the biota of aquatic systems, from phytoplankton to invertebrates and vertebrates. Among these organisms, fishes are especially relevant because they can transport energy from the lower to upper levels of the trophic web; fishes generally occupy the highest levels of the trophic chain and are considered important for assessing the degree of exposure of an environment to toxic elements. Furthermore, as they constitute one of the main sources of animal protein for humans, they are relevant when studying the environmental impact of Hg (Ansel, 2021; Castro-Rendón et al., 2022; Emenike et al., 2022; Buck et al., 2019; UNEP, 2019).

Mercury is a silvery-white color metal, stable at liquid form and highly toxic to the environment and living beings. This element can occur in elemental, inorganic and organic forms. Exposure to inorganic Hg can occur through professional occupation, and exposure to inorganic Hg occurs mainly through ingestion of food and water. Depending on its form and characteristics, Hg concentrations can vary in the ecosystem, as well as its accumulation, toxicity and biological effects on the human body. The main source of mercury exposure for humans is food. The forms of Hg most found in foods, although at very low levels, are the Hg^{2+} cation and organic methylmercury (CH_3Hg), with fish meat being the one with the highest concentrations. It is important to highlight that CH_3Hg is the form of mercury of greatest concern, as it easily crosses the blood-brain and placental barriers and has an extremely high absorption efficiency (>90%) when ingested in food. This explains, in part, the greater relative risk associated with exposure to CH_3Hg compared to inorganic Hg species (Nava et al., 2023; Di Bella et al., 2018; Di Bella et al, 2020, Di Bella et al., 2017).

Mercury contamination of fish and seafood is a global concern and various forms of investigation of these contaminants in aquatic environments are necessary to protect consumers (Nava et al., 2023). Organic and inorganic pollutants are ubiquitous elements in aquatic environments due to stream transport, surface runoff and atmospheric deposition (Di Bella et al., 2020). Many different human activities on land, water and air contribute to the contamination of water bodies, sediment and, consequently, aquatic organisms. To estimate organic and inorganic contamination in the aquatic environment, different types of organisms can be used as environmental biomarkers (Arias et al., 2007). Seaweed and filter-feeding mollusks can be used to assess heavy metals; bivalve mollusks particularly have high tolerance to pollutants, are widely distributed, and have a long life cycle, which allows a high capacity to accumulate heavy metals in several orders of magnitude, which can be representative of the pollution of an area (Di Bella et al., 2018). Furthermore, top predators, such as tuna and pirarucu, are well suited for environmental pollution studies dues its position in the food chain (Di Bella et al., 2017).

Recent ecotoxicological studies have indicated different levels of Hg contamination in aquatic ecosystems in the Amazon basin. Mercury is the main environmental pollutant naturally present in high concentrations in soils in the region (Albuquerque et al., 2021). Anthropogenic activities, such as deforestation, especially of riparian forests, and the installation of reservoirs for energy generation, caused increased Hg contamination of aquatic ecosystems from the Amazonian soils (Albuquerque et al., 2020). Illegal artisanal and small-scale gold mining is an important source of Hg contamination, especially with the changes in technological base of gold extraction to increase production scale (Bandeira JR; Carvalho, 2023, Karagas et al., 2012; Gonzalez et al., 2013; Gonzalez et al., 2019); this technique causes deforestation and heavy metal deposition in the environment (Lobo et al., 2016).

The presence of high concentrations of heavy metals in animal tissues is an important public health concern. Due to its environmental persistence and bioaccumulative properties, Hg is considered highly toxic to humans, ecosystems, and wildlife (Beehrooz & Poma, 2021. While low levels of toxic metals are tolerable, higher levels of these metals can accumulate in humans via biomagnification; beyond certain concentrations, they can cause severe toxicity. This is of particular importance in the Amazon region, owing to high concentrations of Hg in the ecosystem. Traditional riverine communities regularly consume large quantities of fish, which can be the main protein source in their diets (Albuquerque et al., 2020; Bourdineaud et al., 2015; Lima et al., 2000; Oliveira et al., 2010). In addition to the environmental and human health impacts, the consumption of fish contaminated with Hg also has economic consequences, being necessary cost-effective methods to guarantee food security (Trasande et al., 2016).

The comparability, reliability, and traceability of chemical measurements is increasingly being recognized and demanded, given their importance in terms of both research robustness and obtaining superior quality analytical data. Unreliable data can lead to catastrophic errors, and consequently, substantial financial losses (Ribani et al., 2004).

Thus, ecotoxicological monitoring studies are fundamental in determining toxic elements (Voegborlo et al., 1999) and verifying the concentrations of substances used and generated in socioeconomic activities that will be disposed into water, soil, air, and biota (Olivero-Verbel et al., 2016; Olivero-Verbel et al., 2015). In aquatic environments, Hg and other heavy metals can be distributed in soil, plants, animals, and sediments (Costa et al., 2008; Shepis et al., 2016).

To obtain analytical data for Hg, some basic requirements must be fulfilled, such as appropriate sample collection, pretreatment for analysis, selection of the measurement method, and appropriate preparation method for test samples. Moreover, when conducting Hg analysis, precautions to prevent sample contamination must be enforced (Suzuki et al., 2004).

Different types of spectroscopy are typically used to quantify mineral elements in biological samples, such as Atomic Absorption Spectrometry, Optical Plasma Emission Spectrometry, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which provide the best results in terms of analytical capacity (De Muynck et al., 2008). While spectrometers can determine the concentration of different mineral elements, specific equipment can quantify Hg in different types of samples, for example the Thermal Decomposition Amalgamation Atomic Absorption Spectroscopy (TDA-AAS), performed here by a direct mercury analyzer (model DMA-80, Milestone Srl, Sorisole, Italy). The TDA-AAS allows the analysis of samples of any matrix without prior preparation, for example, acid digestion (Gomes et al., 2013; Luna et al., 2019). The method is specific for determining total Hg concentrations in liquid and solid samples using the principles of thermal decomposition, amalgamation, and atomic absorption at 254 nm. Using three cells with different optical paths, it expands the working range, allowing a higher detection limit of up to 0.01 ng of Hg; it can be applied in diverse areas, such as petrochemical analysis, environmental analysis, and food analysis (USEPA, 2007).

The ICP-MS technique is costly and time-consuming, which is a general feature of methods that involve the slow and dangerous process of sample digestion, typically involving acids. Another important factor specifically related to Hg is its high volatility, and the digestion of samples for Hg determination in open systems is not indicated, requiring the use of a digester, such as a microwave (Tseng et al., 1997). A more efficient and cost-effective analytical method that excludes the sample digestion phase and presents reliable results is necessary for environmental monitoring programs and research in the Amazon region. Thus, we evaluated the direct mercury analyzer (DMA-80) for determining total Hg in muscle tissue samples from different species of fish, aiming to determine whether the results obtained are reliable compared to those obtained using the gold standard method of ICP-MS.

2. Material and methods

2.1. Samples

A total of 106 paired dried samples of muscle tissue were collected from four different fish species of different trophic levels and feeding habitats from the Amazon region. Table 1 shows the fish species included in sampling.

Muscle samples were obtained from fishes from the Lower Amazon region, following a previously described methodology (Albuquerque et al., 2021). The fish were packed in hermetically sealed plastic bags and immediately transported to the laboratory at 4°C. Samples were obtained from the same dorsolateral muscle cut from a single fish specimen collected directly after capture. After freezing the samples, the tissues were dried via lyophilization (benchtop equipment, model L-101, Liobras, São Carlos, São Paulo, Brazil). The material was placed inside the drying chamber of the lyophilizer until completely dry, usually for 24 h. From the already lyophilized tissue, the samples were homogenized, and two equal subaliquots were taken which were subjected to two different Hg measurement techniques.

The samples were carefully chosen to include a wide range of Hg concentrations from very low to high, which can be observed in some fish species in the Amazon region.

2.2. Analyses using ICP-MS

The sub-samples were sent to the Laboratory of Mineral Analysis, Research Infrastructures Area of the University of Santiago Compostela-USC/Spain for ICP-MS analysis, working under an ISO9001 quality management system. Approximately 1.0 g of sample was subjected to acid digestion using a mixture of 5 ml of concentrated Nitric Acid (Suprapur grade, Merck, Barcelona, Spain) and 3 ml of 30% w/v Hydrogen Peroxide (Merck) in a microwave system (Ethos; Milestone, Sorisole, Italy) with 1000 W of power according to a four-steps digestion ramp: 1 (3 min, 90°C); 2 (15 min, 165°C); 3 (3 min, 180 °C); 4 (10 min, 180°C), followed by a cooling period. The digested samples were transferred to polypropylene test tubes and diluted to a final volume of 25 ml with ultrapure water, and an aliquot was then taken for ICP-MS analysis (ICP-MS/MISS; VGElemental Plasma Quad Option, Model 7700).

2.3. ICP-MS quality control

Analytical quality control was used throughout this study. Blank and test samples were processed simultaneously, and the values obtained were subtracted from the sample readings to calculate the final values. The limits of detection were calculated as thrice the standard deviation of the reagent blanks, and were based on the average weight of the sample. In all cases, the LDs obtained were sufficiently low to determine Hg levels in the samples examined. The precision of the determination was evaluated by comparison with the analytical recovery of certified reference materials (DORM-3 National Research Council Fish Protein, Ottawa, Ontario, Canada). We observed good agreement between the analyzed values and certified reference material of fish protein for Hg (DORM-3) obtained from the National Research Council of Canada, with reference values of 0.382 ± 0.060 mg/kg, a detection limit of 0.001 (mg/kg), and a determined level of 0.348 ± 0.021 (mg/kg). Calibration curves can be accessed in the Supplement material 1.

2.4. Analyses using DMA-80

Approximately 0.1 g of sample was subjected to analysis using DMA-80. The analyses were performed at the Experimental Biology and Bioprospection Laboratory of the Federal University of Western Pará (LabBio/UFOPA). For Hg analysis, duplicate samples were weighed directly into Hg analyzer sample cells. The samples were heated at 850°C for 180 s to decompose the Hg under a stream of clean air which was collected in a gold amalgam tube. For releasing Hg in the UV photodetector for measurement at 253.65 nm, the amalgamated tube was subsequently heated to 155°C. (Jebara et al., 2021)

In this technique, the samples were subjected to the heating process, starting a heating ramp up to 850°C in a quartz or nickel container, called nacelle, using compressed air as oxidizing gas. Hg vapors were directed to a catalyst containing a gold amalgam that selectively retains Hg, following which the products of combustion are selectively removed. After rapid heating, the amalgamation cell released Hg vapor, and the oxygen flow transports it to an absorption cell. The Hg content was quantified by determining the absorption at 253.7 nm (USEPA, 2007).

2.5. DMA-80 quality control

To ensure the reliability of the results, the certified reference material BCR-463 Tuna Fish (SIGMA) was used, with reference values of 2.85 ± 0.16 mg /kg. We obtained a value of 2.80 ± 0.07 upon analyzing the reference material, with a detection limit of 0.01 ng.

2.6. Statistical analysis

A mean comparison analysis was performed using a Paired Equivalence test and regression analysis. Subsequently, the data were subjected to Bland-Altman analysis to assess the agreement between the two quantitative methods. Considering the maximum allowed limits stipulated by the Brazilian (1 mg/kg), European and (0,5 mg/kg) and EUA (0,3 mg/kg) agencies for Hg concentration in fish tissue (EFSA, 2004, ANVISA, 2013, USEPA, 2001), the quantitative data were converted to nominal data classified as positive/negative depending on if the detected concentrations were above or below the allowed limit. This analysis was performed to evaluate the specificity and sensitivity of the direct Hg analyzer compared to the ICP-MS, which remains the gold standard for Hg detection.

3. Results

Table 2 shows the values obtained using the two devices; the average value of Hg concentration obtained using the DMA-80 (1.90 µg/kg ± 0.18 SEM) was higher than that obtained using ICP-MS (1.55 ± 0.13 µg/kg), showing a difference when analyzed using the paired t-test ($p>0.05$).

The linear regression analysis between the Hg concentrations obtained using the two devices (figure 1) was mostly within the 95% prediction interval. The high coefficient of determination (95.8%) reinforces the agreement between the values obtained using the devices.

Figure 2 shows the Bland-Altman analysis of the difference between the two tested methods for Hg (DMA-80 and ICP-MS) divided by their average concentrations. Bland-Altman analysis resulted in a positive bias of 6.48% (± 27.3% SD; -47.13 to 60.10 95% CI) for DMA-80 when compared to the results obtained using the ICP-MS method. The results showed that in fish with higher Hg concentrations, DMA-80 had higher Hg concentrations, increasing the difference between methods as the Hg concentration increased in the samples.

Table 3 shows the results of the specificity, sensitivity, and agreement analyses of both methods considering ICP-MS as the gold standard. For this analysis, we used two different conditions: legally permitted limits of Hg in fish tissue adopted by the European Union (0.5 µg/kg) (EFSA, 2004) or the Brazilian government (1.0 µg/kg) (ANVISA, 2013) and the maximum recommended methylmercury in fish by the United States (US) Environmental Protection Agency (EPA) (0.3 ppm) (USEPA, 2001) A sample was considered positive (for Hg contamination) or negative depending on whether it presented a concentration above or below the legally acceptable limit, respectively.

The results from this analysis show that for all the maximum recommended limits for Hg contamination, DMA-80 efficiently detected the contaminated samples and presented high specificity and sensitivity when compared to ICP-MS, which is the gold standard for Hg detection.

4. Discussion

As was demonstrated, the mean value of the Hg concentration obtained using DMA-80 was higher than that obtained using ICP-MS, presenting a statistical difference using the paired t-test ($p<0.05$). We suggest two possible explanations for the lower mean values obtained using ICP-MS.

The first is related to the premise that ICP-MS is an analytical technique based on the separation of ionic species by the mass/charge ratio that specifically evaluates Hg specimens. Therefore, samples with unknown specimens of Hg that are not certified for ICP-MS detection can show values of Hg that are lower than the levels actually present. This event was cited in a study carried out by (Sannac et al., 2012), in which the inorganic Hg specimens in samples were not recorded because they did not present certification, assuming that only certified samples, such as Hg^{2+} and $MeHg^{+}$, would be present. DMA-80, however, does not feature speciation of the sample and performs the reading of total Hg. Thus, such differences in Hg concentration results may have been due to the varied concentrations of different Hg species in the samples.

A second possibility suggested that the difference in Hg concentrations is related to the known loss of analytes that can occur during the digestion step that precedes ICP-MS analysis. Hg and its compounds are extremely volatile. They can be lost during sampling, storage, preparation, or analysis even if the samples are subjected to relatively low temperatures (Kasper et al., 2015; Micaroni et al., 2000).

Considering the results from the certified reference material used in the equipment, the ICP-MS results showed a reference concentration that was 8.9% lower than the expected value. DMA-80 results also showed a lower Hg concentration than the expected reference value, but the difference was much lower than that obtained for ICP-MS. DMA-80 showed a 1.8% decrease in the Hg concentration of the certified reference material in comparison with the expected Hg values. Thus, the recovery of Hg in the CRM by ICP-MS was 91%, and that by DMA-80 was 98%, which may be related to Hg loss by volatilization or the measurement of only certified Hg specimens by ICP-MS.

Even with the difference observed between the concentrations resulting from ICP-MS and DMA-80, the linear regression analysis of the Hg obtained using the two devices resulted in a high coefficient of determination (95.8%), which reinforces the agreement between the devices. The agreement between results is important because even though the difference in analysis methods can potentially influence the obtained results, such differences are not sufficient to conclusively establish the superiority of one method over another. The correlation coefficient ($r = 0.979$) also revealed a very strong correlation between the results obtained using the two methods. The regression equation Hg ICP-MS = 0.2088 + 0.7098 Hg DMA is described here and can be used to convert the results obtained using DMA-80 to ICP-MS (Zhang and Zhou, 2020).

Few studies have compared ICP-MS with other methods for measuring Hg concentrations. Silva et al. (2021) compared the total Hg values obtained via ICP-MS and

Atomic Fluorescence Spectrometry Coupled to Cold Vapor Aeration in the liver of marine tetrapods. The results obtained using the two methods were not significantly different; however, when comparing fresh or freeze-dried samples, a difference in Hg content was noted due to Hg loss during lyophilization.

(Bussan et al., (2015) coupled DMA and ICP-MS systems, demonstrating several advantages over DMA alone for Hg determination, including increased sensitivity, lower detection limits, reduced potential for sample contamination, and applicability to stable Hg isotope marker studies. To the best of our knowledge this is the first study that compare both devices.

We also created a chart based on the Bland-Altman Analysis to check the agreement between the Hg analysis methods and found a positive bias towards DMA-80, although with a high confidence interval. Samples with a lower Hg content most often produced very similar results for both analytical methods. The differences responsible for the elevated SD were sourced from the samples with high Hg content.

Countries advocate a maximum detection limit for Hg in marketed foods to protect their populations from the deleterious effects of ingesting this metal. For example, values of 1.0 mg/kg and 0.5 mg/kg have been established as the maximum tolerable concentration of Hg by Brazil and the European Union, respectively, and the maximum recommended CH_3Hg in fish by the EPA (0.3 mg/kg) (ANVISA, 2013., EFSA, 2004., USEPA, 2001). DMA-80 is often characterized by not having a low detection limit for Hg when compared to ICP-MS; however, via the specificity, sensitivity, and concordance analyses conducted in this study, we demonstrated that DMA-80 was efficient in determining the legal maximum values required by European countries and Brazil, attaining a specificity and sensitivity above 95%. With these specificity and sensitivity tests, we suggest that DMA-80 is a safe, accessible, and cost-effective alternative to ICP-MS, lacking time-consuming and costly steps such as acid digestion.

In addition to DMA-80 analysis (Prazeres et al., 2018) and ICP-MS (Albuquerque et al., 2020), several other analytical methods have been used in the Amazon region to determine Hg in fish tissue samples, including Cold Vapor Atomic Absorption Spectrometry (Filho et al., 1999)), Cold Vapor Atomic Fluorescence Spectrometry (Hacon et al., 2020), and Graphite Furnace Atomic Absorption Spectrometry (de Queiroz et al., 2018).

Most studies have used single-element methods. However, such techniques are expensive, time-consuming, and require pre-treatments such as acid digestion (Han et al., 2019; Rey-Crespo et al., 2013). DMA allows analysis in liquid and solid matrices without the need for prior sample preparation (such as acid digestion), facilitating its application in routine tests (Nortje, 2008; Torres et al., 2012). It is quick, sensitive, and cost-effective (Han et al., 2019), employing the principles of thermal decomposition, amalgamation, and atomic absorption. The system provides a much faster analysis with minimal amount of sample and excellent Hg recovery, and is more cost-effective compared to atomic absorption and mass spectrometry techniques (Carbonell et al., 2009; Han et al., 2019;

Ribeiro and Germano, 2015; Torres et al., 2012). Therefore, this method can be particularly useful for government agencies (for monitoring purposes), especially in regions with a history of environmental deterioration wherein active mineral exploration is still being conducted.

5. Conclusions

Hg concentration in fish tissue obtained using the direct Hg analyzer were highly correlated with those obtained using ICP-MS with high (>95%) sensitivity and specificity to detect fish samples in which the Hg concentration exceeded the maximum allowed concentration according to European, USA, and Brazilian legislation. The direct mercury analyzer had a positive bias of 6.5% against ICP-MS, which was more evident in samples with high Hg concentrations.

Acknowledgements

This research was supported by Coordenação de Aperfeicoamento de Pessoal de Nível Superior (CAPES). FEA Alburquerque is grateful to Fundação Amazônia de Amparo a Estudos e Pesquisas (FAPESPA) for his Post-Doc fellowship. AHH Minervino is grateful to CNPq for his research productivity fellowship.

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Table 1. Fish species from the Amazon that were included in the paired muscle sampling.

Name	Scientific name	Feeding habit	N
Acari	*Pterygoplichthys pardalis* (Castelnau, 1855)	Detritivores	32
Piranha	*Pygocentrus nattereri* (Kner, 1958)	Omnivores	17
Caparari	*Pseudoplatystoma fasciatum* (Linnaeus, 1766)	Carnivores	19
Tucunaré	*Cichla ocellaris* (Bloch & Schneider, 1801)	Carnivores	38

Table 2. Mercury concentration in dried fish tissue analyzed using direct mercury analyzer (DMA-80) or inductive plasma-coupled mass spectrometer (ICP-MS).

Methods	**N**	**M**	**SEM**	**SD**	**Var**	**CV**	**Min**	**Med**	**Max**	**Amp**
DMA-80 (mg/kg)	106	1.90^{a}	0.18	1.82	3.23	95.96	0.015	1.31	6.78	6.77
ICP-MS (mg/kg)	106	1.55^{bq}	0.13	1.32	1.75	84.88	0.047	1.32	5.30	5.25

Different letters indicate significant differences by paired t-test ($p<0.05$). N, number of samples; M, mean; SEM: Standard Error of the Mean; SD: Standard Deviation; Var: Variance; CV: Coefficient of Variation; Min minimum value; Med, median; Max: Maximum value; Amp: Amplitude.

Table 3. Specificity, sensitivity, and concordance between the two Hg detection methods performed on fish tissue.

Europe legal Hg limit in fish (0.5 mg/kg)[a]			
	ICP/MS +	ICP/MS -	Total
DMA +	78	1	79
DMA -	0	27	27
Total	78	28	106
Sensitivity (95% CI)	1.0 (0.95 to 1.0)		
Specificity (95% CI)	0.96 (0.82 to 1.0)		
Positive Predictive Value (95% CI)	0.99 (0.93 to 1.0)		
Negative Predictive Value (95% CI)	1.0 (0.87 to 1.0)		
Brazilian legal Hg limit in fish (1.0 mg/kg)[b]			
	ICP/MS +	ICP/MS -	Total
DMA +	61	0	61
DMA -	2	43	45
Total	63	43	106
Sensitivity (95% CI)	0.97 (0.89 to 1.0)		
Specificity (95% CI)	1.0 (0.92 to 1.0)		
Positive Predictive Value (95% CI)	1.0 (0.94 to 1.0)		
Negative Predictive Value (95% CI)	0.96 (0.85 to 0.99)		
USA recommended maximum CH_3Hg in fish (0.3 mg/kg)[c]			
	ICP/MS +	ICP/MS -	Total
DMA +	83	1	84
DMA -	0	22	22
Total	83	23	106
Sensitivity (95% CI)	1.0 (0.95 to 1.0)		

Specificity (95% CI)	0.95 (0.78 to 0.99)
Positive Predictive Value (95% CI)	0.98 (0.93 to 1.0)
Negative Predictive Value (95% CI)	1.0 (0.84 to 1.0)

[a]EFSA, 2004; [b]ANVISA, 2013; [C] USEPA, 2001. CH_3Hg: Methylmercury. CI: confidence interval.

CHAPTER III

Heavy metal acumulation in muscle of cattle raised Western Pará, Amazon and Heavy metal content and health risk assessment.

Abstract

The region of the Lower Amazon, in the State of Pará, northern Brazil, presents itself as a wide zone of mineral exploration, either in artisanal or industrial form, which implies the direct deposition of potentially toxic elements from these activities in the soils or in the hydrographic basins of the region. In the present study, we considered to evaluate the con centration of toxic metals, such as mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) in muscle tissue samples from cattle belonging to three municipalities in the Lower Amazon region: Oriximiná, Itaituba, Monte Alegre. The concentrations of the non-essential elements (As), (Cd), (Hg) and (Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS). Among the toxic metals studied, we verified that cattle presented Hg concentrations above the maximum recommended levels (MRL) established by the Brazilian government, with 10% of the animals analyzed with concentrations above the allowed and animals with excess Hg in the muscle were identified in all the municipalities studied. In this study, the results of the calculation of daily intake of toxic metals (EDI), which represents the daily intake of toxic metals through the daily consumption of beef (71 g per person), in general, indicated that the concentrations of metals were slightly below the reference values (RfDo). The results of the analysis of the target risk quotient (THQ) and the multiple contaminant exposure data (TTHQ) revealed that the values of the four toxic elements were not associated with risks by isolated exposure (THQ<1). The city of Monte Alegre presented the highest concentrations of THQ and TTHQ for the metals As and Pb. However, in Itaituba and Oriximiná, the concentrations of toxic elements resulted in the highest THQ values of Hg and Cd, leading to a TTHQ of 0.06 to 0.02, respectively. The results of the present research indicate that beef from animals raised in the region is safe for consumption.

1. Introduction

The Amazon region is currently established, from an abundant collection of scientific productions, as an ecosystem potentially contaminated by the element mercury. Studies on mercury concentrations are strongly directed to bioaccumulation in the tissues of fish in the region as well as in its potential consumers, the populations residing in the cities located within the environment attacked by this heavy metal (Meneses et al., 2022).

The Tapajós River basin, state of Pará, is one of the prominent regions in mercury contamination. In this region the exploitation of mineral resources is quite evident. With the mining activity present, the use of mercury by man during the processes of mining and extraction of gold ore, results in a high and significant environmental contamination and cases of human intoxication, already existing in the state (Crespo Lopes et al., 2021).

However, it is important to note that the contamination of this element is not restricted to aquatic environments, since the natural cycle of mercury allows this heavy metal to reach the atmosphere as well as the soils of the regions (Brito et al., 2021). It is estimated that the fires in the Amazon are responsible for emitting 13% of the atmospheric mercury in Brazil, which is equivalent to 8.7 tons per year (Lacerda et al., 2007).

Mercury is not the only heavy metal with potential harm to the health of Amazonian populations (Albuquerque et al., 2021). Arsenic, cadmium and lead, are toxic metals known to be present in the region, whether due to anthropic or natural action (Albuquerque et al., 2020). The main example of As contamination in the Amazon basin is that described in the area of the municipality of Santana, in the State of Amapá, where As is associated with manganese (Mn) ores exploited in the last 50 years (Rodrigues and Mlafaia, 2010). Since 2001, Seyler and Boaventura have been calculating arsenic levels in Amazonian rivers. The highest values of arsenic are in the Andean rivers, such as the Solimões and the Madeira. In Óbidos, the Amazon River already had values higher than 0.8 mg/m3 of arsenic, indicating that about 0.5 tons of element are taken daily to the sea, that is, more than 200 tons annually.

The contamination by Pb in the Brazilian Amazon, especially in the state of Pará, for example, is the object of research in the soil, water, sediment compartment, in the traditional practices of hunting and fishing and in feeding since the geological nature of the region of the middle and lower Tapajós between the municipalities of Santarém and Itaituba has in its constitution the Pb in the form of galena (Faria et al., 2004). In addition to the natural presence is added the use of lead as a component of paints, batteries, varnishes, greases, waxes and among other products that when discarded can harm the environment with this element (Santana et al., 2017).

The main sources of cadmium release into the air and emissions of anthropogenic origin may exceed those of natural origin. Cadmium and cadmium compounds have negligible vapor pressures, but can exist in the air, as suspended particles, in industrial emissions, in the combustion of fossil fuels or in soil erosion (Coutinho, 2018). In relation to aquatic systems, it is estimated that rivers carry about 30 thousand tons of Cd from weathering and erosion processes to the oceans, in addition, between 4 and 13 thousand tons of cadmium are deposited in aquatic environments throughout the planet by atmospheric deposition of emissions from natural and anthropogenic sources (Nava-Ruiz & Mendez-Armenta, 2011).

Although the topic related to mercury contamination in fish is widely addressed in the scientific literature, in cattle, this study is still limited. There are vast studies of the concentration of toxic metals in fish from the Amazon region, and we found no reports of contamination that has its meat products as one of the most consumed by the population, thus representing a risk route to human exposure to toxic metals. In addition, there are also frequent studies that relate the effects of these chemical elements on human health or on some specific environmental compartment, such as soil, sediments, vegetation or water (Marques et al., 2022). Obviously, when these compartments are contaminated, there is a possibility of increased levels of these elements in animal organisms (Albuqurque et al., 2020).

It is noteworthy, in the region of the lower Amazons, the dynamism of the rivers during the periods of flood and drought, exposes the cattle to two distinct environments called terra firme, a generic term, used in the Amazon region, to designate places that do not suffer influence of the floods caused by the rivers, and the floodplain, used to designate areas subject to periodic flooding caused by river floods, which contribute to new sediment deposits in the soil, and expose cattle to direct consumption of their waters (Cravo et al., 2002).

Thus, we aimed to determine the presence of toxic metals in bovine muscle in different cities in the western region of Pará subject to different types of metal contamination and to perform a risk analysis to human health of the consumption of beef from animals slaughtered in the region.

2. Material and Methods

2.1 Study site and sample collection

For the analysis of toxic metals, samples of muscle tissue from cattle were used. Samples of muscle tissue were obtained from different butcher shops located in three municipalities in the western region of the state of Pará. In Oriximiná, a region/area where bauxite has been exploited since 1969, in Itaituba, where the largest small-scale gold mining sites called "garimpos" are located; and Monte Alegre, which is free of mining activities. Ten samples of muscle tissue from the caudal region, weighing 100g each totaling 30 samples of each tissue, were collected in each municipality, which were later stored at an average temperature of 4°C and sent to the Animal Health Laboratory of the Federal University of Western Pará.

TABLE 1 - Municipalities and number of tissue samples collected

Cities	Muscle Samples
Itaituba	10
Monte Alegre	10
Oriximiná	10
Total	30

2.2 Sample preparation

Once in the laboratory, each specimen was measured and weighed. Subsamples of approximately 1g were accurately weighed and digested in a mixture of 5ml of concentrated nitric acid (TMA, Hiperpure, PanReac, Spain) and 3ml of 30% w/v hydrogen peroxide (PanReac, Spain) in a microwave-assisted digestion system (Ethos Plus; Milestone, Sorisole, Italy). Digested samples were transferred to polypropylene sample tubes and diluted to 15ml with ultrapure water according to previously described procedures and conditions (Minervino et al., 2018; Rey-Crespo et al., 2013).

2.3 Toxic element Analysis

The concentrations of the non-essential elements arsenic (As), cadmium (Cd), mercury (Hg), lead (Pb) in the digested samples were determined by inductively coupled plasma mass spectrometry (ICP-MS; VG PQ Excel, Thermo Elemental, USA). A detailed description of the analytical conditions is provided elsewhere (Albuquerque et al., 2020b; Luna et al., 2019; Rey-Crespo et al., 2013). Analytical quality control was applied throughout the study. Blank samples were processed at the same time as test samples, and the values obtained were subtracted from sample readings for the calculation of the final values. The limits of detection (LOD) were calculated as three times the standard deviation of the reagent blanks and were based on the mean sample weight. In all cases, the obtained LODs were low enough to determine all essential and trace metals at the usual levels in the studied samples. The good agreement between the measured and the certified values demonstrated the high accuracy of the method. The mean recoveries were 91% and 94% respectively. The precision of the analytical method, calculated as the relative standard deviation (RSD) of 10 different extractions of the same sample, ranged between 5.4 and 9.6%. Principal Component Analysis (PCA) was performed with the aid of the CANOCO program (version 4.5) (ter Braak and Šmilauer, 2002).

2.4 Human health risk assessment of heavy metals in cattle muscle

To assess the risk of consuming contaminated cattle to human health, different methods were used, for review, see (Chien et al., 2002; USEPA, 2000; Varol and Sünbül, 2018; Yi et al., 2017). To determine the risk of heavy metals from the consumption of bovine muscle, the calculations were made following the data used in this study: the average adult body weight was 70 kg, the average daily consumption of beef in the Lower Amazon was 71 g/day, and an average per capita consumption rate of beef in Brazil of 25 kg per year, i.e. 71 g/person/day (USDA, 2020)

2.4.1 Estimated daily intake (EDI)

$$EDI = \frac{CxCons}{Bw}$$

where C is the concentration of heavy metals in bovine muscle (mg/kg fresh weight), Cons is the average daily consumption of beef in the region and the national daily intake rate (71 g/day) and Bw represents the body weight of adults (70 kg).

2.4.2 Determination of hazard quotient (HQ)

$$HQ = \frac{EDI}{RfDo}$$

(2)

Where *RfDo* is the oral reference dose for a given element (As 0.0003, Cd 0.001, Hg 0.005 and Pb 0.004 mg/kg Bw/day*)*

2.4.3 Determination of target hazard quotient (THQ)

$$THQ = \frac{EFr\ x\ EDtot\ x\ FIR\ x\ C}{RfDo\ x\ Bw\ x\ ATn} x10^{-3}$$

(4)

Where; *FrS* is the frequency of exposure (350 days/year); *Edtot* is the duration of exposure (70 years); *FIR* is the rate of food intake (g/day), while 10-3 is the conversion factor of the unit; *C* is the concentration of heavy metals in bovine muscle (mg/kg fresh weight); *RfDo* is the oral reference dose for a given element (As 0.0003, Cd 0.001, Hg 0.005 and Pb 0.004 mg/kg Bw/day*)*; *Bw* is the average adult body weight (70 kg); *and ATn* is the average exposure time for non-carcinogens (365 days/year × number of years of exposure, assuming 70 years) (US EPA, 1989).

2.4.4 Determination of total target hazard quotient (TTHQ)

In this study, the total THQ was expressed as the aritmetic sum of the individual THQ values for each of the metals analyzed (As, Pb, Hg, Cd) (Chien et al., 2002) :

Total THQ (TTHQ) = THQ (metal 1) + THQ (metal 2) + THQ (metal 3) + THQ (metal 4)

3. Result

Concentration of toxic metals in the muscle of cattle

The concentrations of metals in cattle muscle from the municipalities of Western Pará are presented in table 2. According to the results, the concentration range of toxic elements expressed in mg/kg of dry weight was As: 0.0003-0.059; Cd: 0.0004-0.037; Hg: 0.001-0.035; Pb: 0.011-0.242.

TABLE 2 - Concentrations of toxic elements (expressed in mg/kg dry weight) in muscle of cattle from Western Pará, Lower Amazonas, Brazil.

Cities	Metal concentration in muscle tissue (mg/kg)			
	As	Cd	Hg	Pb
	Mean±SEM (range)	Mean±SEM (range)	Mean±SEM (range)	Mean±SEM (range)
Itaituba	0.01±0.001 (0.004-0.013	0.004±0.001 (0.0004-0.014)	0.01±0.01 (0.003-0.035)	0.04±0.01 (0.011-0.125
Monte Alegre	0.009±0.01 (0.0003-0.059)	0.011±0.01 (0.001-0.031)	0.004±0.001 (0.001-0.011)	0.07±0.03 (0.011-0.242

Oriximiná	0.006±0.002 (0.001-0.019)	0.015±0.004 (0.001-0.037)	0.005±0.001 (0.001-0.014)	0.02±0.004 (0.004-0.043
MRL Brazil$^{\alpha}$	*0.5*	*0.05*	*0.03*	*0.1*
MRL EU$^{\beta}$	*0.5*	*0.05*	*0.01*	*0.1*

MRL: maximum recommended levels. EU: *European Union.* $^{\alpha}$*Anvisa (Decreto nº 55.871/1965 e Instrução Normativa SDA nº42 (PNPC 2015).* $^{\beta}$*Commissiion regulation (EC) nº 466/2001*

Principal component analysis (PCA) of the distribution of toxic metals

The PCA was also used to study the relationships between the variables. The results of the loads of the different bioaccumulations of the four metals in the muscle samples of cattle are presented in the reduced space of the two main components (Fig. 1). The first axis represents a gradient of the metals As and Pb, explaining 45.8% of the variability of the data. The second axis represents an Hg gradient, explaining 24.22% of the variability of the data sampled from the cities considered in this study. The PCA Loadings (Table 3) shows the main variables that contribute significantly to each axis. For the first axis it is observed that As has the greatest contribution to this gradient, followed by Pb. For the second axis, the greatest contribution is observed by Pb. It is noteworthy that the highest values of As concentration occurred in the sample collected in Monte Alegre and the highest values of Hg were in the samples collected in Itaituba.

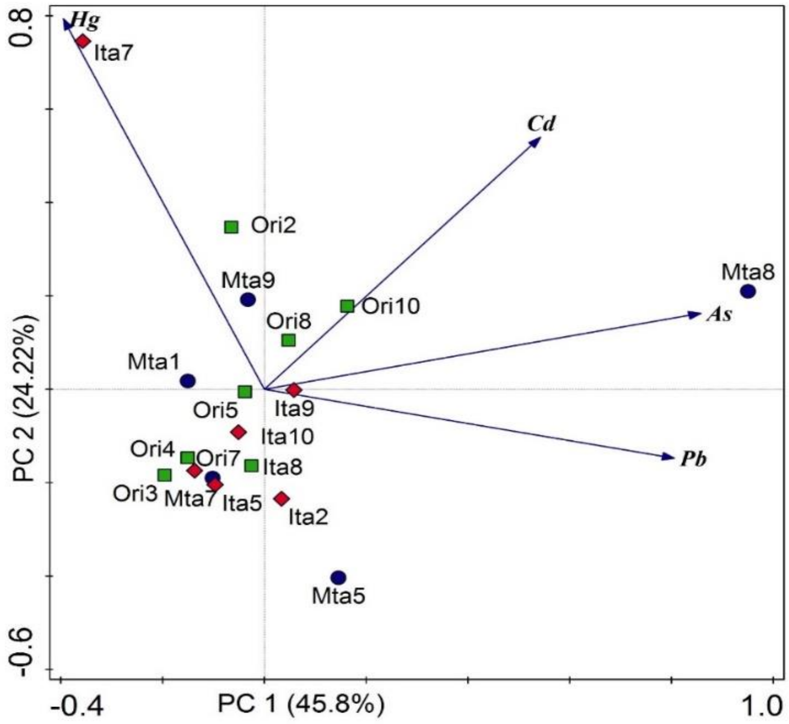

Figure 1 – Ordering diagram of the analysis of principal components of heavy metals sampled from the muscles of cattle from three large centers in western Pará. Legend: Ori – Oriximiná; Mta – Monte Alegre; Ita – Itaituba.

Table 3 – PCA loadings, in bold are the variables that have significant contribution

Metals	PC 1	PC 2
As	**0.65037**	-0.00361
Cd	**0.43893**	0.51714
Hg	-0.13484	**0.83566**
Pb	**0.60513**	-0.18501

3.2. Human health risk assessment

3.2.1. Estimated daily intake of metals

In this study, the results of the calculation of the EDI (Table 4), which represents the daily intake of toxic metals through the daily consumption of beef (71 g per person), in general, indicated that the concentrations of metals were slightly below the reference values (RfDo).

Table 4. Estimated daily intake (mg/kg person/day) of toxic metals.

Citie	Metal			
	As	Cd	Hg	Pb
Itaituba	0,0000071	0,00000406	0,00000913	0,0000375
Monte Alegre	0,00000913	0,0000112	0,00000406	0,0000669
Oriximiná	0,00000609	0,0000152	0,00000507	0,0000223

3.2.2 Determination of hazard quotient (HQ)

The results of the HQ (Table 5) indicated that all toxic metals had a value lower than 1 and that the adverse effects of an ingestion are unlikely.

Table 5. Risk quotient (HQ) for toxic metals evaluated in cattle muscles for consumption in the West of Pará State.

Citie	Metal			
	As	Cd	Hg	Pb

Itaituba	0,02	0,004	0,02	0,009
Monte Alegre	0,030	0,011	0,008	0,017
Oriximiná	0,020	0,015	0,010	0,006

3.2.3. Target risk quotient (THQ)

The results of the analysis of the target risk quotient (THQ) and the data of exposure to multiple contaminants (TTHQ) are presented in Table 6. According to table 6, the values of the four toxic elements were not associated with risks due to isolated exposure (THQ<1). However, the results of TTHQ were analogous to the values found in the THQ calculations.

Table 6. Target risk coefficient (THQ) and target total risk quotient (TTHQ) in beef (expressed in mg/kg dry wet) for the scenario of the West of the state of Pará.

Citie	THQ				TTHQ
	As	Cd	Hg	Pb	
Itaituba	0,02	0,004	0,02	0,01	0,06
Monte Alegre	0,03	0,01	0,01	0,017	0,07
Oriximiná	0,02	0,02	0,01	0,006	0,02

3. Discussion

The evaluation of the mean concentrations of metals evaluated in bovine muscle revealed values below the maximum limits established by the European Union (MRL EU) and by Brazil (MRL Brazil), in the three municipalities submitted to research. The results are optimistic in the face of a well-known scenario of environmental contamination in the Amazon region, resulting from mineral exploration, and contrast with the high averages found in studies related to the bioaccumulation of toxic metals in species in the region, such as fish mainly (Albuquerque, 2020; Albuquerque, 2021). We suggest that the concentrations found do not reveal a bioaccumulative status in the tissues studied due to the average slaughter time of the bovine species, which in the Amazon region is 2 years, not being sufficient for the bioaccumulation of these elements.

Hashemi (2018) evaluated the concentrations of heavy metals in muscle tissues of cattle submitted to an environment with pollution status in southern Iran and did not verify the bioaccumulation of Cd and Hg, however, the values of the mean Pb were above the MRL-EU. Pb values were also above the established (FAO/WHO) in a study conducted by Akele et al. (2022) in bovine muscle living in pollution-prone environments. In Brazil, research related to the concentration of heavy metals in cattle points to increases in the values of these elements, only in cases of acute intoxication (Barbosa et al., 2015), but studies that point to bioaccumulation were not found in our reviews (Santana et al., 2015).

It is important to emphasize that, when analyzing the samples individually, 30% of the animals from the municipality of Itaituba presented values above those established for the Hg element (MRL EU/ MRL Brazil), and, although these excesses were not reflected in the general average, we consider this highlight important, given the uniqueness of the result.

The calculations of daily intake (EDI) of bovine muscle for the elements As, Cd, Hg and Pb, revealed values below the established limits (RfDo). Beef is among the three most consumed food types in the Amazon region, alongside chicken and fish and, in the latter, studies on the risk of consumption have been carried out in various aquatic environments known to be subjected to activities that emit potential toxic metals, such as in the Aegean Sea (Dogruyol et al., 2023) and Caspian Sea (Amerizadeh et al., 2023), revealed an EDI above the established limits (FAO/WHO) and classified as having serious risks to human health with potential carcinogenic risk. In the Amazon region, Sousa-Araujo et al., (2022) demonstrated an EDI for As, Hg and Pb, above RfDo in almost half of the fish species analyzed and with carcinogenic potential to consumer populations. The present study does not intend, with the discussions presented, to compare food security between fish and cattle, but to highlight the potential for distinct bioaccumulation between species, influenced by factors such as time and place of exposure as well as the trophic level in the food chain.

The results obtained from the determination of the Hazard Quotient (HQ) showed values lower than 1 (HQ <1), suggesting that poisoning or adverse effects resulting from the ingestion of these metals in beef are unlikely. The determination of the Target Hazard Quotient (THQ), through which risks arising from an isolated consumption of heavy metals estimated for 365 days (1 year) for 70 years were verified, revealed no exposure (THQ<1) to harmful effects resulting from the ingestion of these elements. The Total Target Hazard Quotient (TTHQ), which deduces the joint risk of all elements, also demonstrated the absence of potential adverse effects. THQ and TTHQ data have been instrumental in verifying populations exposed to toxic metals from food.

These estimates have already revealed health risks to populations in Iran (Raissy et al., 2022; Hashempour-baltork et al., 2023; Barani et al., 2023), in studies with dairy products in Armenia (Pipoyan et al., 2022), in fruits (Kilic, 2024), and from groundwater consumption in Cambodia (Phan et al., 2010). For the Amazon region, the present study is the first to evaluate these data that point to the risks of ingestion of toxic metals resulting from the ingestion of bovine muscle tissue.

In view of this scenario, we present results that suggest the potential food security in the consumption of beef in the municipalities surveyed, with regard to the toxic metals verified, even in places subjected to intense mineral exploration that emit residues proven to be contaminated with these metals and that can directly affect the cattle production chain since, pastures have their contamination due to the atmospheric deposition of industrial activities, such as mining, in addition to the potential for effluent pollution that may be responsible for the contamination of water used by animals (Marçal et al., 2015).

As previously mentioned, the present study is the first that seeks to verify the risks of consumption of toxic metals present in bovine tissues in the Amazon region, being fundamental as a tool for controlling and preventing the risks of foodborne diseases. We also suggest that cattle can be used as bioindicators of exposure to potentially toxic elements, which may pose potential risks to the health of Amazonian populations. The proximity of coexistence and the similarity in physiological terms with human beings, in addition to the coincidence of living habitats, make animals such as cattle have the potential to be used as indicators of environmental contamination.

5.Conclusion

The results of the HQ indicated that the adverse effects of an intake are unlikely. The results of the analysis of the target risk quotient (THQ) revealed that values of the four toxic elements were not associated with risks by isolated exposure.

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CHAPTER IV

Concentrations and health risk assessment of trace elements in bovine liver tissues from the legal Amazon, Brazil.

Abstract

The objective of the present study was to determine the concentrations of essential elements such as cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) in liver tissue of cattle consumed in municipalities of the western region of Pará. The underlying hypothesis of the study assumes that historical mining activities in the region would have impacts on the concentrations of essential elements in cattle and that there would be variations according to the area evaluated. We also assessed the risks arising from the daily consumption of these elements. The daily intake of bovine liver tissue calculations were higher than those established for the copper element in all municipalities evaluated. The EDI of Se and Zn remained above the values when the hepatic tissues from Oriximiná were evaluated. The HQ regarding the ingestion of hepatic tissue revealed a risk regarding the adverse effects of Cu in all the municipalities evaluated. The total target risk quotient (TTHQ) revealed that all municipalities, were exposed to the risks of excessive consumption of these minerals, indicating that the degree of accumulation of essential elements in cattle in the Amazon region presents a significant risk for the consumption of the local population.

Introduction

The state of Pará has the second largest cattle herd in Brazil with 23,921,005 head of cattle, reaching the seventh consecutive year of growth (IBGE, 2021). Agribusiness is responsible for an average of 21% of the composition of the GDP of the municipalities of Pará, composing the economic base of most of them and a source of employment for a substantial portion of the population. The countryside absorbs about 1,500,292 million people, which corresponds to 42.68% of the workers in the state of Pará (FAEPA, 2022).

Cattle ranching in Pará occurs in a context simultaneous to that of mineral exploitation. With more than R$ 250 billion billed during 2021, mining activity reached a growth of more than 19% compared to the collection of the Brazilian sector in 2020. The data also show that the highest increase occurred in Pará, which totaled R$ 94.6 billion in the months analyzed, representing 43% of the Brazilian industry's revenue (IBRAM, 2021). In the western region of Pará, it is important to highlight that ore exploration is carried out by bauxite and gold mining activities.

In view of the coexistence between mining and cattle ranching activities in the western region of the state of Pará, we intend to verify a correlation between the potential harmful effects of mining activities through biomonitoring. The organisms used in biomonitoring are known as bioindicators and can be any form of life used to measure and monitor properties and attributes of a system (Vieira et al., 2021).

The proximity of coexistence and the similarity in physiological terms with human beings, in addition to the coincidence of living habitats, make animals such as cattle have the potential to be used as indicators of environmental contamination, since pastures often have their contamination resulting from atmospheric deposition. industrial activities, such as minings, in addition to the potential for effluent pollution that may be responsible for the contamination of water used by animals (Sousa et al., 2009).

Thus, the objective of the present study was to determine the concentrations of essential elements such as cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) in liver tissue of cattle consumed in the western region of Pará. The underlying hypothesis of the study assumes that historical mining activities in the region have had impacts on the concentrations of essential elements in cattle and that there will be variations according to the area evaluated.

2. Methodology

2.1. Place of study, collection and preparation of samples

A total of 90 samples of bovine tissue were collected at four different sites in the regions of Itaituba, Monte Alegre, Oriximiná, Placas, Rurópolis, and Uruará. The samples were packed in plastic bags and immediately transported to the laboratory at 4ºC. Once in the laboratory, each sample was measured and weighed. Subsamples of approximately 1g were accurately weighed and digested in a mixture of 5 ml of concentrated nitric acid (TMA, Hiperpure, PanReac, Spain) and 3 ml of 30% w/v hydrogen peroxide (PanReac, Spain) in a microwave-assisted digestion system (Ethos Plus; Milestone, Sorisole, Italy). The digested samples were transferred to polypropylene sample tubes and diluted to 15ml with ultrapure water according to previously described procedures and conditions (Minervino et al., 2018; Rey-Crespo et al., 2013).

2.2 Trace element analysis

Subsamples of approximately 1 g were precisely weighed and digested in a mixture of 5 ml of concentrated nitric acid (TMA, Hiperpure, PanReac, Spain) and 3 ml of hydrogen peroxide at 30% w/v (PanReac, Spain) in microwaves in an assisted digestion system (Ethos Plus; Milestone, Sorisole, Italy). The digested samples were transferred to polypropylene sample tubes and diluted to 15 ml with ultrapure water. The concentrations of the essential trace elements cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) were determined by inductively coupled plasma mass spectrometry (ICP-MS; VG PQ Excel, Thermo Elemental, USA). Analytical quality control was applied throughout the study. The blank values were processed together with the samples, and the obtained values were subtracted from the sample readings before calculating the final results. The limits of detection (LOD) were calculated as three times the standard deviation of the reagent blanks and were based on the mean weight of the analyzed sample.

2.3 Human health risk assessment of heavy metals in liver

To assess the risk to human health of the consumption of contaminated bovine tissue, different methods were used, for review see (Chien et al., 2002; USEPA, 2000; Varol & Sünbül, 2018; Yi et al., 2017). To determine the risk of essential metals, the calculations were made following the data used in this study: the average adult body weight was 70 kg, the average daily consumption of liver in Brazil was 26 kg per year 71 g/day (AgroConab, 2023), that is, 71 g/person/day. The following indices were calculated according to their respective equations:

2.4.1 Estimated daily intake (EDI)

$$EDI = \frac{CxCons}{Bw}$$

(1)

Where C is the concentration of essential metals in the tissue (mg/kg fresh weight), Cons is the average daily liver intake in the region and the national daily intake rate (71 g/day Bw) and Bw represents the body weight of the adult (70 kg).

2.4.2 Determination of the hazard quotient (HQ)

$$HQ = \frac{EDI}{RfDo}$$

(2)

Where RfDo is the reference oral dose for a given element (Cr = 0.003; Co = 0.0003; Cu = 0.04; Fe = 0.7; Mn = 0.14; Mo = 0.005; Ni = 0.02; Se = 0.005; Zn = 0.3) (Lima et al., 2022; Nędzarek et al., 2022).

2.4.4 Determination of the Target Hazard Quotient (THQ)

$$THQ = \frac{EFr\ x\ EDtot\ x\ FIR\ x\ C}{RfDo\ x\ Bw\ x\ ATn} x 10^{-3}$$

(3)

Where; EFr is the frequency of exposure (350 days/year); EDtot is the exposure time (70 years); FIR is the ratio of dietary intake (g/day), while 10-3 is the unit conversion factor; C is the concentration of essential metals in bovine tissues (mg/kg fresh weight); RfDo is the reference oral dose for a given element (see equation 2 mg/kg person/day); *Bw is the average adult body weight (70 kg); and ATn is the mean time of exposure for non-carcinogens (365 days/year × number of years of exposure, assuming 70 years).*

2.4.5 Determination of the Total Target Hazard Quotient (TTHQ)

In this study, the total THQ is calculated to estimate the additive effects of exposure to all metals accumulated in cattle tissues (Chien et al., 2002):

THQ total (TTHQ) = THQ (Cr) + THQ (Co) + THQ (Cu) + THQ (Fe) + THQ (Mn) + THQ (Mo) + THQ (Ni) + THQ (Se) + THQ (Zn) (5)

3. Results

3.1 Concentrations of essential elements

The concentrations of essential elements in beef liver from the municipalities of Western Pará are shown in Table 1. According to the results, the concentration range of toxic elements expressed in mg/kg dry weight was Cr: 0.020-0.794; Co: 0.005-0.933; Cu: 3600-952.448; Faith: 129.078-2900.144; Mn: 0.612-18.321; Mo: 0.086-4816; Ni: 0.001-0.068; Se: 0.123-2582; Zn: 85.546-421.606. There are no reference values for maximum levels of essential elements in liver (MRL) established by the Brazilian government

Table 1. Concentrations of essential elements (expressed in mg/kg dry weight) in liver of cattle from Western Pará, Lower Amazon

Metal	Mean/SEM (Range)					
	Itaituba	Monte Alegre	Oriximiná	Placas	Rurópolis	Uruará
Cr	0.075±0.018 (0.028-0.191)	0.212±0.085 (0.026-0.724)	0.128±0.025 (0.046-0.287)	0.170±0.045 (0.058-0.567)	0.070±0.018 (0.024-0.267)	0.093±0.046 (0.020-0.496)
Co	0.295±0.044 (0.013-0.483)	0.230±0.078 (0.037-0.833)	0.198±0.043 (0.005-0.453)	0.274±0.030 (0.161-0.439)	0.251±0.025 (0.118-0.403)	0.210±0.030 0.087-0.326
Cu	391.662±84.142 (16.170-875.792)	153.711±35.661 (27.114-378.859)	148.091±46.165 (3.600-455.924)	348.733±63.328 (4.539-594.952)	373.095±63.155 (74.921-952.448)	361.84±68.93 7.461-791.926
Fe	265.261±32.226 (145.282-398.778)	432.809±54.374 (215.502-646.656)	740.672±249.439 (129.078-2900.144)	378.795±132.294 (161.698-1637.053)	249.287±18.329 (146.617-403.179)	337.27±76.52 (163.9-977.8)
Mn	10.038±0.964 (3.078-13.487)	9.643±0.679 (7.866-14.381)	9.556±1.114 (0.612-13.284)	10.355±0.799 (8.083-16.475)	8.877±0.483 (5.975-12.501)	9.142±1.086 (6.50-18.32)
Mo	3.286±0.354 (0.294-4.413)	3.050±0.271 (2.079-4.816)	2.398±0.419 (0.086-4.042)	3.248±0.134 (2.629-4.209)	3.205±0.112 (2.627-3.828)	3.248±0.144 (2.535-3.737)
Ni	0.041±0.005 (0.016-0.068)	0.020±0.002 (0.013-0.027)	0.025±0.004 (0.009-0.053)	0.026±0.005 (0.012-0.061)	0.015±0.005 (0.004-0.067)	0.012±0.005 (0.001-0.050)
Se	1.521±0.182 (0.123-2.245)	0.347±0.091 (0.160-1.016)	1.202±0.095 (0.577-1.473)	1.568±0.184 (0.590-2.582)	1.388±0.125 (0.690-2.013)	1.267±0.108 (0.608-1.800)
Zn	149.679±11.479 (105.452-221.598)	149.995±17.824 (86.546-243.807)	203.930±28.133 (128.489-421.606)	173.209±10.538 (124.435-218.403)	161.228±15.813 (112.883-289.123)	152.97±10.69 (115.6-242.4)

3.3 Principal Component Analysis (PCA) of the Distribution of Essential Metals in the Liver

In the ordering diagram, it is observed that the samples were not grouped, i.e., there was no spatial stratification in the distribution of these minerals collected in the liver. The first axis is a gradient of Cr, Fe, Co Cu Se, Mo and Zn and these minerals were a gradient that explains the variability of the data 27.28%. O The second axis explains 17.02% forming a gradient of Ni and Mn. The samples of Fe and Cr show higher concentrations in the municipalities of Monte Alegre and Oriximiná, while the municipalities of Placas, Uruará and Rurópolis have higher concentrations of Zn, Cu, Se and Mo and Itaituba higher concentrations of Mo, Se, Co, Ni and Mn the samples collected in Monte Alegre show a high similarity in the concentrations of Fe and Cr.

Figure 2 – Ordering Diagram. Concentration of essential metals in the liver of cattle

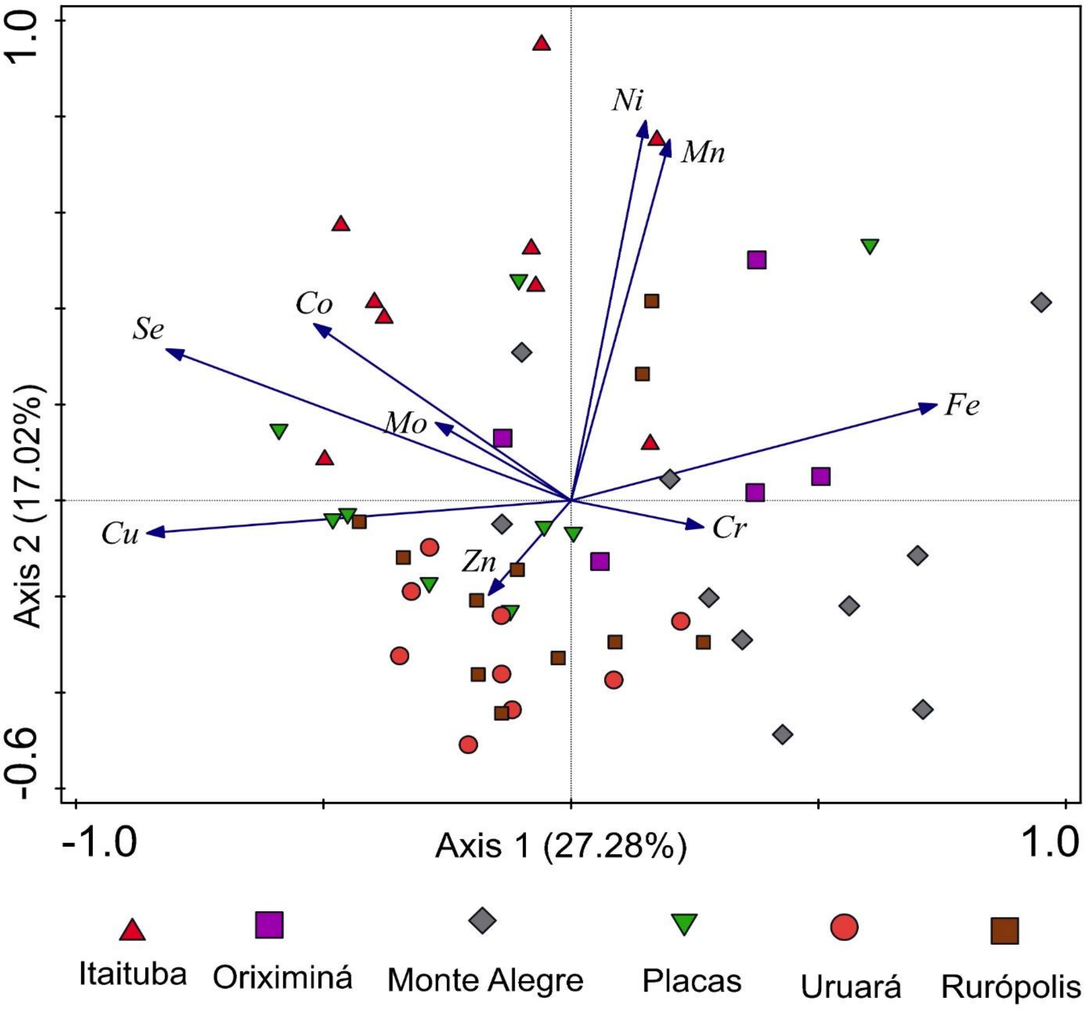

Table 2- Loadings da PCA of the concentration of essential metals in the liver of cattle. In bold are the variables that have a significant contribution

	PC 1	PC 2
Cr	**0.29984**	0.1547
Co	0.26251	**0.54142**
Cu	**0.47391**	0.099169
Fe	**0.43547**	0.19259
Mn	0.30315	**0.44299**
Mo	0.12257	0.22675
Ni	**0.37648**	**0.49375**
Se	**0.42214**	**0.37744**
Zn	0.009605	0.045847

3.4 Human exposure and health risk assessment through beef consumption

3.1.1 Estimated daily intake (EDI)

In this study, the results of the EDI calculation presented in Tables 3 represent the daily intake of essential metals through the daily consumption (71 g per person) of bovine liver. In general. When evaluating the EDI for liver tissue, it was found that Cu values were above RfDo in all municipalities. Values above RfDo were also found for the elements Fe, Se and Zn in the Municipality of Oriximiná.

Table 3. Estimated Daily Intake (EDI) of Essential Metals in Liver

Citie	Metal								
	Cr	Co	Cu	Fe	Mn	Mo	Ni	Se	Zn
Itaituba	0,00008	0,0003	0,40	0,27	0,01	0,003	0,00004	0,002	0,2
Monte Alegre	0,0002	0,0002	0,1559	0,4390	0,0098	0,0031	0,0000	0,0004	0,1521
Oriximiná	0,0001	0,0002	0,1502	0,7513	0,0097	0,0024	0,0000	0,244	0,689
Placas	0,0002	0,0003	0,3537	0,3842	0,0105	0,0033	0,0000	0,0016	0,1757
Rurópolis	0,0001	0,0003	0,3784	0,2528	0,0090	0,0033	0,0000	0,0014	0,1635
Uruará	0,0001	0,0002	0,3670	0,3421	0,0093	0,0033	0,0000	0,0013	0,1552

3.1.2 Determination of the hazard quotient (HQ)

Table 4. Risk quotient (HQ) for essential elements evaluated in liver of cattle for consumption in the West of Pará State.

The results of the HQ (Table 4) for essential elements evaluated in liver for consumption in the West of the State of Pará indicated that Cu presented a value greater than 1, which makes possible the adverse effects of the ingestion of this element in all the municipalities evaluated.

Citie	Metal								
	Cr	Co	Cu	Fe	Mn	Mo	Ni	Se	Zn
Itaituba	0,03	0,997	9,93	0,384	0,078	0,667	0,002	0,309	0,506
Monte Alegre	0,07	0,78	3,90	0,63	0,07	0,62	0,001	0,07	0,51
Oriximiná	0,043	0,669	3,755	1,073	0,069	0,486	0,001	0,244	0,689
Placas	0,06	0,93	8,84	0,55	0,08	0,66	0,00	0,32	0,59
Rurópolis	0,024	0,849	9,461	0,361	0,064	0,650	0,001	0,282	0,545
Uruará	0,032	0,710	9,175	0,489	0,066	0,665	0,001	0,257	0,517

*3.1.3 Determination of target hazard quotient (THQ)*Table 5. Target risk coefficient (THQ) in bovine liver (expressed in mg/kg dry wet) for the scenario of the West of the state of Pará.

Citie	Metal								
	Cr	Co	Cu	Fe	Mn	Mo	Ni	Se	Zn
Itaituba	0,03	1,00	1	0,38	0,08	0,67	0,002	0,31	0,51
Monte Alegre	0,07	0,78	3,9	0,63	0,07	0,62	0,001	0,07	0,51
Oriximiná	0,04	0,67	3,76	1,073	0,07	0,49	0,001	0,24	0,69
Placas	0,06	0,93	8,84	0,55	0,08	0,66	0,00	0,32	0,59
Rurópolis	0,02	0,85	9,46	0,36	0,06	0,65	0,00	0,28	0,55
Uruará	0,03	0,71	9,18	0,49	0,07	0,67	0,00	0,26	0,52

3.1.4 Determination of total target hazard quotient (TTHQ)

The results of TTHQ in the liver are shown in the table12. The city of Itaituba had the highest concentrations of TTHQ.

Table 12 - Total target risk quotient (TTHQ) in bovine liver (expressed in mg/kg dry wet) for the scenario of the West of the state of Pará.

Origem	TTHQ
Itaituba	12,90
Monte Alegre	6,64
Oriximiná	7,03

Placas	12,014
Rurópolis	11,912
Uruará	12,236

4. Discussion

Brazilian legislation does not establish recommended maximum limits for the concentration of essential elements in cattle liver tissue. In this way, our evaluations are restricted to the analysis of risks arising from the averages obtained. When verifying the daily intake (EDI) of essential metals of bovine liver, it is possible to perceive values above those established for the element Cu, in all the municipalities studied, in addition to Se and Zn in the municipality of Oriximiná. Taking into account that EDI is totally influenced by the mean concentration of metals in the tissues studied, we consider two suggestions that justify the increase of the variable studied.

For the increase in Cu EDI, the liver is considered to be the tissue with the highest concentrations of the element, where it binds to proteins synthesized in this organ such as albumin and transcuprin (Faia et al., 2017). Excessive consumption of copper is a rare condition, and its accumulation in the body may be due to disorders in its metabolism, such as Wilson's disease or hepatolenticular degeneration, an alteration in the metabolism of copper characterized by its progressive intracellular accumulation in tissues, especially the liver and brain (Limongi, 2013).

Concentrations of Se and Zn have been correlated by some authors as parameters for observing environmental pollution, for example, Licata et al. (2004) verified the concentration of these metals as an indication of contamination in cow milk. The concentrations of Zn and Se found in the present study are higher than those found by Lopes-Alonso et al. (2007) in pig liver in Galicia in northwestern Spain. Oriximiná is a municipality recognized for the exploitation of bauxite, where between 1979 and 1989 it suffered from the direct release of tailings from the exploitation of this ore in its hydrographic basin.

We do not intend to state that there is a direct correlation between the history of contamination and the levels of Se and Zn, after all, the behavior of these metals in water cycles is not fully understood (Albuquerque et al., 2021), the main route through which these elements could reach animal production in this region. However, taking into account that the low levels of these elements in the studies by Licata et al. (2004) and Lopes-Alonso et al (2007) occur in areas with low levels of pollution, our data deserve special attention since they are found in a region with a history of environmental contamination.

In our reviews, we did not find a pathological type associated with increased Zn intake, but its deficiency is closely related to dibetes mellitus, since the element is essential for insulin synthesis (Ripa and Ripa, 1995). New data have been accumulated in the scientific literature in recent years that allow a more adequate risk assessment of selenium with reference to human health, and populations with average selenium intake of less than 13–19 µg/day appear to be at risk of a severe cardiomyopathy, Keshan disease (Vicent et al., 2017).

When we evaluated the target risk coefficient (HQ), we noticed that Cu presented values above 1 in all the municipalities evaluated. This behavior was reflected in the total risk coefficients (THQ), which evaluates ingestion over a period of 70 years. We must take into account that, although our results point to a risk of consumption for the element copper, it is important to emphasize that this element undergoes metabolization that keeps it in homeostatic balance in the body, in addition to performing three essential functions within metalloproteins such as providing structural support, serving as an enzymatic cofactor and becoming an intermediate in the transport of electrons (Wang et al., 2020; Jomova et al., 2022). In this way, the risks of ingestion can be mitigated as a result of metabolization in a healthy organism.

The TTHQ pointed to the risk of consumption in all municipalities evaluated in the present study. As previously mentioned, essential metals undergo organic metabolization, however, our data are important because they occur in areas subject to extensive industrial exploitation, and we point to the importance of studies that correlate such activities with the real increase of essential metals in tissues consumed by the local population. We also highlight that our studies were carried out using hepatic tissue, the tissue that accumulates the most metals in the body due to its synthesis and metabolization function, which, despite not being one of the main food sources in the Amazon region, already highlights the importance of monitoring essential metals in the locality.

Conclusion

The daily intake of bovine liver tissue calculations were higher than those established for the copper element in all municipalities evaluated. The EDI of Se and Zn remained above the values when the hepatic tissues from Oriximiná were evaluated**.** The HQ regarding the ingestion of hepatic tissue revealed a risk regarding the adverse effects of Cu in all the municipalities evaluated. The total target risk quotient (TTHQ) revealed that all municipalities, were exposed to the risks of excessive consumption of these minerals, indicating that the degree of accumulation of essential elements in cattle in the Amazon region presents a significant risk for the consumption of the local population.

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2. FINAL THOUGHTS

In view of the coexistence between mining and cattle ranching activities in the western region of the state of Pará, it is important to continuously verify the correlation between the potential harmful effects of mining activities through biomonitoring on central species in the diet of the region's population. The present thesis demonstrated that results from biomonitoring can be reliably performed in equipment such as the DMA-80, which is affordable to use and cost compared to the ICPM-S, which represents the gold standard for the evaluation of toxic metal concentrations. We also proved that there is a percentage risk of heavy metal contamination in the consumption of beef, especially in the regions that are subject to mining activities and we warn of the risks of consumption of trace elements, accumulated in beef, although essential to human health, may offer risk factors when accumulated above the concentrations predicted in the tissues evaluated.

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PROGRAMA DE PÓS-GRADUAÇÃO EM SOCIEDADE, NATUREZA E DESENVOLVIMENTO

ATA DE DEFESA DE TESE

Aos 29 dias do mês de fevereiro do ano de 2024, às 14 horas, na sala 447 do BMT, instalou-se a banca examinadora de tese de doutorado do discente **Osvaldo Gato Nunes Neto** do PPGSND/UFOPA. A banca examinadora foi composta pelo Dr Antonio Humberto Hamad Minervino, PPGSND/UFOPA, orientador e presidente da banca, e pelos professores Dra. Adriana Caroprezo Morini, UFMS (examinador externo), Dr. Gustavo da Silva Claudiano, UFOPA (examinador externo ao programa), Dr. Waldiney Pires Moraes, PPGSND/UFOPA (examinador interno) e Dr. Luis Reginaldo Ribeiro Rodrigues, PPGSND/UFOPA (examinador interno). Deu-se início à abertura dos trabalhos, por parte do professor Antonio Humberto Hamad Minervino, que, após apresentar os membros da banca examinadora e esclarecer a tramitação da defesa, solicitou à candidata que iniciasse a apresentação da tese, intitulada "BIOMONITORAMENTO AMBIENTAL DE METAIS TÓXICOS E ESSENCIAIS EM ECOSSISTEMAS AMAZÔNICOS UTILIZANDO TECIDOS ANIMAIS", informando ao discente que possui de 30 a 60 minutos para a apresentação. Concluída a exposição, o Prof. Antonio Humberto Hamad Minervino, presidente da banca, passou a palavra à examinadora externa, Adriana Caroprezo Morini, para arguir o candidato, e, em seguida, aos professores examinadores Gustavo da Silva Claudiano; Luis Reginaldo Ribeiro Rodrigues e Waldiney Pires Moraes, para que fizessem o mesmo, nesta ordem. Após o término das arguições o orientador solicitou que o público se retira-se da sala para que ocorresse a reunião exclusiva entre os membros da banca para o julgamento do trabalho. Após a deliberações da banca examinadora, o orientador convocou o candidato e o público ouvinte de volta para divulgação do resultado. A banca examinadora decidiu por unanimidade pela **aprovação** do candidato, conforme as normas vigentes na Universidade Federal do Oeste do Pará. A versão final da tese deverá ser entregue ao programa, no prazo de trinta dias após a defesa, contendo as modificações sugeridas pela banca examinadora. Nada mais havendo por constar, lavrou-se e fez-se a leitura da presente ata que segue assinada pelos membros da Banca Examinadora, Presidente da Banca e Doutorando. Santarém (PA), vigésimo nono dia do mês de fevereiro do ano de dois mil e vinte e quatro, às dezoito horas.

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Chemosphere 351 (2024) 141146

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Comparative analysis between mercury levels in fish tissues evaluated using direct mercury analyzer and inductively plasma-coupled mass spectrometer

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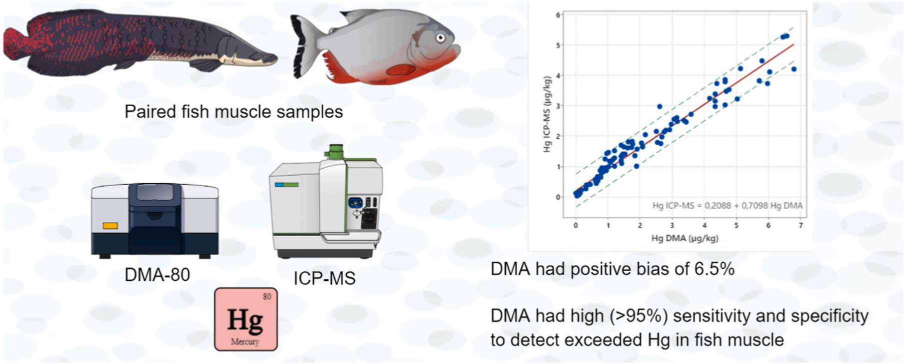
HIGHLIGHTS

- Anthropogenic activities may increase Hg contamination in aquatic ecosystems.
- We compared a Direct Mercury Analyzer (DMA) and ICP-MS for [Hg] in paired fish muscle.
- DMA had a positive bias of 6.5% against ICP-MS.
- Hg concentration were highly correlated ($r = 0.979$).
- DMA had high (>95%) sensitivity and specificity to detect contaminated fish samples.

GRAPHICAL ABSTRACT



DMA had positive bias of 6.5%

DMA had high (>95%) sensitivity and specificity to detect exceeded Hg in fish muscle

ARTICLE INFO

Handling Editor: Dr Patryk Oleszczuk

Keywords:
Mercury
Fish
DMA-80

ABSTRACT

Recent ecotoxicological studies have indicated mercury (Hg) contamination in aquatic ecosystems in the Amazon Basin. Although Hg contamination can be associated with small-scale gold mining, the soils of the Amazon region have naturally high Hg concentrations, and can be transported to aquatic ecosystems via deforestation and mining activities. Biomagnification of Hg can pose risks to the local human population; therefore, its concentration in fish tissues must be monitored consistently. Fast and sensitive Hg determination is required for

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https://doi.org/10.1016/j.chemosphere.2024.141146
Received 25 March 2023; Received in revised form 18 November 2023; Accepted 6 January 2024

ICP-MS
Amazon
Environmental contamination

continuously monitoring ecosystems impacted by mineral exploration. The direct mercury analyzer (DMA-80) is widely used for determining total Hg levels in tissue samples; it is fast and cost-effective, without requiring sample preparation. Here, we determined the sensitivity and specificity of Hg detection accomplished using DMA-80, and whether these results are reliable compared to those obtained using Inductively Coupled Plasma Mass Spectrometer (ICP-MS), which is the gold standard. We obtained 106 paired dried samples of muscle tissue from fish species occupying different trophic levels in the Lower Amazon region, and analyzed them using both equipment (DMA-80 and ICP-MS). The results obtained using DMA-80 had an overall Hg mean of 1.90 ± 0.18 mg/kg which was higher ($p < 0.05$) than the mean of those obtained using ICP-MS (1.55 ± 0.13 mg/kg). Linear regression analysis comparing the Hg levels obtained using both devices was within the 95% prediction interval, and a high coefficient of correlation showed agreement between the devices ($r = 0.979$; 0.069 to 0.986, 95% CI). Bland-Altman analysis showed that DMA-80 had a positive bias of 6.5% in relation to ICP-MS, which is more evident in samples with high Hg concentrations. DMA-80 was efficient in determining whether the Hg levels exceeded the maximum allowed levels required by the European Union, USA, and Brazil, showing a specificity and sensitivity of above 95%.

1. Introduction

Mercury (Hg) is a metal capable of associating with most living organisms that constitute the biota of aquatic systems, from phytoplankton to invertebrates and vertebrates. Among these organisms, fishes are especially relevant because they can transport energy from the lower to upper levels of the trophic web; fishes generally occupy the highest levels of the trophic chain and are considered important for assessing the degree of exposure of an environment to toxic elements. Furthermore, as they constitute one of the main sources of animal protein for humans, they are relevant when studying the environmental impact of Hg (Ansel, 2021; Castro-Rendón et al., 2022; Emenike et al., 2022; Buck et al., 2019; UNEP, 2019).

Mercury is a silvery-white color metal, stable at liquid form and highly toxic to the environment and living beings. This element can occur in elemental, inorganic and organic forms. Exposure to inorganic Hg can occur through professional occupation, and exposure to inorganic Hg occurs mainly through ingestion of food and water. Depending on its form and characteristics, Hg concentrations can vary in the ecosystem, as well as its accumulation, toxicity and biological effects on the human body. The main source of mercury exposure for humans is food. The forms of Hg most found in foods, although at very low levels, are the Hg^{2+} cation and organic methylmercury (CH_3Hg), with fish meat being the one with the highest concentrations. It is important to highlight that CH_3Hg is the form of mercury of greatest concern, as it easily crosses the blood-brain and placental barriers and has an extremely high absorption efficiency (>90%) when ingested in food. This explains, in part, the greater relative risk associated with exposure to CH_3Hg compared to inorganic Hg species (Nava et al., 2023; Di Bella et al., 2017, 2018, 2020).

Mercury contamination of fish and seafood is a global concern and various forms of investigation of these contaminants in aquatic environments are necessary to protect consumers (Nava et al., 2023). Organic and inorganic pollutants are ubiquitous elements in aquatic environments due to stream transport, surface runoff and atmospheric deposition (Di Bella et al., 2020). Many different human activities on land, water and air contribute to the contamination of water bodies, sediment and, consequently, aquatic organisms. To estimate organic and inorganic contamination in the aquatic environment, different types of organisms can be used as environmental biomarkers (Arias et al., 2007). Seaweed and filter-feeding mollusks can be used to assess heavy metals; bivalve mollusks particularly have high tolerance to pollutants, are widely distributed, and have a long life cycle, which allows a high capacity to accumulate heavy metals in several orders of magnitude, which can be representative of the pollution of an area (Di Bella et al., 2018). Furthermore, top predators, such as tuna and pirarucu, are well suited for environmental pollution studies dues its position in the food chain (Di Bella et al., 2017).

Recent ecotoxicological studies have indicated different levels of Hg contamination in aquatic ecosystems in the Amazon basin. Mercury is the main environmental pollutant naturally present in high concentrations in soils in the region (Albuquerque et al., 2021). Anthropogenic activities, such as deforestation, especially of riparian forests, and the installation of reservoirs for energy generation, caused increased Hg contamination of aquatic ecosystems from the Amazonian soils (Albuquerque et al., 2020). Illegal artisanal and small-scale gold mining is an important source of Hg contamination, especially with the changes in technological base of gold extraction to increase production scale (Bandeira Junior and Carvalho, 2023; Karagas et al., 2012; Gonzalez et al., 2013; Gonzalez et al., 2019); this technique causes deforestation and heavy metal deposition in the environment (Lobo et al., 2016).

The presence of high concentrations of heavy metals in animal tissues is an important public health concern. Due to its environmental persistence and bioaccumulative properties, Hg is considered highly toxic to humans, ecosystems, and wildlife (Behrooz and Poma, 2021). While low levels of toxic metals are tolerable, higher levels of these metals can accumulate in humans via biomagnification; beyond certain concentrations, they can cause severe toxicity. This is of particular importance in the Amazon region, owing to high concentrations of Hg in the ecosystem. Traditional riverine communities regularly consume large quantities of fish, which can be the main protein source in their diets (Albuquerque et al., 2020; Bourdineaud et al., 2015; Lima et al., 2000; Oliveira et al., 2010). In addition to the environmental and human health impacts, the consumption of fish contaminated with Hg also has economic consequences, being necessary cost-effective methods to guarantee food security (Trasande et al., 2016).

The comparability, reliability, and traceability of chemical measurements is increasingly being recognized and demanded, given their importance in terms of both research robustness and obtaining superior quality analytical data. Unreliable data can lead to catastrophic errors, and consequently, substantial financial losses (Ribani et al., 2004).

Thus, ecotoxicological monitoring studies are fundamental in determining toxic elements (Voegborlo et al., 1999) and verifying the concentrations of substances used and generated in socioeconomic activities that will be disposed into water, soil, air, and biota (Olivero-Verbel et al., 2015, 2016). In aquatic environments, Hg and other heavy metals can be distributed in soil, plants, animals, and sediments (Costa et al., 2008; Shepis et al., 2016).

To obtain analytical data for Hg, some basic requirements must be fulfilled, such as appropriate sample collection, pretreatment for analysis, selection of the measurement method, and appropriate preparation method for test samples. Moreover, when conducting Hg analysis, precautions to prevent sample contamination must be enforced (Suzuki et al., 2004).

Different types of spectroscopy are typically used to quantify mineral elements in biological samples, such as Atomic Absorption Spectrometry, Optical Plasma Emission Spectrometry, and Inductively Coupled Plasma Mass Spectrometry (ICP-MS), which provide the best results in terms of analytical capacity (De Muynck et al., 2008). While spectrometers can determine the concentration of different mineral elements, specific equipment can quantify Hg in different types of samples, for example the Thermal Decomposition Amalgamation Atomic Absorption Spectroscopy (TDA-AAS), performed here by a direct mercury analyzer (model DMA-80, Milestone Srl, Sorisole, Italy). The TDA-AAS allows the analysis of samples of any matrix without prior preparation, for example, acid digestion (Gomes et al., 2013; Luna et al., 2019). The method is specific for determining total Hg concentrations in liquid and solid samples using the principles of thermal decomposition, amalgamation, and atomic absorption at 254 nm. Using three cells with different optical paths, it expands the working range, allowing a higher detection limit of up to 0.01 ng of Hg; it can be applied in diverse areas, such as petrochemical analysis, environmental analysis, and food analysis (USEPA, 2007).

The ICP-MS technique is costly and time-consuming, which is a general feature of methods that involve the slow and dangerous process of sample digestion, typically involving acids. Another important factor specifically related to Hg is its high volatility, and the digestion of samples for Hg determination in open systems is not indicated, requiring the use of a digester, such as a microwave (Tseng et al., 1997). A more efficient and cost-effective analytical method that excludes the sample digestion phase and presents reliable results is necessary for environmental monitoring programs and research in the Amazon region. Thus, we evaluated the direct mercury analyzer (DMA-80) for determining total Hg in muscle tissue samples from different species of fish, aiming to determine whether the results obtained are reliable compared to those obtained using the gold standard method of ICP-MS.

2. Material and methods

2.1. Samples

A total of 106 paired dried samples of muscle tissue were collected from four different fish species of different trophic levels and feeding habitats from the Amazon region. Table 1 shows the fish species included in sampling.

Muscle samples were obtained from fishes from the Lower Amazon region, following a previously described methodology (Albuquerque et al., 2021). The fish were packed in hermetically sealed plastic bags and immediately transported to the laboratory at 4 °C. Samples were obtained from the same dorsolateral muscle cut from a single fish specimen collected directly after capture. After freezing the samples, the tissues were dried via lyophilization (benchtop equipment, model L-101, Liobras, São Carlos, São Paulo, Brazil). The material was placed inside the drying chamber of the lyophilizer until completely dry, usually for 24 h. From the already lyophilized tissue, the samples were homogenized, and two equal subaliquots were taken which were subjected to two different Hg measurement techniques.

The samples were carefully chosen to include a wide range of Hg concentrations from very low to high, which can be observed in some fish species in the Amazon region.

2.2. Analyses using ICP-MS

The sub-samples were sent to the Laboratory of Mineral Analysis, Research Infrastructures Area of the University of Santiago Compostela-USC/Spain for ICP-MS analysis, working under an ISO9001 quality management system. Approximately 1.0 g of sample was subjected to acid digestion using a mixture of 5 ml of concentrated Nitric Acid (Suprapur grade, Merck, Barcelona, Spain) and 3 ml of 30% w/v Hydrogen Peroxide (Merck) in a microwave system (Ethos; Milestone, Sorisole, Italy) with 1000 W of power according to a four-steps digestion ramp: 1 (3 min, 90 °C); 2 (15 min, 165 °C); 3 (3 min, 180 °C); 4 (10 min, 180 °C), followed by a cooling period. The digested samples were transferred to polypropylene test tubes and diluted to a final volume of 25 ml with ultrapure water, and an aliquot was then taken for ICP-MS analysis (ICP-MS/MISS; VGElemental Plasma Quad Option, Model 7700).

Table 1
Fish species from the Amazon that were included in the paired muscle sampling.

Name	Scientific name	Feeding habit	N
Acari	*Pterygoplichthys pardalis* Castelnau, 1855	Detritivores	32
Piranha	*Pygocentrus nattereri* Kner, 1958	Omnivores	17
Caparari	*Pseudoplatystoma fasciatum* Linnaeus, 1766	Carnivores	19
Tucunaré	*Cichla ocellaris* Bloch and Schneider, 1801	Carnivores	38

2.3. ICP-MS quality control

Analytical quality control was used throughout this study. Blank and test samples were processed simultaneously, and the values obtained were subtracted from the sample readings to calculate the final values. The limits of detection were calculated as thrice the standard deviation of the reagent blanks, and were based on the average weight of the sample. In all cases, the LDs obtained were sufficiently low to determine Hg levels in the samples examined. The precision of the determination was evaluated by comparison with the analytical recovery of certified reference materials (DORM-3 National Research Council Fish Protein, Ottawa, Ontario, Canada). We observed good agreement between the analyzed values and certified reference material of fish protein for Hg (DORM-3) obtained from the National Research Council of Canada, with reference values of 0.382 ± 0.060 mg/kg, a detection limit of 0.001 (mg/kg), and a determined level of 0.348 ± 0.021 (mg/kg). Calibration curves can be accessed in the Supplement material 1.

2.4. Analyses using DMA-80

Approximately 0.1 g of sample was subjected to analysis using DMA-80. The analyses were performed at the Experimental Biology and Bioprospection Laboratory of the Federal University of Western Pará (LabBio/UFOPA). For Hg analysis, duplicate samples were weighed directly into Hg analyzer sample cells. The samples were heated at 850 °C for 180 s to decompose the Hg under a stream of clean air which was collected in a gold amalgam tube. For releasing Hg in the UV photodetector for measurement at 253.65 nm, the amalgamated tube was subsequently heated to 155 °C (Jebara et al., 2021).

In this technique, the samples were subjected to the heating process, starting a heating ramp up to 850 °C in a quartz or nickel container, called nacelle, using compressed air as oxidizing gas. Hg vapors were directed to a catalyst containing a gold amalgam that selectively retains Hg, following which the products of combustion are selectively removed. After rapid heating, the amalgamation cell released Hg vapor, and the oxygen flow transports it to an absorption cell. The Hg content was quantified by determining the absorption at 253.7 nm (USEPA, 2007).

2.5. DMA-80 quality control

To ensure the reliability of the results, the certified reference material BCR-463 Tuna Fish (SIGMA) was used, with reference values of 2.85 ± 0.16 mg/kg. We obtained a value of 2.80 ± 0.07 upon analyzing the reference material, with a detection limit of 0.01 ng.

2.6. Statistical analysis

A mean comparison analysis was performed using a Paired Equivalence test and regression analysis. Subsequently, the data were subjected to Bland-Altman analysis to assess the agreement between the two quantitative methods. Considering the maximum allowed limits stipulated by the Brazilian (1 mg/kg), European and (0.5 mg/kg) and EUA (0.3 mg/kg) agencies for Hg concentration in fish tissue (EFSA, 2004; ANVISA, 2013; USEPA, 2001), the quantitative data were converted to nominal data classified as positive/negative depending on if the detected concentrations were above or below the allowed limit. This analysis was performed to evaluate the specificity and sensitivity of the direct Hg analyzer compared to the ICP-MS, which remains the gold standard for Hg detection.

3. Results

Table 2 shows the values obtained using the two devices; the average value of Hg concentration obtained using the DMA-80 (1.90 mg/kg ± 0.18 SEM) was higher than that obtained using ICP-MS (1.55 ± 0.13 mg/kg), showing a difference when analyzed using the paired *t*-test ($p > 0.05$).

The linear regression analysis between the Hg concentrations obtained using the two devices (Fig. 1) was mostly within the 95% prediction interval. The high coefficient of determination (95.8%) reinforces the agreement between the values obtained using the devices.

Fig. 2 shows the Bland-Altman analysis of the difference between the two tested methods for Hg (DMA-80 and ICP-MS) divided by their average concentrations. Bland-Altman analysis resulted in a positive bias of 6.48% (±27.3% SD; −47.13 to 60.10 95% CI) for DMA-80 when compared to the results obtained using the ICP-MS method. The results showed that in fish with higher Hg concentrations, DMA-80 had higher Hg concentrations, increasing the difference between methods as the Hg concentration increased in the samples.

Table 3 shows the results of the specificity, sensitivity, and agreement analyses of both methods considering ICP-MS as the gold standard. For this analysis, we used two different conditions: legally permitted limits of Hg in fish tissue adopted by the European Union (0.5 mg/kg) (EFSA, 2004) or the Brazilian government (1.0 mg/kg) (ANVISA, 2013) and the maximum recommended methylmercury in fish by the United States (US) Environmental Protection Agency (EPA) (0.3 ppm) (USEPA, 2001) A sample was considered positive (for Hg contamination) or negative depending on whether it presented a concentration above or below the legally acceptable limit, respectively.

The results from this analysis show that for all the maximum recommended limits for Hg contamination, DMA-80 efficiently detected the contaminated samples and presented high specificity and sensitivity when compared to ICP-MS, which is the gold standard for Hg detection.

4. Discussion

As was demonstrated, the mean value of the Hg concentration obtained using DMA-80 was higher than that obtained using ICP-MS, presenting a statistical difference using the paired *t*-test ($p < 0.05$). We suggest two possible explanations for the lower mean values obtained using ICP-MS.

The first is related to the premise that ICP-MS is an analytical technique based on the separation of ionic species by the mass/charge ratio that specifically evaluates Hg specimens. Therefore, samples with unknown specimens of Hg that are not certified for ICP-MS detection can show values of Hg that are lower than the levels actually present. This event was cited in a study carried out by Sannac et al. (2012), in which the inorganic Hg specimens in samples were not recorded because they did not present certification, assuming that only certified samples, such as Hg^{2+} and $MeHg^{+}$, would be present. DMA-80, however, does not feature speciation of the sample and performs the reading of total Hg. Thus, such differences in Hg concentration results may have been due to the varied concentrations of different Hg species in the samples.

A second possibility suggested that the difference in Hg concentrations is related to the known loss of analytes that can occur during the digestion step that precedes ICP-MS analysis. Hg and its compounds are extremely volatile. They can be lost during sampling, storage, preparation, or analysis even if the samples are subjected to relatively low temperatures (Kasper et al., 2015; Micaronida et al., 2000).

Considering the results from the certified reference material used in the equipment, the ICP-MS results showed a reference concentration that was 8.9% lower than the expected value. DMA-80 results also showed a lower Hg concentration than the expected reference value, but the difference was much lower than that obtained for ICP-MS. DMA-80 showed a 1.8% decrease in the Hg concentration of the certified reference material in comparison with the expected Hg values. Thus, the recovery of Hg in the CRM by ICP-MS was 91%, and that by DMA-80 was 98%, which may be related to Hg loss by volatilization or the measurement of only certified Hg specimens by ICP-MS.

Even with the difference observed between the concentrations resulting from ICP-MS and DMA-80, the linear regression analysis of the Hg obtained using the two devices resulted in a high coefficient of determination (95.8%), which reinforces the agreement between the devices. The agreement between results is important because even though the difference in analysis methods can potentially influence the obtained results, such differences are not sufficient to conclusively establish the superiority of one method over another. The correlation coefficient ($r = 0.979$) also revealed a very strong correlation between the results obtained using the two methods. The regression equation Hg ICP-MS = 0.2088 + 0.7098 Hg DMA is described here and can be used to convert the results obtained using DMA-80 to ICP-MS (Zhang and Zhou, 2020).

Few studies have compared ICP-MS with other methods for measuring Hg concentrations. Silva et al. (2021) compared the total Hg values obtained via ICP-MS and Atomic Fluorescence Spectrometry Coupled to Cold Vapor Aeration in the liver of marine tetrapods. The results obtained using the two methods were not significantly different; however, when comparing fresh or freeze-dried samples, a difference in Hg content was noted due to Hg loss during lyophilization.

Bussan et al. (2015) coupled DMA and ICP-MS systems, demonstrating several advantages over DMA alone for Hg determination, including increased sensitivity, lower detection limits, reduced potential for sample contamination, and applicability to stable Hg isotope marker studies. To the best of our knowledge this is the first study that compare both devices.

We also created a chart based on the Bland-Altman Analysis to check the agreement between the Hg analysis methods and found a positive bias towards DMA-80, although with a high confidence interval. Samples with a lower Hg content most often produced very similar results for both analytical methods. The differences responsible for the elevated SD were sourced from the samples with high Hg content.

Countries advocate a maximum detection limit for Hg in marketed foods to protect their populations from the deleterious effects of ingesting this metal. For example, values of 1.0 mg/kg and 0.5 mg/kg have been established as the maximum tolerable concentration of Hg by Brazil and the European Union, respectively, and the maximum recommended CH_3Hg in fish by the EPA (0.3 mg/kg) (ANVISA, 2013., EFSA, 2004; USEPA, 2001). DMA-80 is often characterized by not

Table 2
Mercury concentration in dried fish tissue analyzed using direct mercury analyzer (DMA-80) or inductive plasma-coupled mass spectrometer (ICP-MS).

Methods	N	M	SEM	SD	Var	CV	Min	Med	Max	Amp
DMA-80 (mg/kg)	106	1.90[a]	0.18	1.82	3.23	95.96	0.015	1.31	6.78	6.77
ICP-MS (mg/kg)	106	1.55[b]	0.13	1.32	1.75	84.88	0.047	1.32	5.30	5.25

Different letters indicate significant differences by paired *t*-test ($p < 0.05$). N, number of samples; M, mean; SEM: Standard Error of the Mean; SD: Standard Deviation; Var: Variance; CV: Coefficient of Variation; Min minimum value; Med, median; Max: Maximum value; Amp: Amplitude.

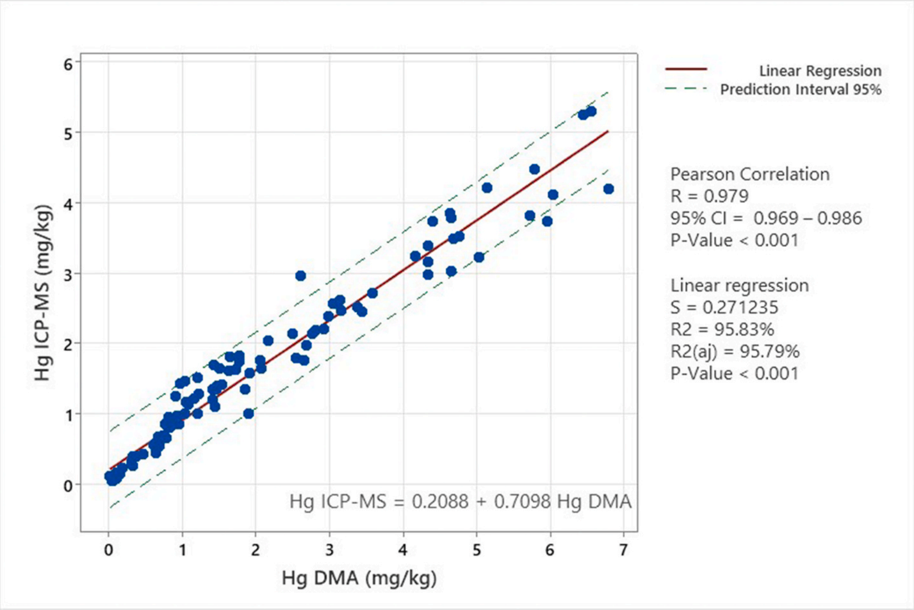

Fig. 1. Linear regression analysis between the Hg concentration determined using the two devices. Hg DMA: Hg concentrations obtained using Direct Mercury Analyzer (DMA-80); Hg ICP-MS: Hg concentrations obtained using Inductively Coupled Plasma Mass Spectrometry (ICP-MS); R: Correlation coefficient; R2: Determination coefficient; R2(aj.): Adjusted R2; Cl: confidence interval.

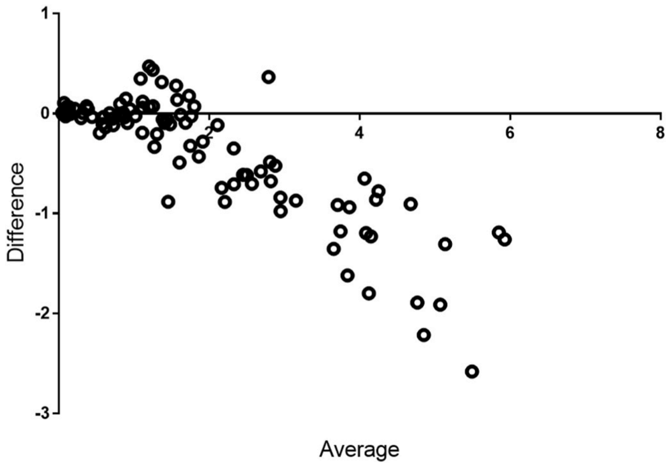

Fig. 2. Bland-Altman Analysis Graph that shows agreement between the two Mercury (Hg) analysis methods (difference of Hg concentration measured using ICP-MS minus Hg concentration measured using DMA-80 divided by the mean value of results obtained using both methods).

Table 3
Specificity, sensitivity, and concordance between the two Hg detection methods performed on fish tissue.

Europe legal Hg limit in fish (0.5 mg/kg)[a]			
	ICP/MS +	ICP/MS -	Total
DMA +	78	1	79
DMA -	0	27	27
Total	78	28	106
Sensitivity (95% CI)	1.0 (0.95–1.0)		
Specificity (95% CI)	0.96 (0.82–1.0)		
Positive Predictive Value (95% CI)	0.99 (0.93–1.0)		
Negative Predictive Value (95% CI)	1.0 (0.87–1.0)		
Brazilian legal Hg limit in fish (1.0 mg/kg)[b]			
	ICP/MS +	ICP/MS −	Total
DMA +	61	0	61
DMA −	2	43	45
Total	63	43	106
Sensitivity (95% CI)	0.97 (0.89–1.0)		
Specificity (95% CI)	1.0 (0.92–1.0)		
Positive Predictive Value (95% CI)	1.0 (0.94–1.0)		
Negative Predictive Value (95% CI)	0.96 (0.85–0.99)		
USA recommended maximum CH_3Hg in fish (0.3 mg/kg)[c]			
	ICP/MS +	ICP/MS −	Total
DMA +	83	1	84
DMA −	0	22	22
Total	83	23	106
Sensitivity (95% CI)	1.0 (0.95–1.0)		
Specificity (95% CI)	0.95 (0.78–0.99)		
Positive Predictive Value (95% CI)	0.98 (0.93–1.0)		
Negative Predictive Value (95% CI)	1.0 (0.84–1.0)		

[a] EFSA, 2004.
[b] ANVISA, 2013.
[c] USEPA, 2001. CH_3Hg: Methylmercury. CI: confidence interval.

having a low detection limit for Hg when compared to ICP-MS; however, via the specificity, sensitivity, and concordance analyses conducted in this study, we demonstrated that DMA-80 was efficient in determining the legal maximum values required by European countries and Brazil, attaining a specificity and sensitivity above 95%. With these specificity and sensitivity tests, we suggest that DMA-80 is a safe, accessible, and cost-effective alternative to ICP-MS, lacking time-consuming and costly steps such as acid digestion.

In addition to DMA-80 analysis (Prazeres et al., 2018) and ICP-MS (Albuquerque et al., 2020), several other analytical methods have been used in the Amazon region to determine Hg in fish tissue samples, including Cold Vapor Atomic Absorption Spectrometry (Filho et al., 1999)), Cold Vapor Atomic Fluorescence Spectrometry (Hacon et al., 2020), and Graphite Furnace Atomic Absorption Spectrometry (de Queiroz et al., 2018).

Most studies have used single-element methods. However, such techniques are expensive, time-consuming, and require pre-treatments such as acid digestion (Han et al., 2019; Rey-Crespo et al., 2013). DMA allows analysis in liquid and solid matrices without the need for prior sample preparation (such as acid digestion), facilitating its application in routine tests (Nortje, 2008; Torres et al., 2012). It is quick, sensitive, and cost-effective (Han et al., 2019), employing the principles of thermal decomposition, amalgamation, and atomic absorption. The system provides a much faster analysis with minimal amount of sample and excellent Hg recovery, and is more cost-effective compared to atomic absorption and mass spectrometry techniques (Carbonell et al., 2009; Han et al., 2019; Ribeiro and Germano, 2015; Torres et al., 2012). Therefore, this method can be particularly useful for government agencies (for monitoring purposes), especially in regions with a history of environmental deterioration wherein active mineral exploration is still being conducted.

5. Conclusions

Hg concentration in fish tissue obtained using the direct Hg analyzer were highly correlated with those obtained using ICP-MS with high (>95%) sensitivity and specificity to detect fish samples in which the Hg concentration exceeded the maximum allowed concentration according to European, USA, and Brazilian legislation. The direct mercury analyzer had a positive bias of 6.5% against ICP-MS, which was more evident in samples with high Hg concentrations.

CRediT authorship contribution statement

Osvaldo Gato Nunes Neto: Formal analysis, Investigation, Methodology, Writing – original draft. **Salatiel Ribeiro Dias:** Formal analysis, Investigation, Methodology, Resources. **Fábio Edir Amaral Albuquerque:** Investigation, Methodology, Resources, Software, Writing – original draft. **Marta Miranda:** Conceptualization, Data curation, Formal analysis, Supervision, Writing – review & editing. **Marta Lopez-Alonso:** Conceptualization, Formal analysis, Supervision, Writing – review & editing. **Ricardo Bezerra Oliveira:** Data curation, Formal analysis, Methodology, Resources, Validation. **Diana Pinto:** Data curation, Resources, Software, Validation, Visualization. **Antonio Humberto Hamad Minervino:** Conceptualization, Funding acquisition, Project administration, Supervision, Validation, Writing – review & editing.

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.

Data availability

We will publish the data as a data paper

Acknowledgements

This research was supported by Coordenação de Aperfeicoamento de Pessoal de Nível Superior (CAPES). FEA Alburquerque is grateful to Fundação Amazônia de Amparo a Estudos e Pesquisas (FAPESPA) for his Post-Doc fellowship. AHH Minervino is grateful to CNPq for his research productivity fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141146.

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