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CHARLES DOS SANTOS BARROS

# **BIOMARCADORES DE ESTRESSE OXIDATIVO EM** *Plagioscion squamosissimus* **E** *Macrobrachium amazonicum,* **DA AMAZÔNIA ORIENTAL, BRASIL**

MACAPÁ - AP

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências Ambientais (PPGCA) da Universidade Federal do Amapá, como requisito parcial à obtenção do título de Mestre em Ciências Ambientais.

Orientador: Dr. Gabriel Araujo da Silva

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Examinador 3 - Dr. Daímio Chaves Brito – Universidade Estadual do Amapá (UEAP)

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**SUMÁRIO**

## 1 INTRODUÇÃO GERAL

Ação antrópica é uns todos principais causadores de efeitos deletérios em um ecossistema, um exemplo disso foi com a exploração de minério (manganês) no município da Serra do Navio em 1957 pela Indústria e Comércio de Minérios S/A (ICOMI) na qual transportava o mesmo até a área portuária do município de Santana, a literatura descreve que foi a primeira experiência de exploração de minério industrial na Amazônia, a mesma provocou diversas expectativas na área social e econômica (MONTEIRO, 2003).

No entanto, por meio do processo de pelotização e com o objetivo comercial, o arsênio que anteriormente era insolúvel na forma de hidróxidos de ferro e de manganês, tornou-se solúvel em condições ambientais. Os rejeitos desse processo foram depositados em uma barragem artificial localizada no município de Santana, formada por escavações que alcançaram o nível freático. Neste contexto, iniciou-se um problema socioambiental, principalmente após a identificação de concentração elevada de arsênio nos igarapés do Elesbão I e II (LIMA et al., 2007). Tais concentrações também foram descritas por meio de relatórios técnicos desenvolvidos pela empresa Anglo American, caracterizando-se a comunidade do Elesbão como uma área contaminada.

Segundo dados do IBGE (2010) a população de Santana é composta por 101.262 habitantes, sendo 2.600 moradores da comunidade do Elesbão, cujas as principais atividades econômicas são: coleta de açaí, produção de tijolos (olaria) e pesca. Na região predomina-se o clima quente e úmido com duas estações bem definidas (estações mais chuvosas e menos chuvosa) durante todo o ano. A estação mais chuvosa inicia-se fins de dezembro até agosto, e a estação menos chuvosa de setembro a dezembro (LIMA et al., 2007).

Diversos estudos já foram desenvolvidos na referida área, no entanto, existem divergências entre os resultados. Tais divergências foram produtos na geração do manuscrito intitulado "**Evaluation of arsenic contamination from mining exploration in the eastern amazon"** submetido a revista Ecotoxicology and Environmental Safety.

Assim, visando uma resposta para a população local, utilizamos bioindicadores de contaminação ambiental, com o propósito de avaliarmos se área em questão encontra-se impactada. Para tal, desenvolvemos estudos na área da ecotoxicologia aquática, na qual avaliamos biomarcadores de estresse oxidativo em amostras de peixes e camarões. Este

trabalho justifica-se pelo fato de poucos estudos utilizarem biomarcadores e bioindicadores com espécimes de peixes e camarões na região amazônica, além das referidas amostras serem utilizadas para alimentação pela população local.

A problemática que norteia esse estudo é qual das espécies *Plagioscion squamosissimus* ou *Macrobrachium amazonicum* é mais sensível as mudanças do meio aquático, oriundas de duas áreas, sendo a primeira caracterizada pela presença da atividade humana e o despejo do esgoto não tratado (igarapé 01) e a segunda (igarapé 02) pela região onde havia menos atividade humana perto do rio ou de seus efluentes, localizada no rio Amazonas no município de Santana-AP. Como hipótese, levando em consideração o nível trófico dos camarões e sua biomassa, sugere-se que sejam mais sensíveis as mudanças do meio em relação aos peixes.

Neste cenário sabemos que vários parâmetros biológicos podem ser afetados por agentes químicos, no entanto, por meio da determinação quantitativa desses parâmetros podemos mitigar os impactos negativos ao ambiente. A utilização de biomarcadores serve como uma ferramenta no controle da saúde ambiental. Os organismos aquáticos geralmente são expostos a concentrações elevada de poluentes químicos. Assim, surgir-se a preocupação com o equilíbrio do ecossistema e consequentemente para identificar tal problema utiliza-se bioindicadores de contaminação ambiental.

Tais poluentes podem afetar o equilíbrio dinâmico, levando ao aumento do nível de espécies reativas de oxigênio (ERO), e ocasionando danos aos constituintes celulares, ou seja, estresse oxidativo (KEY; WIRTH; FULTON, 2006). A avaliação de biomarcadores aquáticos são essenciais para o equilíbrio do ecossistema, uma vez que, qualquer modificação no ambiente será facilmente identificada. A utilização de biomarcadores podem ter como finalidade a elucidação da causa-efeito e dose-efeito para fins de monitorização biológica e por conseguintes medidas de mitigação poderão ser tomadas. A presente dissertação tem como objetivo geral avaliar biomarcadores de estresse oxidativo: não enzimático (glutationa reduzida) e subproduto da peroxidação lipídica (substâncias reativas ao ácido tiobarbitúrico) em *Plagioscion squamosissimus* e *Macrobrachium amazonicum*, além de contaminantes inorgânicos presentes no musculo das amostras.

As mesmas foram coletadas pela equipe do Laboratório de Química Orgânica e Bioquímica da Universidade Estadual do Amapá (LabQOBioq - UEAP) e pescadores da região, para tal

utilizamos apetrechos de pesca, tais como: anzol, redes de emalhar, tarrafa, rede de arrasto (malha 20 mm) e armadilhas regionais denominadas “matapi”, em toda a extensão dos igarapés. Os espécimes foram acondicionados em sacos apropriados com aeração e transportados vivos até o LabQOBioq – UEAP, onde foram feitas as análises.

A presente dissertação foi estruturada em forma de agregados de artigos, sendo constituída com uma introdução geral. E, os artigos estão divididos das seguintes formas:

- Capítulo I: **YIELD AND CHARACTERIZATION OF THE CENTESIMAL COMPOSITION OF AMAZONIAN ESTUARINE FISH**. Nesse artigo avaliamos as amostras quantos aos aspectos nutricionais. Artigo aceito para publicação na revista Journal of Agricultural Science and Technology A & B USA.

- Capítulo II: **EVALUATION OF ARSENIC CONTAMINATION FROM MINING EXPLORATION IN THE EASTERN AMAZON.** Neste capítulo é feita uma revisão sobre a contaminação por arsênio no municipio de Santana. Submetido a revista Ecotoxicology and Environmental Safety.

- Capítulo III: ***Macrobrachium amazonicum* IS A GOOD BIOINDICATOR OF ENVIRONMENTAL CONTAMINATION IN AQUATIC ENVIRONMENTS?** Avaliamos os biomarcadores de estresse oxidativo e as concentrações dos contaminantes inorgânicos. Artigo submetido a revista PLOS ONE.

- Capítulo IV: **ESTRESSE OXIDATIVO EM *Plasgiossion squamossimus* PROVENIENTE DA AMAZÔNIA ORIENTAL.** Assim como no capítulo anterior, avaliamos os biomarcadores de estresse oxidativo e as concentrações dos contaminantes inorgânicos, mas tais variáveis são avaliadas no peixe. Submetido ao Novos cadernos – NEA.

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# 3 CAPÍTULO 01. YIELD AND CHARACTERIZATION OF THE CENTESIMAL COMPOSITION OF AMAZONIAN ESTUARINE FISH

*Artigo aceito para publicação no periódico "Journal of Agricultural Science and Technology A & B USA"*

**Yield and Characterization of the Centesimal Composition of Amazonian Estuarine Fish**

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## 3.1 Abstract

Fish, as one of the fishery resources, is an important constituent of the diet of the Amazon population, as it is the source of several nutritional components. The present work aimed to characterize the centesimal composition of *P. squamosissimus* fillet (n = 10) and *M. amazonicum* meat (n = 82), species acquired in the estuarine region of the state of Amapá, Brazil. Carcass yield, as well as protein, moisture, lipids and ashes were determined according to the methodologies proposed by the Adolf Lutz Institute, as well as carbohydrate and caloric determinations. The analyzes were performed in triplicate per sample. After comparing with the literature, it was possible to conclude that *P. squamosissimus* presented a fillet yield of 31,11 ± 0,61%, high protein content (15,99±1,26 g $100g^{-1}$) and humidity (79,40±1,10 g $100g^{-1}$), moderate contents of mineral residues (1,10 ± 0,07 g $100g^{-1}$) and carbohydrates (0,96 ± 0,90g $100g^{-1}$), low lipid contents (2,29 ± 0,65 g $100g^{-1}$), as well as low caloric values (384.760,64 J $100g^{-1}$) and *M. amazonicum* a meat yield of 44,12 ± 8,34%, high levels of (1,82 ± 1,61 g $100g^{-1}$) and mineral residues (1,76 ± 0,78 g $100g^{-1}$), moderate moisture contents (73,38 ± 0,78 g $100g^{-1}$), low lipid levels (0,43 ± 0,08 g $100g^{-1}$), as well as low caloric values

(440.491,52 J $100g^{-1}$). The results obtained in this work can serve as a subsidy in nutritional diets for humans, thus allowing an adequate dietary use of these species.

**Keywords:** Nutritional assessment; Bromatological variables; Nutritional value; Chemical parameters of food.

## 3.2 Introduction

Fishing is one of the oldest activities used by man to meet his food needs. Fish is an important constituent of the human diet because it represents a source of several nutritional components. Although variable, the centesimal composition of fish meat is very close to the composition of poultry, cattle and pigs [1, 2, 3].

*Plagioscion squamosissimus* is commonly known as white hake, corvina, hake and piauí hake. It is a marine species adapted in fresh water, that is distributed by rivers of the Guianas, by the Central Amazon, region of the Low Amazon, by the estuary of the Caeté River, Bay of Marajó, coast of Amapá, reentrances Maranhenses and rivers of the northeast region of Brazil. The individuals of this species feed on small fish, crustaceans (shrimp, copepods) and aquatic insects [4, 5, 6].

*Macrobrachium amazonicum* is commonly known as cinnamon shrimp, shrimp, calamari, and shrimp. It is endemic to South America and has a wide geographical distribution: Brazil, Bolivia, Paraguay and northern Argentina. This species inhabits environments with different levels of salinity. There is no known information about the eating and feeding habits of *M. amazonicum* in a natural environment [7, 8, 9, 10].

There are few data on the processing of fish, and related to the yield of fillet and meat of Amazonian aquatic species. In general, in literature it is common to find results for Nile tilapia, but the anatomy of each species and the slaughter weight directly influence its yield [11, 12, 13].

Knowledge about the chemical composition of foods is advantageous and necessary for the consumer to better meet the demand for macronutrients and other nutritional components, for influencing the increase of their acceptance and for providing competition with other sources of nutrients are of paramount importance both nutritionally and economically [11, 14].

Although data and nutritional tables on fishes, the use of this information on centesimal constituents should be made with caution, since the food is produced or captured in different regions and its values vary according to the intrinsic and extrinsic factors, such as age, sex, species, food, seasonality, part of the fish in which the sample was obtained and the place of capture [14]. The aim of this study was to evaluate the yield of fillet and meat, and the centesimal composition of *P. squamosissimus* and *M. amazonicum*, obtained in the estuarine region of the state of Amapá, Brazil.

### 3.3 Material and Methods

Samples of *P. squamosissimus* (n = 10) were obtained from the Bailique archipelago, and *M. amazonicum* (n = 82) samples were collected from the city of Santana. Both were donated by artisanal fishermen from the state of Amapá, Eastern Amazonia, Brazil.

The fish were previously washed, then weighed separately on a commercial scale to obtain the total weight. The removal of the fillet of *P. squamosissimus* was done using a method described in Souza [15] with some adjustments, where with the whole fish already eviscerated, the fillet was first removed with skin and then separated the skin of the fillet, with the aid of a knife, and at the end, the respective fillets without skin, of the heavy specimens again. For *M. amazonicum* specimens, they were first weighed to obtain the total weight and then removed the head and the carapace, obtaining the meat material, which afterwards were weighed again to obtain the weight of this. The quantification of the fillet and meat yield was made through the following mathematical calculation: % Yield of filet and meet$= \frac{WF}{WT} X\ 100$, at where WF = weight of fillets e WT = total weight.

The moisture determinations were made through gravimetry and according to analytical methods recommended by the Instituto Adolfo Lutz (IAL) [16], with adaptations. The porcelain crucibles were first dried for 1h in an oven at 105 °C, after cooling in a

desiccators with silica, these were weighed in analytical balance to obtain the initial weights. The analyzes were done in triplicates. A small amount with approximately 5 g of fillet sample was weighed into the respective crucibles. After 24 hours in a greenhouse, at 105 °C, the crucibles plus the samples were cooled in a desiccators for another 24 hours and weighed again. The quantification and determination of the humidity values were made through the weight loss ratio through water evaporation and the calculation described below: $\%M = \frac{(MCI+MSI)-MTAG}{MSI} x100$, at where %M = percentage of moisture, MCI = mass of the initial crucible, MSI = mass of the initial sample e MTAG = total mass after greenhouse.

The determination of mineral residues was done as described in the analytical methods recommended by IAL [16], with adaptations. With porcelain crucibles previously dried at 105 °C for 1h and cooled in a desiccators for 24h, they were weighed to verify initial weight, then weighed approximately 3 g of sample in analytical balance. After the 24h oven drying time, the crucibles were transferred to the samples for incineration in muffle at 560 °C for a period of 6h. The samples were cooled in the desiccators for 24 hours and weighed to verify the final weight and subsequent mathematical calculations, such as:$\%WM = \frac{(MTAM-MCI)}{MSI} x100$, at where %WM = percentage of mineral waste, MTAM = total mass after muffle, MCI = mass of the initial crucible e MSI = mass of the initial sample.

The total lipids were determined by continuous and direct extraction in a Soxhlet type apparatus, after drying the sample in an oven, as described by IAL [16], with modifications. A filter paper cartridge containing the sample was analyzed and the whole was weighed on an analytical balance. The extraction was done during 6h in Soxhlet extractor apparatus with hexane ($C_6H_{14}$) at approximately 65 °C. At the end of the extraction the cartridges were taken to the stove for drying for 1h and the cooling took place in a desiccators for 24h until it obtained a constant weight value. The determination of total lipids was done by the

calculation described below: $\%LT = \frac{(MTCI-MTCF)}{MSI} x100$, at where %LT = percentage of total lipids, MTCI = mass of the initial cartridge, MTCF = mass of the final cartridge e MSI = mass of the initial sample.

The determination of total proteins was done as described in the analytical methods proposed by IAL [16], with adaptations and in triplicates of samples, in which it was divided into three stages. The first consisted of the digestion of approximately 500 mg of the sample in Kjeldahl tubes in 7 mL of concentrated sulfuric acid ($H_2SO_4$) with 2,5 g of catalytic mixture (containing 7 g of potassium sulfate ($K_2SO_4$), 7 g of copper sulfate ($CuSO_4$) and 0,7 g of manganese dioxide ($MnO_2$)). Digestion occurred in a digester block (SL – 25/42, SOLAB, Piracicaba, São Paulo, Brazil) in which the tubes were coupled with the mixture to be digested. This process finally culminated in a blue colored liquid with an apparent precipitate at the bottom of the kjeldahl tube.

The second stage was carried out in a nitrogen distiller (SL – 74, SOLAB, Piracicaba, São Paulo, Brazil), where the samples were neutralized with 25 mL of 50% Sodium Hydroxide (NaOH), the ammonia released, condensed in a 4% boric acid ($H_3BO_3$) indicator solution, with three drops of mixed indicator contained in a 250 mL erlemeyer. The distillation was done until the turn of pink color to blue-green occurs and for a longer time so that it can be retained in the solution, all the nitrogen contained in the sample.

The final step consisted in titrating the distillate from the sample with $H_2SO_4$ (0,05M) in a 50 mL burette, until the turn from blue-green to pink. The quantification of the protein content was done through the calculations as: $\%N = \frac{(Va-Vb)xNxFx14x100}{(MD)}$, at where %N = percentage of nitrogen, Va = volume of the $H_2SO_4$ spent in the sample titration, Vb = volume of the $H_2SO_4$ spent on the titration of the blank, N = normality, F = correction factor of $H_2SO_4$, the number 14 = milligrams of nitrogen or 1 mEq of nitrogen and MD = mass to be digested. Continue: $\%PMD = \%NMDx6,25$, at where %PMD = percentage of protein in the

dry mass, %NMD = percentage of nitrogen in the dry mass e 6,25 = total nitrogen conversion factor in crude protein for fish. Finishing: $\%PT = \frac{((100 - \%M - \%LT)\%PMS)}{100}$, onde %PT = percentage of total proteins, %M= percentage of moisture, %LT = percentage of total lipids e %PMD = percentage of protein in the dry mass. It is worth mentioning that the normality of the concentrated $H_2SO_4$ and the correction factor (1,0 mL) was considered.

Carbohydrates were quantified by difference, at where %C = (100 - (%Moisture + %Waste Mineral + %Total lipids + %Total proteins)), as described by the Brazilian Table of Food Composition (TACO) [17] and the results expressed in g $100g^{-1}$. The caloric value was calculated as follows: VC = (%C x 4 + %LT x 9 + %PT x 4), where VC = caloric value, % C = percentage of carbohydrates, %LT = percentage of total lipids, %PT = percentage of total proteins and caloric coefficients of macronutrients (4, 9, and 4, respectively). Done as described by Watt and Merrill [18], and the results were expressed as J $100g^{-1}$.

### 3.4 Results and Discussion

The results obtained for the meat yield and the centesimal characterization of the analyzed species are presented in table 1, followed by means and the standard deviations for each parameter evaluated through the quantitative analyzes of the samples.

The average result of fillet yield for *P. squamosissimus* was made from specimens with a mean total weight of 200 g and for the meat yield of *M. amazonicum* with an average weight of 3 g. The result of this work is provided for every 100 grams of these fish.

The fillet and meat yield depends on the efficiency of the manual method applied by the worker, the anatomical shape of the body, the size of the head and weight of the viscera, fins and skin, the form of removal of skin from fish fillet and the time of slaughter for fish in general [19, 13, 20].

Souza [15], in its analyzes with Nile Tilapia (*Oreochromis niloticus*), one of the most popular fish raised and marketed in Brazil, obtained a yield of 34,63% for fillet without skin. The percentage of yield found in this study was slightly lower when compared to the analyzes made by Souza [15].

The determination and quantification of the moisture present in food is one of the most important measurements in the centesimal analysis, since the quality of the product and the adequate use of mechanisms in which it can be used in the process of food preservation are closely linked. Foods with high humidity tend to deteriorate more quickly than those with low humidity. Your percentage may vary by species, time of year, age, sex, and nutritional status [14, 21, 2].

According to Andrade *et al.* [14] and Spitz *et al.* [21], the fish muscle can contain on average 64% to 90% of humidity, being the results found in this work in agreement with the described in the literature.

The results obtained in this study for *P. squamosissimus* were described for the same species by Sales and Sales [22] with a value of 78,0 g $100^{-1}$, Sanchez *et al*. [23] with a result of 78,8 g $100^{-1}$ and by TACO [17] which presented a value of 79,2 g $100^{-1}$ of moisture content.

The results of moisture content for *M. amazonicum*, when compared with other literatures, were found to be below that reported by Portela *et al*. [24] and above the values reported by Furuya *et al*. [25]. This difference can be explained as follows: the first author used a methodology different from the one used by this study, since the second author used whole amazonicum specimens in his analyzes, as well as using another methodology to reach his results.

In general, calcium, phosphorus, sodium, potassium, arsenic, manganese, copper, lithium, cobalt, zinc, iron, selenium and iodine are among the essential elements of the human

diet. In addition, the fish is still a source of lipids, proteins and carbohydrates [26, 27, 28, 2, 29].

In fish we can find a wide variety of mineral residues, and according to TACO [17] and Viana *et al*. [2], the variations of minerals in fish oscillate between 1% and 2%, being in this way the results found by this study according to what the mentioned literature reports.

Lourenço *et al.* [30] in their analyzes carried out in the state of Pará, for the same species of fish, found values of 1,1 g $100g^{-1}$ of mineral residues in the in natura specimens, results identical to those found in this study. This can be explained because the fish come from the same region. Analyzing the ash content in *P. squamosissimus* from Brazilian northeastern reservoirs, Sales and Sales [22] found an average result of 1,5 g $100g^{-1}$, a result that was above the average of what was determined by this study. However, our results when compared to those found in TACO [17], which express values of 1,0 g $100g^{-1}$, are slightly above the values described by this literature.

Furuya *et al.* [25] using in its analyzes *M. amazonicum* from a dam at the Itaipu Plant in the Paraná River, municipality of Santa Helena – PR, Brazil, obtained a value for mineral residues of 1,5±0,1 g $100g^{-1}$. Already Portela *et al*. [24] after captive breeding of prawns of the same species, for a period of four months using a balanced diet, obtained an average value for mineral residues of 1,3±0,01 g $100g^{-1}$. The results of this work, using only the meat product (without cephalothorax and carapace) of *M. amazonicum* originated from a natural environment, are above mentioned by these two authors.

The amounts of lipids that can be found in fish meat range from 0,6% to 36%. The classification of fish on the fat content is based on the following relation: less than 2% of lipids, it is considered as a low fat fish; between 2% and 5%, is a moderate fish in fat content, and values higher than 5%, considered a fish with high fat content [31, 32]. According to the

results obtained in this study for *P. squamosissimus* and for *M. amazonicum*, the first species can be classified as a moderate fish in fat content and the second as low in fat content.

Aguiar [33] in his studies, for the same species of fish (*P. squamosissimus*) acquired in the Central Market of Manaus, in the state of Amazonas, obtained an average value of 1,3 g $100g^{-1}$, below what is described by the present study. Sanchez *et al.* [23] in their analyzes for the same species collected in the Barra Bonita reservoir, in the state of São Paulo, obtained an average value of 2,1 g $100g^{-1}$. Therefore, the values found by this study are greater than those cited by both authors.

The average values found by this work for *M. amazonicum* are below the values reported by Portela *et al.* [24] and Furuya *et al.* [25]. For Bragagnolo and Rodrigues-Amaya [34], the difference between the results is due to the fact that fat storage in shrimps occurs in the hepatopancreas, located in the cephalothorax and since the part analyzed in this study was only the meat part (without carapace and cephalothorax) the low values are therefore explained by this reason.

According to Ogawa and Maia [32], the difference between the results found in the literature compared to that found in this study is quite variable due to several factors such as sex, age, time of year in which it is captured, diet in which the species is submitted, type of body muscle analyzed, environment in which it is developed and part of the body in which the sample material is removed for analysis.

Usually lipids, is the second largest biochemical element, after protein, present in several fish species. The lipid composition varies among species and among the same species, according to some factors such as: environmental conditions, feeding, sex, size, reproductive cycle, diet, nutritional status, location and times of the year in which they develop [35, 28].

Proteins are one of the main reasons why fish and shrimp are eaten, taking into account their nutritional value. The criteria adopted in which justify the consumption of proteins is their importance as they are essential for life.

The fish muscle may contain approximately 12% to 25% protein and, in addition to high nutritional value and high digestibility, they also have good functional properties [35, 28, 36]. The values found in this analysis are in accordance with those reported in the literature.

According to Sartori and Amancio [28], fish are considered low value protein when presented values less than 15% and high protein value when presented with values above this percentage. Therefore, the species analyzed in this study present a protein with high protein value, mainly *M. amazonicum*.

In TACO [17] a value is expressed with 18,9% of proteins for *P. squamosissimus* and in the work of Aguiar [33] 19,4%, thus the data found by the analyzes described in this work are therefore in disagreement and below when compared to these last two literatures. For *M. amazonicum* when compared to the literature we found a result below that reported by Furuya *et al*. [25] for *M. amazonicum*. This differential can be explained, since the cited author used in his analyzes whole shrimp and this can be a factor that will influence the results of this variable.

Proteins exert several biological functions and metabolic processes essential for our survival, being also one of the main constituents of the cells, they are composed of chains of amino acids, some of them are not synthesized by our organism, meaning in this way that their supply must come of food. Animal products are good sources of protein, and the essential amino acids found in fish are considered complete and balanced. From the nutritional point of view, fish proteins are of high biological value and have the particularity of having an excellent digestibility [37, 28, 29, 38].

Carbohydrates present in fish, we can find glycogen and mucopolysaccharides, there are still free sugars and phosphosaccharides, this is found in low quantities. The fish muscle has a low caloric value when compared to other protein foods, influenced by the amount of lipids [32, 39, 28].

The carbohydrate content present in fish can vary from 0.3% to 1.0% on average, depending on the species contributing to the characteristic sweetish taste of this nutritional component. Glycogen is one of the polysaccharides belonging to the carbohydrate group, ie an energy reserve component for fish [32, 28, 38].

During the process of "rigor mortis", the fish tends to seek energy reserves for the biochemical reactions that result from their slaughter, where ATP decomposition initially occurs, and glycogen when used as an energy source in the form of ATP, is broken and their units are removed one by one, until their total exhaustion [40, 28].

Both Sales and Sales [22] and Lourenço *et al*. [30] do not mention carbohydrate values for *P. squamosissimus* in their studies. In TACO [17] and Aguiar [33] the results for *P. squamosissimus* were 0%, being therefore the values found in this present study in disagreement with the cited literature. Portela *et al.* [24] and Furuya *et al.* [25] with whole *M. amazonicum* do not mention the percentage of carbohydrates found in their analyzes.

This work presented caloric values close to those found by Sales and Sales [22] and described in TACO [17] for *P. squamosissimus*, as well as values below the results found by Aguiar [33] for *Plagioscion spp*. acquired in the state of Amazonas.

Furuya *et al*. [25] in their analyzes with whole *M. amazonicum* captured in the Paraná River, in Paraná, Brazil, obtained values of 483795,92 J $100g^{-1}$, so it can be said that the values of the cited literature are above the found by this work, because in their analyzes were used whole *M. amazonicum*, in which it directly influences the value of the collies since the

majority of fats present in the species are located in the cephalothorax region, according to Bragagnolo and Rodrigues-Amaya [34].

### 3.5 Conclusion

We can conclude that the skinless fillet of *Plagioscion squamosissimus* presented high levels of protein, moisture and carbohydrates, moderate contents of mineral residues, low lipid contents, as well as low caloric values and *M. amazonicum* without head and carapace, presented high levels of proteins and carbohydrates, moderate levels of mineral residues and humidity, low levels of lipids and calories. In spite of the great commercial consumption of the studied fish, this work presents the first centesimal determination of the meat material (without head and carapace) of *M. amazonicum* from the Amazonian estuarine region, an important producing center of this species.

Therefore, from a nutritional point of view, the analyzed species are considered to have good dietary patterns because: they have nutritional components that can be used in diets in which they require high protein content and low fat content; the species may further serve as a basis for nutritional diets of humans, allowing through the results found to be suitably used dietetically; and may also serve as a subsidy for subsequent researches that offer products elaborated from the analyzed species, thus allowing a greater insertion of the fish in daily life and in human food.

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Table 1. Yield of fillet of *Plagioscion squamosissimus* (n = 10) and meat of *Macrobrachium amazonicum* (n = 82), as well as characterization of the centesimal composition of these species, and whitefish (*P. squamosissimus*) from the Bailique archipelago, and the shrimp (*M. amazonicum*) from the municipality of Santana, in the metropolitan region of Macapá, in the state of Amapá, northern Brazil. Both were acquired through donations made by artisanal fishermen.

| EVALUATED PARAMETERS | *P. squamosissimus* | *M. amazonicum* |
|---|---|---|
| Yield (%) | 31,11±0,61 | 44,12±8,34 |
| Moisture (g 100g $^{-1}$) | 79,40±1,10 | 73,38±0,78 |
| Mineral wastes (g 100g $^{-1}$) | 01,10±0,07 | 01,76±0,78 |
| Total lipids (g 100g $^{-1}$) | 02,29±0,65 | 00,43±0,08 |
| Total Proteins (g 100g $^{-1}$) | 15,99±1,26 | 22,81±1,72 |
| Carbohydrates (g 100g $^{-1}$) | 00,96±0,90 | 01,92±1,61 |
| Calorific value (J 100g $^{-1}$) | 384.760,64±29.539,04 | 440.491,52±27.865,44 |

Results were expressed as mean ± standard deviation.

# 4 CAPÍTULO 02. EVALUATION OF ARSENIC CONTAMINATION FROM MINING EXPLORATION IN THE EASTERN AMAZON

*Artigo submetido ao periódico "Ecotoxicology and Environmental Safety"*

# EVALUATION OF ARSENIC CONTAMINATION FROM MINING EXPLORATION IN THE EASTERN AMAZON

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## 4.1 Abstract

Environmental exposure to Arsenic (As) has been increasing over time, either naturally or through anthropic action, this process causes concern in the population as to health risks. Thus, the present study aimed to list the scientific information regarding arsenic contamination in the city of Santana-AP, through a review of the literature in the Capes Periodic Portal, Scielo and PubMed database. Eight studies were identified and analyzed, the only ones being published in this region for the study of As concentration. After analyzing the results, the concentration of As in the water that the community of Elesbão consumes were within the established parameters of <10 μg.$L^{-1}$. However, the concentration in the soil (50 mg.$kg^{-1}$) and hair (1.0 mg.$kg^{-1}$) of this population were above the reference value. This is justified by the level of As total identified in fish and shrimp, causing a possible biomagnification process. Therefore, it is necessary to study all the possible variables of the route of contamination by As in the focus region of the present study, since the divergences of results raise doubts about the level of contamination. Thus, this review will serve as a prerequisite for the evaluation of publications from other regions that have undergone or suffer anthropogenic actions.

**Keywords:** Manganese; Arsenic; Metaloid; Bioaccumulation; environment**.**

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## 4.2 Introducion

The environmental exposure to As has raised the population's concern about the risks that this may pose to human health, especially after cases of mass contamination have been reported in West Bengal, Bangladesh and Mexico (1). Human studies have concluded that after long periods of exposure, high levels of this element can be identified in the skin, hair, nails and bones (2). As well as, As can cause neuropathy, cutaneous lesions, vascular lesions and cancer (3).

A review entitled "Arsenic - health: a relationship requiring vigilance" was developed in 2014 in which the authors discussed the importance of structuring a national health surveillance program taking into account the relative concentrations of As in food consumed in Brazil, However, this study did not attention to the contamination of As by anthropic action or environmental exposure (4).

However, in several countries countless researches are being developed to evaluate the relationship between As and the incidence of diseases in humans. Still, there are few studies in Brazil, concentrating on only four regions: the region of the iron quadrilateral (Minas Gerais) Ribeira Valley (Santa Catarina) (5), São Paulo (6) and Amapá (7). This is the last focus of the present study, justifying the lack of publications on the subject, besides belonging to the Amazon region. Thus, this work will serve as an alert to the possible negative impacts resulting from the exploitation of natural resources in Amazon, especially after the federal government stimulates the exploitation of this resource (8).

As is characterized by being a solid, crystalline, grayish metalloid with the chemical valences of $3^-$, 0, $3^+$ and $5^+$, the As varying their chemical form in water according to the pH and the redox potential of the medium (6). The characteristics of the Amazon River in the region of the study favored the chemical variations of the metalloid in question, since it is classified as a class II river according to resolution 357/05 (CONAMA, Brazilian legislation), presenting a pH range of 6.0 to 9.0, is also characterized as a white (muddy) river. (9) As also occurs naturally in rocks and is obtained as a byproduct of the treatment of copper, lead, cobalt, gold and manganese ores, that is, it is a ubiquitous element present naturally or by anthropic action in soil, water, air and food (7,8).

In the environment, it appears in four oxidation states: arsenide As(-III), elemental arsenic As(0), arsenite As(III) and arsenate As(V), the organic forms are represented in figure 01.

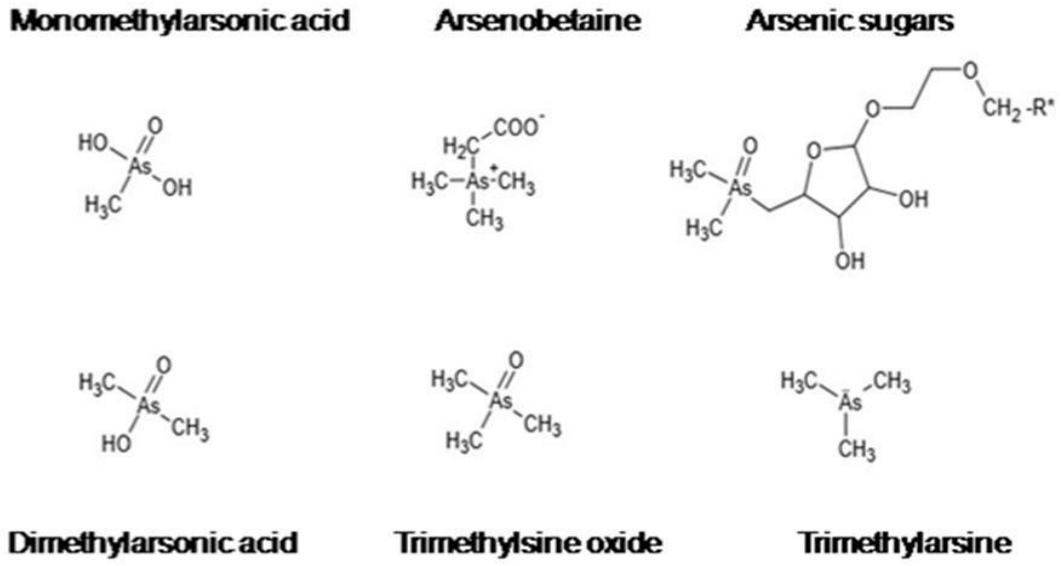



**Figure 01**. The chemical structure of organic compounds of the most common Arsenic.
**Source**: (Adaptado 9,10)

The acute and subacute toxicological effects caused by the inorganic As can be relate to various organs including gastrointestinal tract, skin, cardiovascular system and respiratory system. Inorganic $As^{3+}$ is methylated by the hepatocytes in most mammals, as $As^{5+}$ before being methylated is reduced in the blood, so approximately 70% of the As ingested is excreted in the urine, with a half-life of 10 to 30 hours (9). The pathologies resulting from As contamination are related to the direct consumption of thiols, in addition to the endogenous formation of reactive oxygen species (ROS), such as superoxide anion, hydrogen peroxide, hydroxyl radical, peroxyl radical and singlet oxygen, in which it affects equilibrium homeostatic, by means of the intracellular consumption of antioxidants, causing harmful effects on proteins, lipids, deoxyribonucleic acid and carbohydrates, thus establishing oxidative stress (1015).

Therefore, it is necessary to study all the possible variables of the route of contamination by As in the focus region of the present study, since the divergences of results raise doubts about the level of contamination. Thus, this review will serve as a prerequisite for the evaluation of publications from other regions that have undergone or suffer anthropogenic actions.

### 4.2.1 Arsenic (As) geochemical cycle

Among the anthropogenic sources of As, the mining activity comes first, as well as the metallurgical industry, in addition, have the burning of fossil fuels that is also a source of atmospheric contamination. In agriculture, As was the basis of some pesticides (16).

In this way, the As in the form of $AsO_4^{-3}$ in the presence of iron (Fe) undergoes precipitation in the form of $FeAsO_4$ that can oxidize to $AsO_4^{-3}$, which can undergo a reduction process, becoming $AsO_3^{-3}$ that in the presence of the $H_2S$ will occur the formation of $As_2S_3$, being able to oxidize forming again $AsO_4^{3}$. However, there is the possibility of methylation of $AsO_4^{-3}$ with the formation of monomethylarsenic acid (MMA), which will result in another methylation transforming into dimethylarsic acid (DMA) that will sediment in rocks (17,18) (Figure 02).

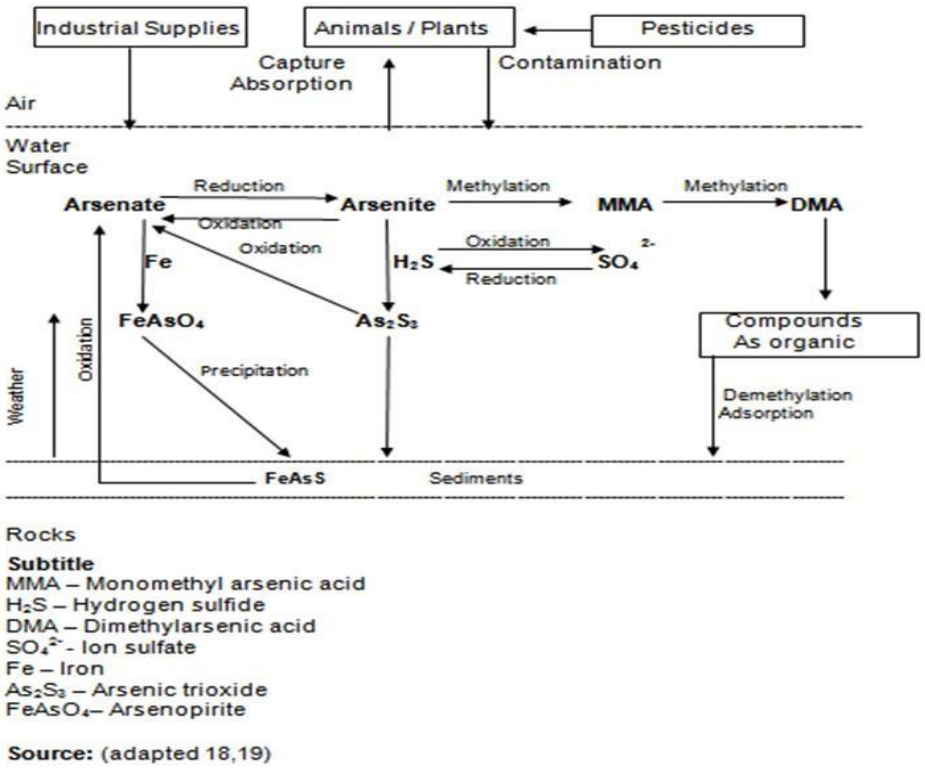



**Figure 02.** As geochemical cycle

The mechanism of pathogenicity of inorganic As has not been fully elucidated, but studies indicate that it involves the induction of DNA damage due to the formation of reactive oxygen species, such damages can produce genetic and epigenetic effects (20), the latter being corroborated by Argos et al. (2015), with evaluation of the association between As exposure (urine and blood sample) and DNA methylation of white blood cells along the epigenome, with a sample of 400 adult participants from rural communities in Bangladesh (21). The

mentioned study observed significant associations between exposure to As and DNA methylation, suggesting that epigenetic modifications may be a pathway to toxicity.

In vitro study with two hamster cell lines demonstrated that As is able to inhibit DNA replication and induce of sister chromatid exchanges, causing chromosomal aberrations (22). The same induced human carcinogenicity in prostate epithelial cells (23).

In this context, in 50 decade, through the exploitation of manganese in the municipality of Serra do Navio, located at latitude 0º55 north and longitude 52º05 west, on the margin of the Amapari river, situated in the state of Amapá, the company Indústria e Comércio de Minérios SA (ICOMI) was shed manganese for 192km on the railroad linking the municipalities of Serra do Navio and Santana (24, 25), during that period of exploration there was environmental contamination by As in the industrial/port area of ICOMI, mainly by the pelletization of As occurred in the municipality of Santana, by heating the manganese at high temperatures, with the objective of beneficiation of the manganese ore, eliminating the low content, consequently the As was released into the atmosphere, where it cooled and condensed with rain, so the substances were dragged into the water table (26).

This possible environmental damage became public in 1998 when the useful domain of the ICOMI area for company Amapá Florestal e Celulose (AMCEL) was transferred, in which an environmental audit was carried out by the company JakkoPoyry Engenharia LTDA, which, when evaluating the hydrogeochemical characteristics of the area identified points with anomalous contents for some metals (Manganese, Iron and Arsenic) (27). Thus, the present study had the objective of evaluating the arsenic contamination from the mining exploration in the Eastern Amazon, through literature review.

## 4.3 Material and methods

We used researches identified in the database of Portal Periodics Capes, Scielo and PudMed, using the descriptors: arsênio Amapá; Arsênio Santana; arsenic Amapá; arsenic Santana; arsenic environment, written in the English or Portuguese languages, published until May 2017. Articles, dissertations, theses and technical reports were included, whose object was to study arsenic in the city of Santana-AP. In total, 67 papers were identified. Considering the inclusion criteria, we analyzed 08 documents listed in table 01.

**Table 01**. Articles and / or dissertations, theses and technical reports used in the bibliographic review

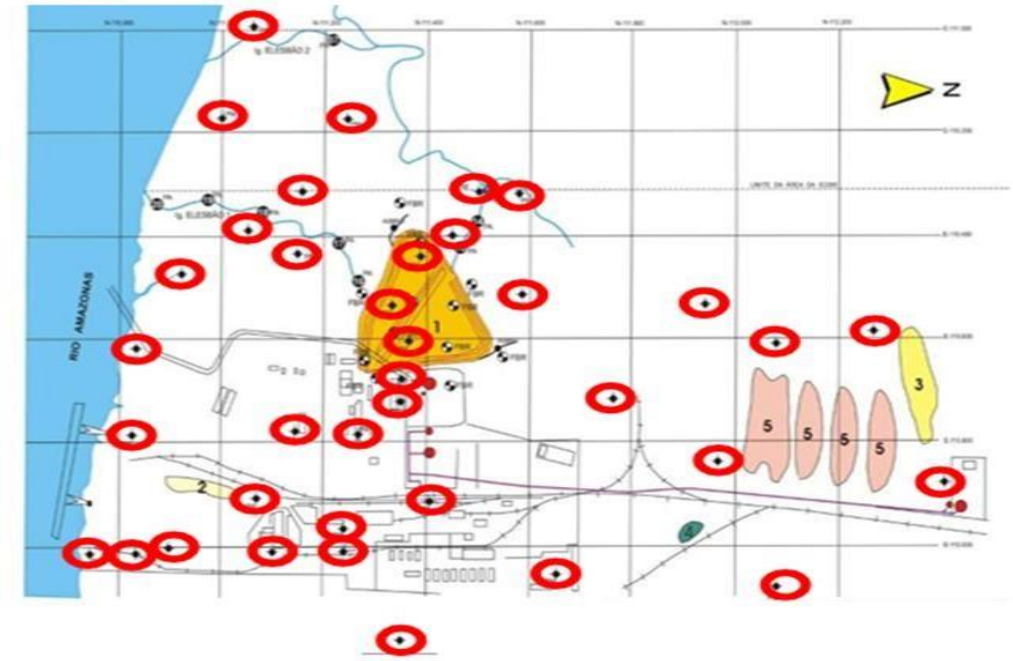
| Nº | Title | Year of Publication | Reference |
|---|---|---|---|
| **1** | ICOMI in Amapá: half a century of mineral exploration | 2003 | 28 |
| **2** | Exposure to mercury and arsenic in Amazonian states: a synthesis of Evandro Chagas Institute studies / FUNASA | 2003 | 29 |
| **3** | Evaluation of total arsenic, traits and bacteriological elements in drinking water in the community of Elesbão, Santana, Amapá state, Brazil | 2007 | 07 |
| **4** | Spectrophotometric determination of arsenic in the city of Santana-AP using the modified silver diethyldithiocarbamate (SDDC) method | 2009 | 30 |
| **5** | Arsenic in thehairoftheindividuals in SantanaAP-Brazil: significanceofresidencelocation | 2010 | 31 |
| **6** | Socioenvironmental damages resulting from improper handling of manganese waste and the implications for the life and health of residents of Vila do Elesbão | 2011 | 32 |
| **7** | Report on the monitoring of surface and sub surface waters | 2012 | 33 |
| **8** | Report on the monitoring of surface and sub surface waters | 2013 | 34 |

## 4.4 Results and discussion

### 4.4.1 Concentration of As in water in Santana (AP)

The study conducted by Santos et al. (2003) entitled "Exposure to mercury and arsenic in Amazonian states: a synthesis of Evandro Chagas/ FUNASA studies", analyzed 33 monitoring wells located in the port and industrial area of ICOMI (Fig. 03), these collection points were the same as those used by the company's water quality monitoring program, in

which the concentrations of As varied from <0.5 to 1970 µg/L, with a mean of 131.4 µg/L (29).




Source: Ampla Engineering (1997, apud 32)

**Figure 03.** Map of the collection points and the location of the old industrial and port facilities of ICOMI in Santana.

In the mentioned study the authors report that anomalous values, restricted only in the area of waste deposition (1976 µg/L, 1320 µg/L, 760 µg/L) were identified. However, when the anomalous values are not considered, the average concentration does not exceed 8.43 mg/L, according to the authors in question the value established by the legislation for drinking water is 10 mg/L. However, this value does not correspond to that described in Administrative Order No. 1469 of 2000 (referenced in the study) (35) and CONAMA No. 003 (2005) (36), both establish the maximum limit of 0.01mg/L or 10 $\mu g.L^{-1}$.

Therefore, the mean values identified (131.4 µg/L and 8.43 mg/L) are above the reference value. So, we assume that the concentrations were written incorrectly. This aggravating factor is worrying, since such a study has been referred to in legal decisions (37).

In the monitoring report of surface and groundwater developed by technical representatives of Anglo American (2012; 2013) (33,34) in the
Elesbão community, high levels of As, with values of 9.7 µg/L were found. This result is within the scope of the legislation. However, the collection points were not the same as those

of the study conducted by Santos et al. (2003) (29), so we cannot infer if the concentration of As in the area of deposition of the tailings decreased.

Figure 3 identifies in the red circles the ICOMI monitoring wells, in the orange color (1) tailings deposit developed by ICOMI, which identified the anomalous values for As concentration (29).

Lima et al., (2007) (7) developed a study on the evaluation of total arsenic of trace elements in drinking water in the community of Elesbão, Santana municipality, in three periods: May 2003 with 50 samples, presenting the average concentration of As 5.93 μg/L; November of 2003 with 52 samples, with an average concentration of As 1.95 μg/L and March of 2004 with 52 samples, with an average concentration of As 2.22 μg/L. Thus, the mean levels of arsenic found in this study are within the values considered normal (38). In this way, the mentioned study concludes that it is not possible to identify route of exposure from the ingestion of the water.

The study in question recognizes that the industrial and port area of ICOMI has negative impacts, making the risk of exposure to As imminent, but they attribute the results of the concentration of As, to regional and environmental factors, such as the possibility of dilution of metals in the Amazon River. However, this study does not question the possibility of adsorption in the sediments, thus, the possible contamination of benthic trophic levels, which may occur the bioaccumulation process or even biomagnification, since these factors were not evaluated.

### 4.4.2 Concentration of As in aquatic species

Biomagnification increases the concentration of a chemical species in living organisms as it accumulates at the highest trophic level. Thus, it is always necessary to evaluate different levels in the food chain.

In the above study, Santos et al. (2003) (29) collected fish samples (n=262) from species consumed in the community of Elesbão, and samples of shrimp from different localities of the community. Fish species were divided into two groups: group 1 (carnivorous) and group 2 (non carnivorous), group 1 content ranged from 12.1 to 156 mg/kg, and group 2 of 10.1 and 348.4 mg/kg. The shrimp samples presented levels ranging from 51.0 to 127.5 mg/kg. Even with the predominant presence of organic (less toxicity) in fish and shrimp, these results represent the concentration of As total, so we can not infer the difference in

concentration of organic and inorganic forms, the latter with reference values in fish and shrimp of 1.0 mg/kg (39,40).

About 90% of total As found in marine aquatic organisms are in the form of organic As and only 10% in inorganic form. In this way, considering that the speciation pattern of As in marine and freshwater fish and shrimps does not present variation (41), we can infer that the inorganic fractions of the results of Santos et al., the As content ranged from 1.21 to 15.6 mg/kg in carnivorous, and in the non-carnivorous group it varied from 1.01 to 34.84 mg/kg. The As levels in the shrimp samples ranged from 5.1 to 12.75 mg/kg. From the inorganic As fractions we can see that the results are above the established values. Thus, further studies are needed to assess the environmental condition of the area in question.

Dör et al. (2014) (28) describe that the high concentration of As in fish is a result of the process of bioaccumulation, that is, when water passes through the gills, the dissolved substances may have a greater affinity to the organism in question, substances tend to accumulate in the tissues. However, when bioaccumulation occurs indirectly, the phenomenon too will be called biomagnification, defined as the accumulation of a xenobiotic or its derivatives in the different trophic levels, that is, bioaccumulate in the fish, and in the human being through the feeding, occurring the process of biomagnification.

In the literature it is shown that most As-containing compounds, whether organic or inorganic, pentavalent or trivalent, end up interfering with cellular homeostasis, inhibiting the action of enzymes, as well as blocking cellular respiration (42). Santos et al., (2003) (29), did not differ As organic and inorganic forms.

### 4.4.3 Concentration of As in human blood and hair

Pereira et al., (2010) (31) evaluated the concentration of As in the hair of individuals from the city of Santana, dividing into two areas: urban (23 individuals) (Central, Nova Brasília and Vila Amazonas) and peripheral (98 individuals) (Vila do Elesbão and Vila Daniel). The analysis was carried out using an atomic absorption spectrophotometer, with samples from 121 donors. These groups presented specific socioeconomic characteristics and different habits. After analyzing the results, 52.17% of the individuals in the urban area and 88.87% in the peripheral region had concentrations of As superior at 1.00 $mg.kg^{-1}$, a level considered as a threshold (30). Although hair is metabolically dead in the epidermis, roots are highly influenced by the health status of living beings, therefore, their analysis is essential in monitoring occupational and environmental exposure to toxic elements (2).

In the Elesbão community, the average number of As in the blood of 1,927 people surveyed reached 5.95 mg/L. In the hair samples, the average of 1,986 individuals was 0.56 $mg.kg^{-1}$, and there was no significant difference values found (29).

After evaluating the data mentioned above, Santos et al. (2003) (29) and Pereira et al., (2010) (31) observed a divergence of results, both evaluating the concentration of As in hair of dwellers living longer 10 years in Santana, especially the residents of the neighborhood Elesbão (region near the contamination area of ICOMI). Thus, the problem that is evident is what the actual concentration of As in the hair samples of the population of the neighborhood of the Elesbão?

### 4.4.4 Concentration of As in the soil of the Municipality of Santana

Pereira et al. (2009) (30) determined by spectrophotometric analysis the concentration of As in the city of Santana (AP) using the modified silver diethyldithiocarbamate (SDDC) method, 19 samples were evaluated. The results showed that 94.74% (mean of As, 682.96 $mg.kg^{-1}$) of the samples had concentrations (50 $mg.kg^{-1}$) of As above the limit published by Casarini et al. (2001) (43) for residential soils evidencing soil contamination by As.

### 4.4.5 Socio-environmental aspects of exposure to As

Facundes (2011) (32) evaluated the socio-environmental damages resulting from the inadequate management of manganese waste and the implications for the life and health of the residents of Vila do Elesbão, who interviewed 33 residents and ex-residents, of whom seven were with visible signs of health problems that resemble some of the adverse effects caused by As-contamination, the most common signs are: severe itching of the body, prominent spots on the skin, back, face, hand and foot injuries.

Such signs, according to those interviewed in the aforementioned study, came from the involuntary exposure to As released with the manganese tailings at Vila do Elesbão by ICOMI. In addition, informants reported headaches, breathing problems, leg pains, stomach problems, kidney and liver problems, eye irritation, body wounds, urinary tract infection, among others. There is the possibility of latency of some days or even 30 years, so that As can produce cutaneous manifestations (44).

In this context, how to explain the low levels of concentration of As in the water consumed by the community and the high levels identified in fish, shrimp, soil and hair besides signs and symptoms of a possible contamination. Bioaccumulation and

biomagnification of As in environments are not well known (45). However, Barwick and Maher (2003) evaluating the biotransference of As in marine grasses in Australia have identified evidence of biomagnification (46). Benthic species are more likely to bioaccumulate, especially in muscle tissue (47).

The question of the concentration of As of this study area, is an environmental problem still unresolved, since the wastes of As with an average concentration of 1,877.7 μg/g, remain stored in Santana and therefore being leached to the surroundings of the industrial and port area of ICOMI (7,28).

### 4.5 Conclusions

The concentration values of As in water for consumption in the Elesbão community were within the established parameters, but in fish and shrimp the total As level was not differentiated (organic/inorganic). Thus, the high level of As in hairs of the Vila do Elesbão individuals is justified, since the majority of the inhabitants live in the fishery and possibly the phenomenon biomagnification between the trophic levels, expressed through the signals and diseases that are manifesting in some residents over time. Thus, further aquatic ecotoxicological studies, clinical and biochemical evaluation of the local population are required, as well as new studies of As concentration in water and soil. All studies will clarify and better understand the factors that affect the health of the exposed population.

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# 5 CAPÍTULO 03. *Macrobrachium amazonicum IS A GOOD BIOINDICATOR OF ENVIRONMENTAL CONTAMINATION IN AQUATIC ENVIRONMENTS?*

*Artigo submetido ao periódicos* "*PLOS ONE*"

*Macrobrachium amazonicum* is a good bioindicator of environmental contamination in aquatic environments?

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## 5.1 Abstract

Anthropic action has come over the years affecting the various types of ecosystems, including the aquatic ecosystems. However, in order to evaluate and diagnose these effects, aquatic ecotoxicology uses different species of living beings in environmental monitoring. Thus, the present study aimed to evaluate the oxidative stress profile of *M. amazonicum* through biomarkers: reduced glutathione (GSH) thiobarbituric acid reactive substances (TBARS), in addition to the concentration of inorganic elements in muscles and water from two areas, one area characterized by the presence of human activity and the discharge of untreated sewage (area 01), the second by region where there was less human activity near the river or of its effluents (area 02), both areas located on the Amazon River in the municipality of Santana-AP. Animals (26 animals from area 01 and 18 from area 02) were collected from December 2017 to November 2018. They were taken alive until the laboratory, after euthanasia, the hepatopancreas were removed for the biochemical analyzes. After analyzing the data, we identified that in area 01 the level of dissolved oxygen (OD = 4.5 mg/L $O_2$) was below the limit established by Brazilian legislation, in addition to high values in Cr muscles (ranged from 0.2725 to 1.1749 μg/g) and Pb (ranged from 2.5066 to 6.2133 μg/g). In the water samples from this area, we observed high levels of Cr (0.0031 mg/L), Cu (0.0153 mg/L) and Pb (0.0541 mg/L). Thus, considering the levels of contaminants, the GSH values when compared between the areas presented statistical differences, with low GSH concentrations for area 01. On the other hand, the TBARS values did not present a significant difference, so we can infer the non-occurrence of lipid peroxidation. The *M. amazonicum* was presented as a good bioindicator of environmental contamination, since the levels of the GSH like oxidative stress biomarker changed from exposure to concentrations of inorganic elements. Thus, the analysis of the GSH like biomarker and the chemical parameters characterized the area 01 as impacted and the 02 as not impacted.

**Keywords:** GSH, TBARS, Biomarker, Metals.

## 5.2 Introduction

Several studies are using species of living beings for the purpose of environmental biomonitoring, mainly due to the response that these organisms give to subtle modifications in their habitats [1-2]. Such responses may affect the dynamic balance, leading to an increase in the levels of reactive oxygen species (ROS), biochemical and physiological effects such as: damage to cellular constituents; inhibit enzymes; interfering with the production of adenosine triphosphate (ATP), generating disorders in the metabolism of lipids, carbohydrates and in the breathing process; besides modifying cellular permeability [3-4] therefore to establish a state of oxidative stress [5].

However, to combat the effects of ROS, the antioxidant defense system acts by enzymatic mechanisms (catalase, superoxide dismutase and glutathione-*S*-transferase) and non-enzymatic ones, such as reduced glutathione (GSH), found intracellularly in high concentrations of proteins in the reduced state (SH), participate in the transport of amino acids and detoxification of toxins, perform in the degradation of endogenous peroxides (potentially toxic) and as a coenzyme in various enzymatic reactions [6-7].

The lipophilic characteristics of most xenobiotics make them easily cross the biological membranes. The organisms can in two ways eliminate them, excreting them in their original form or biotransforming them, that is, transforming them into more hydrophilic compounds [8-9]. In this way, GSH has a central role in the biotransformation and elimination of xenobiotics and in the defense of cells against oxidative stress [10]. Though, when there is an imbalance in this defense system, lipidic oxidation occurs. In order to verify the degree of lipid oxidation the TABRS is used, quantifying malondialdehyde, which is a by-product derived from the $\beta$-rupture of endociclization of polyunsaturated fatty acids, considered to be of the main products formed during the lipid deoxidative process [11].

In Brazil, surface water quality is established by physico-chemical and microbiological parameters according to the Resolution of the National Environmental Council (CONAMA nº 357/2005), which establishes maximum values for certain substances, but this resolution did not include information on the use of ecotoxicological tests in water quality assessment [12]. For that reason, mantioned Resolution was complemented and amended by Resolution No. 430/2011, which provides for the control of toxicity in aquatic environments. Therefore, the development of methodologies that aim to evaluate aquatic ecosystems is essential. Among these methodologies are the uses of biomonitoring [13].

Biomonitoring is a process that uses living organisms or their responses to determine the condition or changes of the environment, is used as a method of observing possible external impacts to the ecosystems and their development over time can be used to determine differences between collection points [14]. The organisms that inhabit the aquatic environments: algae, crustaceans, mollusks, fish, among others, can be targets of environmental monitoring. Thus, *M. amazonicum* (Heller, 1862) is a shrimp endemic in tropical South America, with a large geographic distribution occurring in almost all of Brazil [15], and these are described as useful bioindicators of environmental quality due to the different sensitivity to pollution in which they are found [16-17].

The aim of the work was to evaluate the oxidative stress profile of *M. amazonicum* through biomarkers: reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS), as well as the concentration of inorganic elements in muscles and water from an impacted area and another not impacted in a region of the course of the Amazon river, in the municipality of Santana-AP.

## 5.3 Material and methods

### 5.3.1 Area

The samples were collected from the Amazon River at two areas near the mouth of the Matapi River, impacted and not impacted area (Figure 01), located in the community of Elesbão in the municipality of Santana, Amapá. The geographical coordinates of the collection points were determined by georeferencing (impacted area, S00º03’17.5’’ W51°11’41.2’’, not impacted, S00º03’19.7’’ W51°07’06.8’’). According to official data [18] the population of Santana is composed by 101,262 inhabitants, being 2,600 residents of the community of Elesbão, whose main economic activities are: açaí collection, production of pottery and fishing. In the region the hot and humid climate predominates with two well-defined seasons (more rainfall season and less rainfall season) throughout the year. The rainiest season begins at the end of December through August, and the least rainy season from September to December [19].

**Figure 01**. Collection sites, impacted and not impacted area, near the mouth of the Matapi river, tributaries of the Amazon river, located in the municipality of Santana-AP
**Soure: (**adapted, 18).

### 5.3.2 Samples and laboratory analysis

The samples were not vertebrates or cephalopods and did not come from environmental protection areas. Thus, this study does not fit the Brazilian laws of access to biodiversity and genetic heritage (Law no. 13 123, dated May 20, 2015 and Decree No. 8.772/2016). The samples were acquired by donation of the fishermen of the region, who captured the prawns with the use of fishing equipment, such as: regional traps known as "*matapi*". The collection period was divided in four, being: two with few rains and two with a lot of rain (September, December, March and June). The specimens were packed in appropriate bags with constant aeration for transport and brought to LabQOBioq-UEAP where the analyses were processed. We selected only shrimps with sexual maturity (above 5.5 cm of total length) [20].

Samples of water were also collected at the same sites and times of collection of biological material. The water assessment was carried out with the help of CONAMA resolution no.357 of March 17, 2005, which provides for the physical-chemical parameters of water bodies and recommends the maximum permitted values that characterize the framework within the environmental standard established by Brazilian law. Samples were collected in sterile polyethylene bottles with 1 liter sealing. The values of pH, conductivity, total dissolved solids, salinity, temperature, turbidity and dissolved oxygen were determined by the traditional method [21-22]. Microbiological parameters were determined by the Colilert® method [22].

#### 5.3.2.1 Determination of the contents of GSH and TBARS

The animals were dissected and the hepatopancreas were removed for quantification of oxidative stress markers. The hepatopancreas were homogenized in the ratio of 0.1 g of 1 mL tissue of PBS (NaCl 85,0 $g.L^{-1}$; $Na_2HPO_4$ 15,50$g.L^{-1}$ and $NaHPO_4$ 2.30$g.L^{-1}$ and pH 7.2 adjusted with NaOH (1%). Centrifuged for 10 minutes at 3500 rpm. After centrifugation the supernatant was separated into aliquots for the dosage of total thiols (GSH), lipoperoxidation content (TBARS) the results were expressed in $\mu M.g\ tissue^{-1}$.

The final lipid peroxidation products (lipid peroxides, malondialdehydes and other low molecular weight aldehydes), when reacted with 2-thiobarbituric acid (TBA) and trichloracetic acid (TCA), form Schiff Bases. Such complexes are colored and their concentrations were determined by spectrophotometer at 535 nm [23]. In test tubes, 100µL of

the homogenized, white or standard supernatant was pipetted. To each test tube was added 1mL of 0.73% TBA reagent/15% TCA.

The samples were subjected to heating in a water bath, under controlled temperature (100 ° C), for 60 minutes and then cooling in an ice bath for 10 minutes and centrifuging at 3500 rpm.min$^{-1}$. Spectrophotometer reading at 535nm, in duplicate with values expressed in μM.g tissue$^{-1}$.

The glutathione is reacted with 5,5'-dithio-bis-2-nitrobenzoic acid (DTNB) to form a yellowish-colored thiolate (TNB or 5-thio-2-nitrobenzoic acid), measurable in a spectrophotometer at 420 nm. Addition of 500 μl DTNB (1 mg/mL 1% sodium citrate) in the cuvettes containing 500 μl of the diluted sample in 1.5 mL of phosphate buffer pH 8 (0.2 M) allowed the quantification of total thiols expressed in reduced glutathione (GSH) equivalents, after a water bath, under controlled temperature (37ºC) for 3 minutes and then subjected to ice-bath cooling, the absorbance was determined at 420 nm in light spectrophotometer visible. Analyzes were performed in duplicates and values expressed in μM.g of tissue$^{-1}$.

#### 5.3.2.2 Inorganic elements in muscle

Samples were prepared by chemical digestion in which a sample (1 g) was placed in a digestion tube and 5 ml of concentrated $HNO_3$ were added. The system was heated using high pressure decomposition vessels (digestion pump) at 130°C for 90 min and diluted to 25 mL with distilled water. The sample solution was clarified. A blank was performed in the same manner [24].

The reading of the inorganic elements was carried out in Atomic Absorption with Flame Spectrophotometer (F-AAS) in the Atomic Absorption Laboratory of the Federal University of Amapá (UNIFAP). The following contaminants were evaluated: cadmium (Cd), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), zinc (Zn) and chromium (Cr).

### 5.3.3 Statistical Analyzes

Statistical analyzes were performed using the GraphPrism 5.0 program. The data were submitted to the Shapiro-Wilk normality test. To evaluate the level of significance, the non-

parametric Mann-Whitney U test was used. The results with values of $p \leq 0.05$ were considered significant.

### 5.4 Results and discussion

The physical and chemical parameters were established according to the classification for water of class II (CONAMA 357, 2015), these results, besides the microbiological are presented in table 01.

Table 01. Physical, chemical and microbiological parameters evaluated for water at impact point and not impacted, values are expressed by means of the average of the four collection periods.

| | **Less rainy period** | | **Rainer period** | | |
|---|---|---|---|---|---|
| **Parameters** | **Impacted** | **No impacted** | **Impacted** | **No impacted** | **Reference** |
| pH | 7,5 | 7,6 | 7,2 | 6,9 | 6,0 a 9,0 [a] |
| Condutivity (µS $cm^{-1}$) | 53,1 | 44,2 | 58,1 | 65,1 | < 100 [b] |
| Total solids (mg\L) | 34 | 31,2 | 38,1 | 42,5 | < 500 [a] |
| Salinity | 25,3 | 24,1 | 28,8 | 25,2 | ≤ 0,5 % [a] |
| Temperature | 30,8 | 31,6 | 30,4 | 30 | < 40ºc[a] |
| Turbidity (NTU) | 34 | 36 | 27,8 | 29,0 | < 100 [a] |
| Dissolved oxygen | 4,5 | 6,0 | 6,5 | 6,7 | > 5 [a] |

| | | | | |
|---|---|---|---|---|
| (mg\L $O_2$) | | | | |
| Total coliform | + | - | + | - |
| Thermotolerant Coliform | + | - | + | - |

**Note**: [a] (CONAMA 357, 2005),[b][31]

Among the variables analyzed in the four collection periods it was observed that only the values (4.5 mg/L $O_2$) in the impacted area during the period with less rainfall presented a result below the value established by the current legislation (≥ 5 mg/L $O_2$) (CONAMA 357, 2015).

This result is justified by the inadequate disposal of organic matter coupled to the period with a lower concentration of rainfall, resulting in a greater demand for oxygen dissolved in water by aerobic microorganisms, which is essential for the metabolism of aquatic biota [25]. Amazonian water resources in which they find similar OD results with values varying from 4.5 to 6.1 mg/L $O_2$. The collection point of this study was located in a peripheral area of Santarém, near the mouth of the Maicá river submitted to the discharge of domestic and industrial sewage. Similar results were also evidenced by other group [26], when investigating the influence of precipitation on the water quality of the Purus River, obtained OD concentration below 5 mg/L $O_2$ in the dry season.

In the aquatic ecosystem, the low available oxygen content induces physiological systems to pass through possible adaptations, among which respiratory organs of some species can increase the efficiency of gas exchange [27]. Among these species *M. Amazonicum* is able to withstand several conditions unfavorable to the aquatic environment, among them the reduction of OD content, making it a good bioindicator of environmental contamination [28].

From the qualitative microbiological method, we obtained positive results in the two pluviometric periods for total coliform and thermotolerant in the impacted area. [29], through the seasonal monitoring of water quality of the Araguari/AP river, identified that the samples from the city of Ferreira Gomes-AP presented a high number of thermotolerant coliforms, which shows that effluents from the urban area can potentiate the presence of such pathogens of anthropic origin. [30] when evaluating the water quality and trophic index in a tropical ecosystem river under environmental impact, identified the presence of thermotolerant

coliforms in a sample near the city of Vitória do Jari-AP, with positive correlation with rainfall.

The metals can bioaccumulate at different trophic levels, so the concentrations of the inorganic elements in the *M. amazonicum* muscle from the two collection points presented average values of Cd, Cu and Zn within the parameters established by the current legislation. The concentration of Fe and Mn are not stipulated in Brazilian legislation. In this way, we compared the values between the affected areas (Fe=5.2749 to 16.360 μg/g), not impacted (Fe = 7.4191 to 10.983 μg/g) and were not statistically significant ($p < 0.47$). The results of Mn, impacted (1.0148 to 2.3410μg/g), not impacted (0.3519 to 9.3785 μg/g), when compared are not statistically significant ($p < 0.82$). Fe may be toxic and beneficial to organisms; their concentration in the body must be regulated enough for biological functions, their toxicity being related to ROS formation. Manganese has an essential function in enzymatic systems, metabolizing proteins and providing energy in all animals [32].

Similar results were found by [33], in which the Fe concentration in the *Penaeus semisulcatu*s muscle ranged from 5.9 to 12.5 μg/g and Hendrickx; Osuna and Padilla (1998), with Mn values ranging from 0.4 to 0.6 μg/g. However, in 11 samples from the impacted area the concentration of Cr ranged from 0.2725 to 1.1749μg/g. In 3 samples from said area the concentration of Pb ranged from 2.5066 to 6.2133 μg/g. These values are above the limit established by Brazilian legislation (Cr 0.1 μg/g; Pb 2.0 μg/g) (table 02) [34-35].

Table 2. Concentration (mean of triplicate) in μg / g of inorganic elements in muscle tissue of *M. amazonicum*, from the impacted and non-impacted area. N/D = Not detected.

| **Impacted** | Cd | Cr | Cu | Pb | Zn | Fe | Mn |
|---|---|---|---|---|---|---|---|
| C1 | 0.1828 | **0.3680** | 7.0247 | 0.1116 | 8.9693 | 11.4289 | 1.1703 |
| C2 | 0.2696 | **0.9802** | 7.9313 | 0.1390 | 8.9754 | 6.7544 | 1.2030 |
| C3 | 0.2686 | **0.2725** | 2.3402 | 0.1032 | 8.9860 | 7.9284 | 1.1703 |
| C4 | 0.2644 | **0.3574** | 7.1151 | 0.0999 | 9.7046 | 9.9708 | 1.1785 |
| C5 | 0.1696 | 0.1415 | 6.2430 | 0.0566 | 10.427 | 9.7778 | 1.4485 |
| C6 | 0.2170 | 0.0785 | 7.4578 | 0.1074 | 9.3279 | 9.8856 | 1.3912 |
| C7 | 0.2907 | 0.0588 | 5.1180 | 0.1769 | 7.4745 | 9.8765 | 1.4649 |
| C8 | 0.2683 | **0.5803** | 5.1312 | 0.1537 | 9.0241 | 5.2749 | 1.4321 |
| C9 | 0.3380 | **0.3008** | 9.8413 | 0.0695 | 9.2428 | 5.3178 | 2.3410 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C10 | 0.1328 | **1.1749** | 10.045 | 2.0001 | 8.9633 | 5.4186 | 1.4631 |
| C11 | 0.2525 | **0.6228** | 9.9993 | 0.1916 | 9.3887 | 5.2963 | 1.5019 |
| C12 | 0.2999 | **0.4282** | 6.6666 | 0.0231 | 8.0578 | 6.7758 | 2.0377 |
| C13 | 0.3169 | **0.3680** | 6.7433 | 0.0526 | 9.9720 | 6.7343 | 1.9477 |
| C14 | 0.3380 | **0.8351** | 6.7819 | 0.1158 | 4.6730 | 6.5185 | 2.0705 |
| C15 | 0.1894 | 0.0596 | 6.7904 | 0.0231 | 6.5629 | 6.6686 | 2.0214 |
| C16 | 0.2906 | 0.0578 | 5.9876 | 0.1011 | 9.7046 | 16.232 | 1.0148 |
| C17 | 0.2407 | 0.0814 | 5.7194 | 0.0379 | 8.7810 | 16.146 | 2.1769 |
| C18 | 0.2433 | 0.0894 | 5.9035 | 0.2729 | 10.336 | 16.360 | 2.1932 |
| C19 | 0.1539 | 0.0704 | 5.4479 | 0.0884 | 8.6230 | 16.253 | 2.3324 |
| C20 | 0.1854 | 0.0678 | 5.1795 | 0.0652 | 8.7445 | 7.0117 | 2.3815 |
| C21 | 0.2565 | 0.0897 | 5.8634 | 0.1896 | 10.494 | 7.1404 | 1.3585 |
| C22 | 0.2828 | 0.0678 | 9.3501 | 0.1390 | 11.776 | 6.8616 | 1.6695 |
| C23 | 0.3235 | 0.0980 | 5.7190 | **4.1496** | 11.017 | 7.0117 | 1.7186 |
| C24 | 0.2485 | 0.0256 | 5.5093 | **6.2133** | 8.4407 | 9.5419 | 1.7104 |
| C25 | 0.2328 | 0.0236 | 5.6084 | 0.7793 | 9.0787 | 9.8421 | 1.5549 |
| C26 | 0.3906 | 0.0874 | 5.5664 | **2.5066** | 6.4171 | 9.8208 | 1.6367 |
| **Non-impacted** | | | | | | | |
| C1 | 0.7496 | 0.0389 | 6.9925 | 0.6319 | 10.737 | 9.7349 | 0.3519 |
| C2 | 0.6708 | 0.0567 | 5.5347 | 0.1516 | 10.373 | 10.506 | 0.3601 |
| C3 | 0.6063 | 0.0832 | 7.8645 | 0.1284 | 5.8337 | 10.485 | 0.3519 |
| C4 | 0.7036 | 0.0285 | 5.1749 | 0.0252 | 11.454 | 10.635 | 0.6792 |
| C5 | 0.7168 | 0.0684 | 5.6988 | 0.0947 | 9.1699 | 9.5467 | 0.8184 |
| C6 | 0.8378 | 0.0295 | 4.8901 | 0.0210 | 11.236 | 9.9279 | 0.7365 |
| C7 | 0.7589 | 0.0964 | 5.0664 | 0.1896 | 10.433 | 10.249 | 0.8511 |
| C8 | 0.8009 | 0.0386 | 5.0437 | 0.1769 | 9.4798 | 10.078 | 0.8593 |
| C9 | 0.7325 | 0.0368 | 7.8147 | 0.0379 | 9.9842 | 10.983 | 0.9166 |
| C10 | 0.7970 | 0.0748 | 7.3784 | 0.5687 | 10.792 | 8.9012 | 0.9166 |
| C11 | 0.7930 | 0.0846 | 8.5368 | 0.2317 | 9.7350 | 8.9560 | 1.0066 |
| C12 | 0.8141 | 0.0948 | 9.2378 | 0.0505 | 10.834 | 8.8772 | 1.0148 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| C13 | 0.6183 | 0.0841 | 4.7389 | 0.3160 | 10.178 | 9.5248 | 1.0393 |
| C14 | 0.7115 | 0.0913 | 6.4289 | 0.3581 | 8.8296 | 9.6967 | 9.2230 |
| C15 | 0.7325 | 0.0279 | 6.3894 | 0.1390 | 10.190 | 9.9925 | 9.3785 |
| C16 | 0.7352 | 0.0485 | 4.3685 | 0.1200 | 10.397 | 9.7945 | 1.9462 |
| C17 | 0.7378 | 0.0839 | 3.5684 | 0.3791 | 10.263 | 7.5907 | 0.9340 |
| C18 | 0.6589 | 0.0833 | 10.857 | 0.1053 | 10.130 | 7.4191 | 1.4830 |
| **Limits** | 1.0[a.b] | 0.1 [a] | 30.0 [a] | 2.0[a.b] | 50.0 [a] | * | * |

[a][35][b] [34] * Undefined values

Similar results were identified by [36] when evaluating the concentration of inorganic elements in shrimp (*Xiphopena euskroyeri*) found values of 1.39 to 5.25 μg/g for Cr and 17.45 to 73.11 μg/g for Pb, the authors portray that account that these animals occupy the final level of the food chain with the potential to bioaccumulate metals, moreover, above-established concentrations of Cr and Pb may cause damage to humans. An aggravating factor of this result is the fact that *M. amazonicum* is commercialized and consumed as food in the Amazon region.

[37] obtained high values of Cr (0.68 μg/g) when evaluating the contamination by metals in crustaceans belonging to *Callinectes* sp. The biological activity of trivalent Cr is essential to living beings, but its hexavalent state is considered to be corrosive to the mucosa, it can cause various diseases to terrestrial and aquatic organisms [38-39].

The presence of Pb in the aquatic ecosystem results in erosion and leaching of the soil, as well as, through the atmospheric precipitation from industrial processes, it has affinity with chemical structures that have nitrogen and sulfur atoms, it binds to macromolecules cellular, acting negatively on enzymatic reactions, therefore affects the body's metabolism [40].

In the Amazon region, contamination of water resources by toxic metals is gradually worsening, and treatments are becoming increasingly expensive. Thus, in the four collection periods, the inorganic elements of the non-impacted area have remained within the values established by Brazilian legislation. However, in the impacted area the metals: Cr (0.0031 mg/L), Cu (0.0153 mg/L) and Pb (0.0541 mg/L) were above the reference values (Table 03).

Table 3. Concentration (mg / L) of inorganic elements present in the water sample

| | Cd | Cr | Cu | Pb | Zn | Fe | Mn |
|---|---|---|---|---|---|---|---|
| Impacted | **0.0031** | 0.0197 | **0.0153** | **0.0541** | 0.0957 | 0.2467 | 0.0764 |
| Non-impacted | 0.0011 | 0.0029 | 0.0049 | 0.0012 | 0.0095 | 0.0777 | 0.0046 |
| **Limits** | 0.001 | 0.050 | 0.009 | 0.010 | 0.18 | 0.3 | 0.1 |

[a](CONAMA 357, 2015)

[41] obtained similar results when investigating the effects of the anthropic contribution on the waters of the Rio Negro in the State of Amazonas, where they identified high values for Cd (ranging from 0.25 to 0.34 μg/L), Cu (ranging from 0.61 (P = 0.02 to 0.04 μg/L). [42] found high values of Cu (3.789 μg/g) in the Manaus-AM creeks sediments, located next to a bin, in which the authors attributed that the metal came from local industries, whose effluents and solid residues were leached, increasing the concentration of Cu in the samples. [43] when evaluating the presence of metals in the Tarumã-Açu Manaus Basin, through chemical analyzes of water samples and sediments showed high Cu (0.077 mg/L). In addition, in the estuarine system of Santos and São Vicente, the monitoring performed by [31] evidenced elevated levels of Pb, mainly in the São Vicente estuary with concentrations of 36 μg/L in the water.

The high levels of toxicity of some inorganic elements to the aquatic ecosystem, even in low concentrations, associated to its bioavailability capacity in the trophic chains for long periods, justifies the development of studies that aim to quantify the concentrations of such elements [44]. Exposure to Cd can affect both environmental performance and chemical communication in aquatic biota [45]. Contamination by Pb is most often related to anthropogenic action, such as battery, steel and mining industries, posing a risk to living organisms, among them, reduction of growth, contamination of surface water and aquifers, as well as, direct toxicity to humans, animals and microorganisms [46-47]. Cu toxicity varies with respect to the exposure environment, its chemical form and the exposed organism and species; it is believed that its toxicity consists of affinity with SH (thiols) sulfhydryl groups of many proteins and enzymes [48].

In the evaluation of oxidative stress biomarkers, the levels of GSH in the non-impacted area ranged from 0.57 to 34.45 μM/g tissue. In the impacted area ranged from 0.68 to 30.78 μM/g tissue, these results when compared showed a statistically significant

difference in the Mann Whitney test ($p < 0.0078$) figure 02. These results are probably justified by the high concentration of metals (Cr and Pb) in the hepatopancreas of *M. amazonicum* of the impacted area, since the metal ions are well known as inducers of oxidative stress, they can act by two mechanisms, being: metals with redox potential (Fe, Cu, Cr and V) that generate ROS, $Cr^{6+}$ for example, can participate in the Fenton reaction and therefore produce hydroxyl radicals [49] and metals without redox potential (Hg, Ni, Pb and Cd) that impair antioxidant defense by thiols and enzymes, [50-52]. From the results in question, we can infer that GSH presents as a good biomarker-soluble once that the concentration of Cr and Pb in the impacted area are probably related to the low levels of GSH in this group.

**Figure 02.** Collection sites, impacted and not impacted area, near the mouth of the Matapi river, tributaries of the Amazon river, located in the municipality of Santana-AP, Brazil.

Several studies have already identified the reduction of the concentration of GSH in the presence of metal ions [53-61].

The SRAT levels of the non-impacted area ranged from 45.23 to 304.41μM/g tissue. In the impacted area they ranged from 45.61 to 336.74μM/g tissue, when compared with no significant difference (Figure 03). [62] showed that the presence of cadmium and copper generated increase in SRAT and a significant decrease in GSH. [63], when assessing the arsenic-induced oxidative stress, obtained high TBARS values in how much GSH is reduced. These results diverged from our study because the changes in GSH can lead to cell membrane peroxidation and by subsequent elevation of the concentration of TBARS. According to [64], this process occurs only if the first lines of antioxidant defense (enzymatic and non-enzymatic) do not neutralize the oxidizing agents, thus they were more susceptible to lipid peroxidation. Thus, in the present work was evidenced alteration in the lines of antioxidant defense by the reduction of the levels of GSH, however the levels of SRAT were not altered.

**Figure 3.** GSH levels in the hepatopancreas samples of *M. amazonicum*, from the impacted and non-impacted area. (**) significant difference ($p < 0.001$).

## 5.5 Conclusion

In conclusion, *M. amazonicum* presented as a good bioindicator of environmental contamination, since the biomarker of oxidative stress reduced glutathione expressed changes in levels when the animals were exposed to the sublethal concentration of inorganic elements, statistically diverging the values of the impacted area and not impacted. Thus, in our study GSH was an excellent antioxidant agent, justified by non-lipid peroxidation, identified in the TBARS values, in which there was no significant statistical difference between the collection points. The impacted area was thus characterized by anthropic activity, and high levels of inorganic elements (Cr and Pb) in the muscles, as well as high values for Cd, Cu and Pb in the water.

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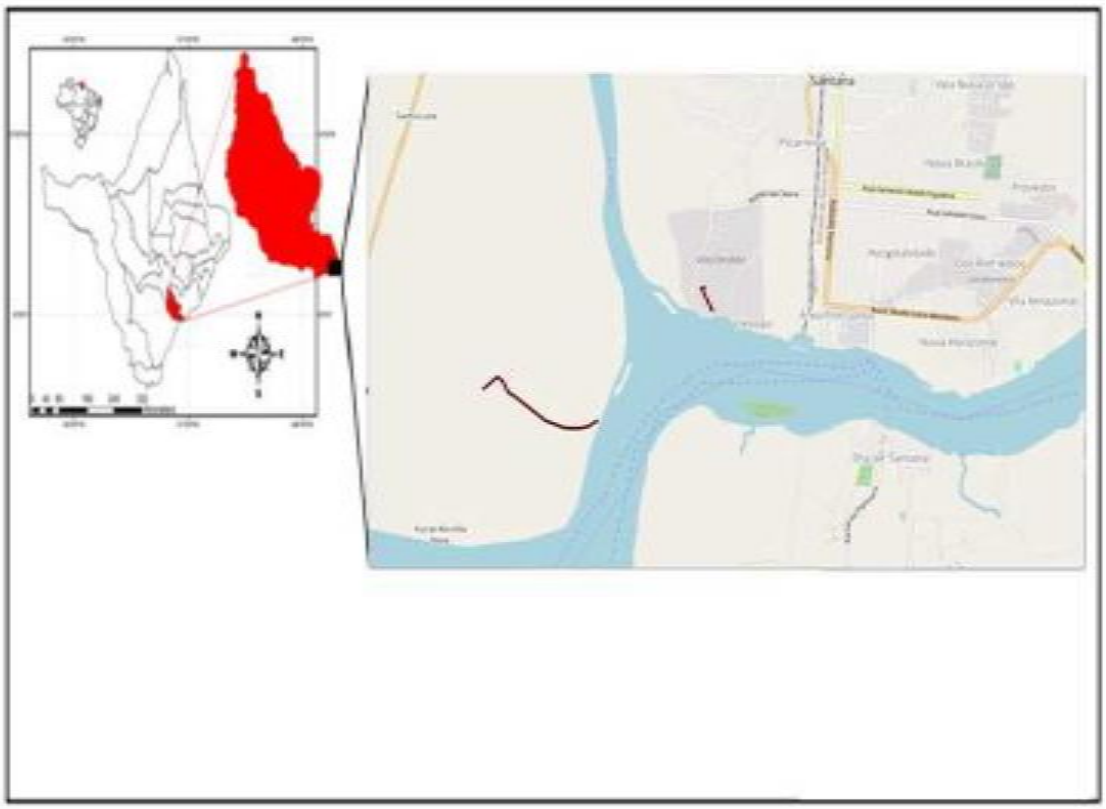

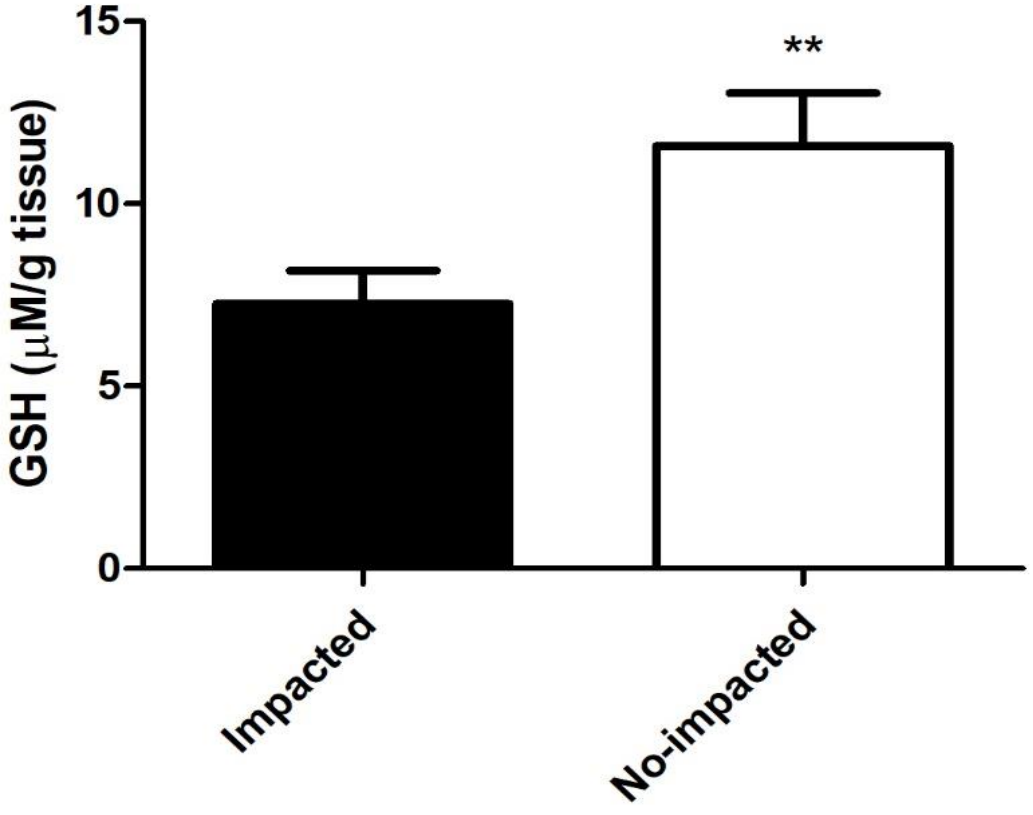
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GSH (μM/g tissue)
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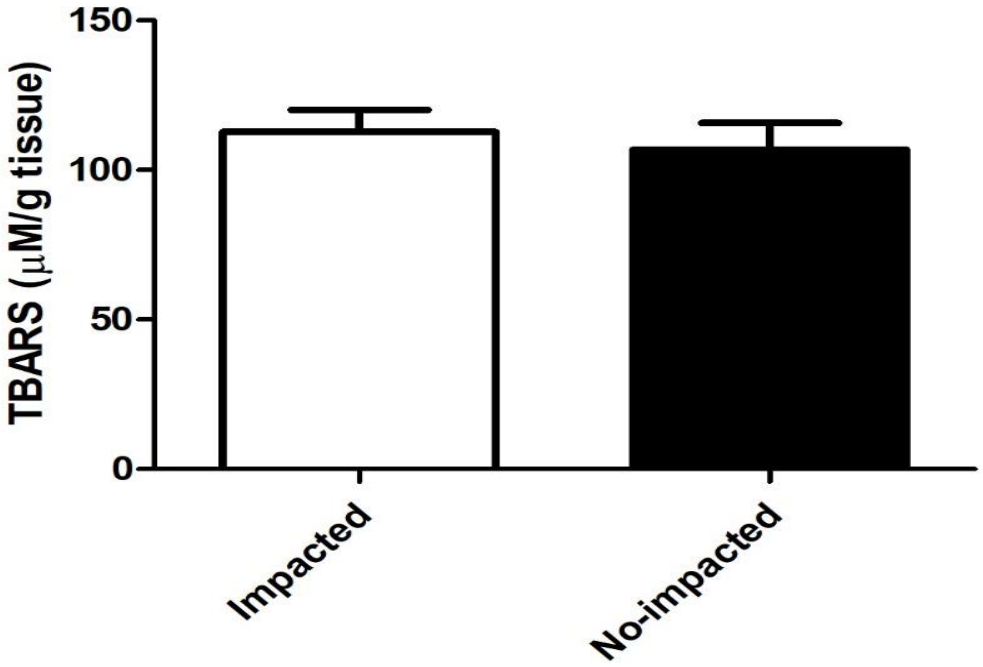
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# 6 CAPÍTULO 04. ESTRESSE OXIDATIVO EM *Plasgiossion squamossimus* PROVENIENTE DA AMAZÔNIA ORIENTAL

*Artigo submetido ao periódico "Novos Cadernos NAEA"*

# ESTRESSE OXIDATIVO EM *Plasgiossion squamossimus* PROVENIENTE DA AMAZÔNIA ORIENTAL

# OXIDATIVE STRESS IN *Plasgiossion squamossimus* FROM THE EASTERN AMAZON

## 6.1 RESUMO

O estudo objetivou-se em avaliar o perfil de estresse oxidativo do *P. squamosissimus* utilizando biomarcadores: glutationa reduzida (GSH) e substâncias reativas ao ácido tiobarbitúrico (SRAT), além da concentração de contaminantes inorgânicos, oriundas de duas áreas, a primeira caracterizada pela presença da atividade humana e o despejo do esgoto não tratado (área 01) e a segunda com menos atividade humana (área 02), localizada no rio Amazonas, Santana-AP. Os peixes foram levados vivos até o laboratório, após a eutanásia, retirou-se o fígado para análise bioquímica. Foram capturados na área 01, 10 e na área 02, 08 indivíduos. Na área 01 a concentração de Cd, Cr e Pb estavam acima do limite. Os valores da GSH apresentaram diferença significativa, com baixa concentração na área 01. Os valores das SRAT não apresentaram diferença estatística entre as áreas. Assim, o GSH foi um excelente agente antioxidante. E, nossos resultados ressaltam a necessidade de avaliação de metais na população da área 01 (impactada). Ao final, foi possível caracterizar a área 01 como impactada e área 02 como não impactada pela ação antrópica.

**Palavras chave**: Biomarcador. Estresse oxidativo. Ecotoxicologia. Metais.

## ABSTRACT

The objective of this study was to evaluate the oxidative stress profile of P. squamosissimus using biomarkers: reduced glutathione (GSH) and thiobarbituric acid reactive substances (SRAT), in addition to the concentration of inorganic contaminants, from two areas, the first characterized by presence of human activity and the discharge of untreated sewage (area 01) and the second with less human activity (area 02), located in the Amazon River, Santana-AP. The fish were taken alive to the laboratory, after euthanasia, the liver was removed for biochemical analysis. Samples were collected in area 01, 10 and area 02, 08 individuals. In area 01 the concentration of Cd, Cr and Pb were above the limit. The GSH values presented a significant difference, with a low concentration in area 01. The SRAT values did not present statistical difference between the areas. Thus, GSH was an excellent antioxidant agent. And,

our results highlight the need for metal evaluation in the population of area 01 (impacted). At the end, it was possible to characterize area 01 as impacted and area 02 as not impacted by

**Keywords**: Biomarker. Oxidative stress. Ecotoxicology. Metals.

## 6.2 INTRODUÇÃO

A contaminação dos ecossistemas aquáticos é um grave problema que vem crescendo ao longo dos anos, principalmente com a elevação da quantidade de poluentes industriais, agrícolas e urbanos, que são descarregados no ambiente, por conseguinte tem elevados efeitos deletérios a biota e também sobre a saúde humana (MCGLASHAN; HUGHES, 2001). Dentre esses contaminantes estão inseridos os metais, que são resistentes à degradação e podem provocar efeitos bioquímicos e fisiológicos nos organismos aquáticos, além da possibilidade de bioacumulação, assim como de biomagnificação, sendo este último responsável pela transferência dos contaminantes nos diferentes níveis tróficos, inclusive a contaminação humana, que de forma direta ou indireta usufruem dos recursos hídricos e pescados (LUSHCHAK, 2011).

Tais efeitos são responsáveis pelo desequilíbrio da homeostasia celular, que poderão elevar o nível de espécies reativas de oxigênio (ERO), ocasionando distúrbios no metabolismo de lipídios, carboidratos, proteínas, permeabilidade celular e danificação do ácido desoxirribonucleico (DNA) (COSTA et al., 2008). Por conseguinte, a instalação de estresse oxidativo (LUSHCHAK, 2011).

Para estabilizar os efeitos das ERO, o sistema de defesa antioxidante irá atuar por meio de vias enzimáticas e não enzimáticas, sendo esta última representada principalmente pela glutationa reduzida (GSH) que tem por objetivo manter os grupos sulfidrilas das proteínas no estado reduzido, além disso, participa no transporte de aminoácidos e detoxificação, atua enzimaticamente degradando peróxidos endógenos, além de coenzima em várias reações enzimáticas (VASCONCELOS et al., 2007).

Assim, com a instalação do estresse oxidativo haverá a formação de peroxidação lipídica, que poderá ser avaliada utilizando as substâncias reativas ao ácido tiobarbitúrico (SRAT), quantificando o malondialdeído que é um produto secundário, derivado da β-ruptura de endociclização de ácidos graxos polinsaturados, considerado uns dos principais produtos formados durante o processo de oxidativo lipídica (KIRSCHNIK; VIEGAS, 2009)

Neste contexto, os organismos que habitam os ambientes aquáticos podem ser alvos de monitoramento ambiental. Os peixes são considerados bioindicadores úteis na determinação da qualidade ambiental, em virtude a sensibilidade diferenciada à xenobióticos. Outro ponto de relevância para estudos ecotoxicológicos em pescados, é que apresentam alto teor protéico, vários micronutrientes e são considerados essenciais na dieta humana, uma vez que, possui grande valor nutricional (NAIGAGA et al., 2011).

Dentre as diversas espécies de peixes destacam-se o *P. squamosissimus,* importante não só como uma espécie comestível, mas também como um modelo adequado para biomonitorização (WUNDERLICH et al., 2015; ROCHA et al., 2016).

*P. squamosissimus* apresenta ampla distribuição geográfica, foi originalmente limitado ao rio Orinoco e bacias do rio Amazonas e rios das guianas (CASATTI, 2005), é considerada endêmica da bacia amazônica (TAVARES et al., 2007; ANTUNES et al., 2012; MELO et al., 2014; MELO et al., 2015), é um peixe sedentário (LOUBENS, 2003; DAGA et al., 2009; BARBOSA; ROCHA; FREDOU, 2012; WUNDERLICH et al., 2015), foi descrito pela primeira vez por Heckel (1840), é conhecido por populares como: pescada branca, corvina, pescada-do-Piauí, corvina-do-rio e pescada-cacunda, sua atividade reprodutiva é registrada ao longo de todo ano (NAKATANI; BAUMGARTNER; BAUMGARTNER, 1997; COOKE; CHAO; BEHEREGARAY, 2012).

As fêmeas geralmente alcançam tamanho e peso mais elevado que os machos, apresentando respectivamente 59 cm e 5,45 Kg, 55,5 cm e 3,73 Kg (LOUBENS, 2003; BARBOSA; ROCHA; FREDOU, 2012). Quanto aos aspectos alimentares, o *P. squamosissimus* é um predador de topo, com comportamento piscívora, na sua dieta estão presentes, camarões independentemente do seu estágio de vida (FERREIRA-FILHO et al., 2014), peixes, gastrópodes, insetos e matéria vegetal (SANTOS et al., 2014; SOUZA et al., 2017).

Neste cenário, o presente estudo objetivou em avaliar o perfil de estresse oxidativo do *P. squamosissimus* por meio dos biomarcadores: GSH e SRAT, além da concentração de contaminantes inorgânicos presentes nos músculos das amostras, oriundas de uma área impactada e não impactada no rio Amazonas no município de Santana-AP.

## 6.3 MATERIAL E MÉTODOS

### 6.3.1 Área de estudo

As amostras foram provenientes do rio Amazonas em dois pontos de coleta próximo a foz do rio Matapi, área impactada e não impactada (figura 01), localizados na comunidade do Elesbão no município de Santana, Amapá. As coordenadas geográficas dos pontos de coletas foram determinadas por GPS (área impactada, S00º 03’17’5 W051º 11’41,2; não impactada, S00º 03’19,7 W051º 07’ 06,8).

**Figura 01**. Locais de coleta, em vermelho representando os igarapés (região impactada, presente no Elesbão e não impactada, próximos a foz do rio Matapi, afluentes do rio Amazonas, localizado no município de Santana-AP


Fonte: (Adaptato: BRITO, 2013).

Na região predomina-se o clima quente e úmido com duas estações bem definidas (estações mais e menos chuvas) durante todo o ano. A estação mais chuvosa inicia-se fins de dezembro até agosto, e a estação menos chuvosa de setembro a dezembro (LIMA et al., 2007).

### 6.3.2 Coleta da amostra

A coleta dos peixes foi realizada pela equipe do Laboratório de Química Orgânica e Bioquímica da Universidade Estadual do Amapá (LabQOBioq - UEAP) e pescadores da região, com a utilização de apetrechos de pesca, tais como: anzol, redes de emalhar, tarrafa,

rede de arrasto (malha 20mm). No período de setembro e dezembro de 2017 e março e junho de 2018, foram capturados 10 espécimes da área impactada e 08 da não impactada. As amostras foram acondicionadas em sacos apropriados com aeração para transporte e levados vivos até o LabQOBioq - UEAP, onde foi processada as análises. Todos os procedimentos foram realizados respeitando os pressupostos do Colégio Brasileiro de Experimentação Animal (COBEA).

### 6.3.3 Análise laboratorial: dosagem das concentrações de GSH e SRAT

Os animais foram dessensibilizados com secção medular e destes separados porções do fígado para a quantificação dos marcadores do estresse oxidativo e tecido muscular para quantificação dos contaminantes inorgânicos. O fígado foi homogeneizado na proporção de 0,5 g de tecido 5 $mL^{-1}$ de PBS (NaCl, 85,0$g.L^{-1}$; $Na_2HPO4$, 15,50$g.L^{-1}$ e $NaHPO_4$, 2,30$g.L^{-1}$) com pH 7,2 ajustado com NaOH (1%). Centrifugado durante 10 minutos com velocidade de 3500 rpm. Após a centrifugação o sobrenadante foi separado em alíquotas para a dosagem de tiois totais, teor de lipoperoxidação e determinação de proteínas totais pelo método de Lowry et al (1951), e os resultados foram expressados em $\mu g.mL^{-1}$.

Os produtos finais da peroxidação lipídica (peróxidos lipídicos, malondialdeídos e demais aldeídos de baixo peso molecular), ao reagirem com o ácido 2-tiobarbitúrico (TBA) e ácido tricloracético (TCA), formam Bases de Schiff. Tais complexos são coloridos e suas concentrações foram determinadas por espectrofotômetro (YAGI, 1976). Em tubos de ensaio, pipetou-se 100µL do sobrenadante homogeneizado, branco ou padrão. A cada tubo de ensaio adicionou-se 1mL de reagente TBA 0,73%/TCA 15%.

Submeteu-se as amostras a aquecimento em banho-maria, sob temperatura controlada (100º C), durante 60 minutos e em seguida ao resfriamento em banho de gelo, por 10 minutos e após centrifugadas à 3500 $rpm.min^{-1}$ efetuou-se a leitura em espectrofotômetro a 535nm, em duplicata com valores expressos em $\mu g.mL^{-1}$.

A glutationa, principal tiol intracelular, reage com o ácido 5,5'-ditio-bis-2-nitrobenzóico (DTNB), formando um tiolato (TNB ou ácido 5-tio-2-nitrobenzóico) de coloração amarelada, mensurável em espectrofotômetro a 420 nm. A adição de 500 µL de DTNB (1 mg/mL de citrato de sódio 1%) nas cubetas contendo 500 µL da amostra diluída em 1,5 mL de tampão fosfato pH 8 (0,2 M) permitiu a quantificação de tióis totais expressos em equivalentes de glutationa reduzida (GSH), após banho-maria, sob temperatura controlada (37° C), durante 3 minutos e, em seguida, submetidas ao resfriamento em banho de gelo, a

absorbância foi determinada a 420 nm em espectrofotômetro de luz visível. As análises foram realizadas em duplicatas e os valores expressos em µmol.mL$^{-1}$.

### 6.3.4 Contaminantes inorgânicos no músculo

Os preparos das amostras ocorreram por meio da digestão química, no qual uma amostra (1 g) foi colocada em tubo de digestão e 5 mL de $HNO_3$ concentrado foram adicionados. O sistema foi aquecido usando vasos de decomposição de alta pressão (bomba de digestão) a 130 ºc durante 90 min. e diluído para 25 mL com água destilada. A solução da amostra foi clarificada. Um branco foi realizado da mesma maneira (TUZEN, 2003).

O processo de leitura das concentrações dos contaminantes inorgânicos foram efetuadas em Espectrofotômetro de Absorção Atômica com Chama (F-AAS) no Laboratório de absorção atômica da Universidade Federal do Amapá (UNIFAP). Os seguintes contaminantes foram avaliados: cádmio (Cd), cobre (Cu), ferro (Fe), chumbo (Pb), manganês (Mn), zinco (Zn) e cromo (Cr).

### 6.3.5 Análise estatística

As analises estatísticas foram efetuadas usando o programa Graph Prism 5.0. Submeteu-se os dados ao teste de normalidade de Shapiro-Wilk. Para avaliarmos o nível de significância, utilizou-se o teste t de Student (dados normais). Foram considerados significantes os resultados com valores de $p \leq 0,05$.

## 6.4 RESULTADOS E DISCUSSÃO

As concentrações dos metais avaliados no presente estudo são apresentadas na tabela 01. Os resultados obtidos demonstraram que os peixes provenientes da área impactada tiveram maior concentração de contaminantes inorgânicos, com valores superiores ao que estabelece a legislação brasileira.

**Tabela 01**. Concentrações (média da triplicata) em µg/g de contaminantes inorgânicos em tecido muscular do *P. squamosissimus,* oriundo da área impactada e não impactada. N/D = Não detectado.

| **Área Impactada** | Cd | Cr | Cu | Pb | Zn | Fe | Mn |
|---|---|---|---|---|---|---|---|

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| P1 | **1,00** | **1,37** | 0,74 | 1,76 | 10,06 | 11,08 | 9,32 |
| P2 | 0,99 | **1,30** | 0,78 | 1,57 | 9,80 | 11,25 | 3,69 |
| P3 | **1,05** | **1,23** | 0,75 | 0,37 | 9,93 | 11,19 | 3,72 |
| P4 | 0,93 | **0,53** | 0,80 | 0,56 | 6,97 | 11,17 | 3,69 |
| P5 | 0,87 | **1,54** | 0,92 | 0,25 | 7,10 | 7,29 | 9,68 |
| P6 | 0,78 | **0,94** | 1,05 | **3,79** | 8,87 | 14,36 | 7,79 |
| P7 | **1,06** | **0,96** | 1,04 | 1,07 | 8,68 | 14,47 | 7,71 |
| P8 | 0,93 | **1,07** | 0,74 | 1,39 | 9,88 | 13,14 | 5,88 |
| P9 | **1,08** | **0,87** | 0,94 | 0,69 | 6,98 | 11,60 | 5,90 |
| P10 | 0,96 | **1,23** | 1,08 | 0,89 | 7,50 | 36,0 | 9,06 |
| **Área Não Impactada** | | | | | | | |
| P1 | 0,67 | 0,014 | 0,01 | 0,88 | 0,23 | 0,09 | 2,37 |
| P2 | 0,50 | 0,023 | 0,03 | 1,06 | 0,40 | 1,03 | 2,56 |
| P3 | 0,78 | 0,013 | 0,01 | 1,00 | 1,30 | 1,24 | 2,98 |
| P4 | 0,77 | 0,076 | 0,04 | 0,90 | 2,02 | 5,74 | 1,99 |
| P5 | 0,56 | 0,068 | 0,08 | 0,63 | 1,43 | 5,67 | 2,44 |
| P6 | 0,70 | 0,078 | 0,01 | 0,57 | 1,89 | 2,45 | 2,67 |
| P7 | 0,59 | 0,080 | 0,03 | 1,04 | 1,56 | 1,26 | 2,45 |
| P8 | 0,72 | 0,060 | 0,02 | 1,02 | 2,00 | 2,79 | 3,01 |
| **Limites** | **1,0**[a,b] | **0,1** [a] | **30,0**[a] | **2,0** [a,b] | **50,0** [a] | * | * |

[a] (BRASIL, 1965); [b] (ANVISA, 1998) * Valores não definidos

Diversos metais podem ocasionar efeitos deletérios na saúde dos peixes e de outros organismos aquáticos, geralmente são encontrados em baixas concentrações. No entanto, existe a possibilidade de bioacumualção e até mesmo de biomagnificação desses contaminantes. Assim, as concentrações dos elementos inorgânicos no músculo do *P. squamosissimus*, oriunda da área impactada obtiveram valores de Cd (0,78 a 1,08 µg/g), Cr (0,53 a 1,54 µg/g) e Pb (0,25 a 3,79 µg/g) acima dos parâmetros estabelecidos pela legislação brasileira (Cd = 1,0 µg/g; Cr = 0,1 µg/g e Pb = 2,0 µg/g).

A concentração de Fe e Mn não estão estipulados pela legislação brasileira. Dessa forma, comparamos os valores entre as áreas, impactada (Fe = 7,2 a 14,4 µg/g) e não impactada (Fe = 0,09 a 5,7 µg/g) com diferença significativa ($p < 0,001$). Os resultados de Mn na área impactada (Mn = 3,6 a 9,6 µg/g) e da não impactada (Mn = 1,9 a 3,1) com diferença significativa ($p < 0,001$).

Gorur et al. (2012), obtiveram concentrações de Fe em amostras de peixes comuns na Turquia variando entre 7,9 a 16,6 µg/g. Rosso et al. (2015), ao avaliarem metais em *Astyanax altiparanae*, *Leporinus friderici* e *Hypostomus strigaticeps*, coletados no córrego Curral de Arame, apresentaram a concentração de Fe entre 39 a 50 mg/Kg. Ambos os valores são

maiores dos identificados em nosso estudo. Assim, tanto o ferro quanto o manganês fazem parte de mecanismos fisiológicos, portanto, seus valores elevados podem ser justificados em um desequilíbrio da homeostasia celular.

Silva et al. (2012), no biomonitoramento de metais em *Astyanax bimaculatus* o Mn apresentou concentração variando de 23,0 a 42,0 mg/Kg, resultado este muito superior ao encontrado no presente estudo.

O Cd possui alta toxicidade e pode afetar a homeostasia celular, ocasionando disfunção fisiológica em peixes e conseguintemente leva a morte (COSTA; HARTZ, 2009). Nos seres humanos, a implicação consiste na incapacidade de excreção, afetando diretamente os rins, além de efeitos gastrointestinais (DURAL; GOKSU; OZAK, 2007).

Valores elevados de Cd foram evidenciados no estudo de Barros et al. (2010), no músculo de*Serrasalmus ssp, Potamorhina spp* e *Cichla spp* provenientes do rio Gelado na Serra dos Carajás – PA, os autores destacam que a principal fonte desse metal são rejeitos liberados pela mineração na água. Resultados semelhantes foram identificados por Lima et al. (2015), na qual encontraram concentrações Cd acima do estabelecido pela legislação brasileira em amostras de músculo do *P. squamosissimus* oriundas do rio Cassiporé –AP, o referido estudo relaciona tais resultados a ação de garimpos e por outras fontes antrópicas na região.

O Cr no ambiente aquático geralmente se apresenta na forma de cromato, no estado de oxidação hexavalente (potencialmente tóxica para a biota aquática). Todas as amostras da área impactada apresentaram concentrações elevadas de Cr com média de 1,1 µg/g. Miranda-filho et al. (2011), ao avaliarem cromo hexavalente em peixes oriundos da Baia de Sepetiba-RJ, obtiveram valores médios no tecido muscular da *Micropogonias furnieri* de 0,52 µg/g, na *Cynoscion ocupa* de 0,46 µg/g. Ambos os valores estão com as concentrações elevadas, no entanto, no estudo em questão, o principal agravante consiste na presença de 100% das amostras da área impactada estarem com valores de Cd acima do que estabelece a legislação vigente, principalmente pelo fato do cromo hexavalente pertencer ao grupo dos agentes comprovadamente carcinogênicos em animais e em humanos (NTP, 2000).

Os valores da concentração de Cr identificados nesse estudo estão semelhantes aos de Forero; Mantilla e Martínez (2009) e Barros et al. (2010), que encontraram altas concentrações variando de 1,8 a 2,1 µg/g em rio que recebe efluente.

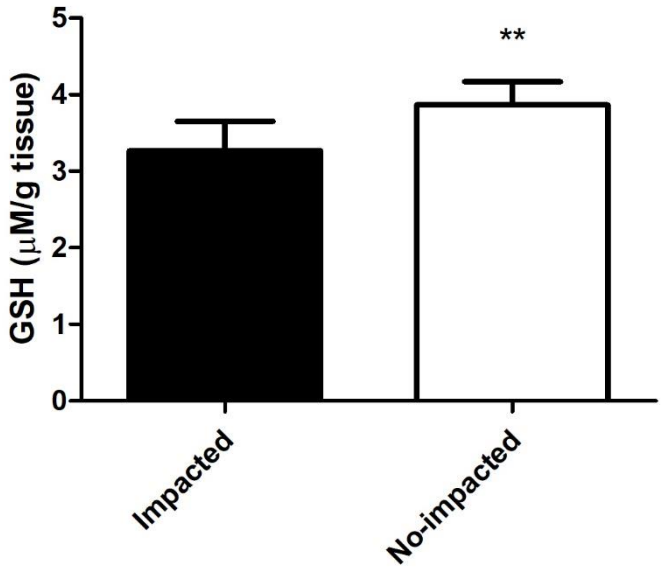
O Pb assim como os outros contaminantes ambientais pode ocasionar diversos danos à saúde humana, a exemplo, anomalias comportamentais e retardo intelectual, não apresenta efeitos benéficos ou nutricionais para os organismos, logo, é considerado extremamente

tóxico (LIMA et al., 2015). A contaminação por Pb na maioria das vezes é ocasionada pela ação antrópica, tais como: industrias de baterias, siderúrgicas e atividades de mineração (ALVES et al., 2008).

A concentração de Pb na área impactada variou 0,25 a 3,79 µg/g, apenas uma amostra apresentou o valor em desacordo com limite máximo permitido (2,0 µg/g). Estudo desenvolvido por Cruz et al. (2015), encontraram resultados semelhantes para a concentração de Pb em músculo de peixes, variando de 0,54 a 3,20 µg/g. Esses resultados estão de acordo com as concentrações de Pb obtidas por Repula et al. (2012), em amostras de músculo de peixes (Tilapias) variando de 0,35 a 2,56 µg/g e Forero; Mantilla e Martínez (2009) em músculo de *Eremophilus mutisii* variando de 3,0 a 3,5 µg/g. Tais contaminantes identificados na área impactada representam risco para a população que depende de forma direta ou indireta da pesca, uma vez que, o músculo constitui a maior massa consumida do peixe. Assim, existe a possibilidade de biomagnificação desses contaminantes para os seres humanos.

Na avaliação dos biomarcadores de estresse oxidativo, os níveis de GSH na área não impactada variou de 2,02 a 5,09 µM/g tecido. Na área impactada variou de 0,75 a 5,44 µM/g tecido, tais resultados quando comparados apresentaram diferença estatisticamente significativa no teste t de Student ($p<0,001$) figura 02.

**Figura 02.** Níveis de GSH nas amostras do fígado do *P. squamosissimus*, oriundos da área impactada e não impactada. Valores expresso em medias ± SEM (erro padrão da média). O asterisco (*) marca diferença significativa (p<0,05).



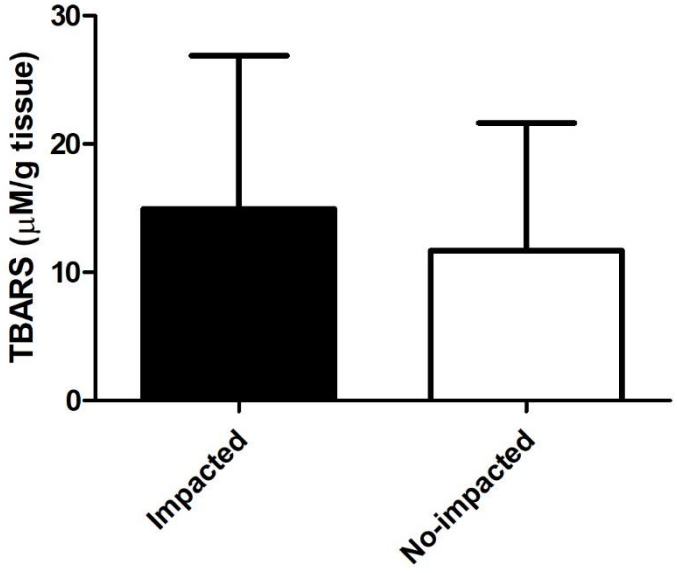

Esses resultados podem ser justificados pela elevada concentração de metais (Cd, Cr e Pb) identificadas no presente estudono tecido muscular do *P. squamosissimus*na área impactada, sendo que, os íons metálicos são indutores de estresse oxidativo, atuando por dois mecanismos, sendo: metais com potencial redox (Fe, Cu, Cr e V) que produzem ERO, por exemplo o Cr hexavalante, que por meio da reação de Fenton produzirá radicais hidroxilas que consequentemente atuarão como oxidantes (SHI; DALAL, 1990) e metais sem potencial redox (Hg, Ni, Pb e Cd) que prejudicam a defesa antioxidante, por meio do consumo de tios e enzimas (SEVCIKOVAN et al., 2011). Diante do exposto, podemos inferir que GSH apresentou-se como um bom biomarcador, considerando que, a concentração de Cd, Cr e Pb na área impactada estão provavelmente relacionadas com os baixos níveis de GSH no referido grupo.

Estudo desenvolvido por Almeida et al. (2009), ao avaliarem biomarcadores de estresse oxidativo em peixes (*Oreochromis niloticus*) expostos ao Cd na concentração de 0,75 mg/L, identificaram que o mesmo afetou as enzimas antioxidantes, com redução significativa da glutationa peroxidase. Silva et al. (2015), encontraram resultados semelhantes ao nosso estudo, ao avaliarem *Astyanax sp*. e *Danio rerio* oriundos de duas áreas (impactada e não impactada) na qual, obtiveram concentrações estatisticamente significativa da glutationa e de

outros biomarcadores do estresse oxidativo quando comparado os grupos. Os autores do referido estudo, atribuem esses efeitos a ação antrópica. Além disso, Gallego; Benavides e Tomaro (1996) e Hansen; Zhang e Jones (2006), identificaram em seus estudos a redução na concentração de GSH na presença de íons metálicos.

Os níveis de SRAT da área não impactada variou de 3,59 a 35,3 µM/g tecido. Na área impactada variou de 2,90 a 40,9 µM/g tecido, quando comparados não apresentaram diferença significativa (figura 03). Senhorin et al., (2014) ao avaliarem o efeito agudo do herbicida glifosato em *Pseudoplatystoma sp* observaram aumento do SRAT no fígado e diminuição da atividade antioxidante. Resultados semelhantes foram evidenciado por Toni et al. (2011), ao avaliaram o estresse oxidativo em peixes (*Cyprinus carpio*) exposto de forma aguda a diferentes concentrações de fungicida, obtiveram aumento significativo nos níveis das SRAT enquanto os níveis da defesa antioxidante enzimáticas e não enzimáticas foram diminuídas. Ambos resultados divergem dos encontrados em nosso estudo, no entanto, a menor disponibilidade no GSH pode levar a peroxidação lipídica da membrana celular e consequentemente elevação da concentração das SRAT.

**Figura 03.** Níveis de SRAT nas amostras do fígado do *P. squamosissimus*, oriundos da área impactada e não impactada. Valores expresso em medias ± SEM (erro padrão da média). O asterisco (*) marca diferença significativa ($p<0,05$).



Contudo, Nagasaraswathi et al. (2013), descrevem que esse processo só ocorrerá se a primeira linha de defesa antioxidantes (enzimáticas e não enzimáticas) não neutralizarem os agentes oxidantes. Assim, o presente trabalho evidenciou alterações nas linhas de defesa antioxidante pela redução dos níveis de GSH, no entanto, não houve modificação estatisticamente significativa nos valores das SRAT.

## 6.5 CONCLUSÃO

A partir das análises do tecido muscular do *P. squamosissimus* oriundo da área impactada foi possível identificar aumento na concentração de Cd, Cr e Pb acima do valor estabelecido pela legislação brasileira em vigor, além disso, tais metais provavelmente estão induzindo a formação de ERO, justificada pela diminuição do GSH nas amostras provenientes da área impactada. No entanto, tais efeitos estão sendo neutralizados pelo referido biomarcador, uma vez que, os níveis das SRAT entre os pontos de coletas, não apresentaram diferença estatística significativa quando comparadas. Desta forma, em nosso estudo o GSH foi um excelente agente antioxidante, pois não identificamos peroxidação lipídica. Nossos resultados ressaltam, portanto, a necessidade de avaliação de tais metais na população que consome a biota oriunda da área impactada.

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## 7 CONCLUSÕES GERAIS

Nosso estudo evidenciou que a área do Elesbão se encontra impactada, com concentração de metais acima do limite estabelecido pela legislação brasileira, tanto em amostras de água, como valores elevados para Cd, Cu e Pb, quando para amostras do tecido muscular do camarão com valores anormais para Cr e Pb, assim como, em amostras no tecido muscular do peixe, com concentração elevadas de Cd, Cr e Pb. Tais metais podem atuar por via redox (oxido-redução) ou consumindo os agentes antioxidantes.

A concentração do GSH em ambas as amostras biológicas quando comparadas entre as duas áreas apresentaram diferença significativa. No entanto, as SRAT não apresentaram diferença significativa, logo podemos inferir a não ocorrência de peroxidação lipídica.

Desta forma, em nosso estudo o GSH foi um excelente agente antioxidante, justificado pela não peroxidação lipídica, identificado nos valores das SRAT, na qual não apresentaram diferença estatística significativa entre os pontos de coletas em ambas as amostras. Nossos resultados ressaltam, portanto, a necessidade de avaliação de tais metais na população que consome a biota oriunda da área impactada, uma vez que, tais metais estão presentes nos dois níveis tróficos avaliados.

Por fim, o GSH apresentou-se como um bom biomarcador em ambas as amostras, no entanto, o *P. squamosissimus* em nosso estudo não foi um bom bioindicar de contaminação ambiental, fato justificado pela dificuldade da captura das amostras durante o desenvolvimento da pesquisa. Umas das características dos bioindicadores é abundância em um determinado ecossistema. Assim, a partir dos resultados podemos observar que nossa hipótese foi aceita, uma vez que, o *M. amazonicum* apresentou-se como um excelente bioindicador de contaminação ambiental. Tanto o *M. amazonicum* quando *P. squamosissimus* apresentaram ótimos valores nutricionais em especial proteicos com baixo teor de gorduras.

ANEXO A- Confirmação do artigo aceito no periódico Journal of Agricultural Science and Technology A & B USA

*From Knowledge to Wisdom*

***Journal of Agricultural Science and Technology A & Journal of Agricultural Science and Technology B*, USA**

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ANEXO B- Confirmação de submissão ao periódico Ecotoxicology and Environmental Safety

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ANEXO C- Confirmação de submissão ao periódico PLOS ONE

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ANEXO D- Confirmação de submissão ao periódico Novos cadernos – NEA.

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