Acta Scientiarum
http://www.periodicos.uem.br/ojs/
ISSN on-line: 1807-863X
Doi: 10.4025/actascibiolsci.v46i1.73039

GENETICS

Erythrocyte changes and ecotoxicological evaluation in freshwater of the Pintado stream in the municipality of Ji-Paraná, Brazil: Anurans of the genus *Leptodactylus* (Leptodactilidae) as a bioindicator organism

Jacqueline Lopes dos Santos[1], Patrícia Pastana de Sousa[1], Natália Faria Romão[1], Matheus Venancio de Paula[2], Ana Carolina Anchieta Adriano[2], Sandro de Vargas Schons[2], Jerônimo Vieira Dantas Filho[1,2*] and Francisco Carlos da Silva[1]

[1]Grupo de Estudo e Pesquisa em Biomonitoramento Ambiental, Centro Universitário São Lucas Ji-Paraná Afya, Av. Eng. Manfredo Barata Almeida da Fonseca, 542, Jardim Aurelio Bernardi, 76907-524, Ji-Paraná, Rondônia, Brazil. [2]Grupo de Pesquisa em Patologia Animal no Bioma Amazônico, Universidade Federal de Rondônia, Rolim de Moura, Rondônia, Brazil. *Author for correspondence. E-mail: jeronimovdantas@gmail.com

ABSTRACT. The main aimed of this study was to conduct out an ecotoxicological assessment through the Micronucleus (Mn) test and other associated anomalies in peripheral erythrocyte cells, using the amphibian of the genus *Leptodactylus* as a bioindicator organism, in addition to analyzing the physicochemical parameters in freshwater of the Pintado stream located in urban perimeter of Ji-Paraná municipality, Rondônia State, Brazil. Sample collections were conducted out at three different points along the stream. The results obtained showed that the animals at points 2 and 3 had a higher frequency of Mn compared to point 1, indicating that there is exposure of the organisms to genotoxic and mutagenic agents has been undergoing changes at the cellular level. The physicochemical results in the water, according to CONAMA Resolution No. 357/2005, that the parameters iron, ammonia nitrogen, dissolved oxygen and electrical conductivity showed high values at monitored points 2 and 3.

Keywords: environmental monitoring; *Leptodactylus fuscus*; *Leptodactylus petersii*; micronucleus test; mutagenicity.

Received on July 23, 2024
Accepted on September 24, 2024

Introduction

Rapid population growth combined with the urbanization process have become the most impactful factors for water pollution. The population tripled in this new millennium, with about 85% of the Brazilian population living in urban areas, a fact that directly impacts water quality (Mello et al., 2020). Although population growth is evident, investments in sanitary infrastructure are still extremely low, and this has a negative impact, since one of the biggest problems in urban centers is wastewater treatment (Ferreira, Graziele, Marques, & Gonçalves, 2021). After the decade 1990s, there was a greater investment in basic sanitation in Brazil, although unevenly, that is, a large part of the population still does not have access to this adequate structure (IBGE, 2012).

Over the years, the need arose to deepen studies on the chemical composition of water and the influence of these compounds on environmental and human health. In this context, the tests that are carried out with bioindicator species serve as monitoring, to know the consequences of these pollutants on the environment, and how they can affect the quality of life of organisms (Parmar, Rawtani, & Agrawal, 2016; Cristo, Silva, Moreira, & Silva, 2017).

The Micronucleus test (Mn) is widely used for this purpose, since its execution is low cost, practical, and analyzes the frequency of defects in the cell's DNA (Hayashi, 2016). Micronucleus appear in daughter cells because of damage to parental cells. They originate from chromosomal fragments that may not integrate the nucleus of that daughter cell at the end of mitosis, a fact that originates a nuclear membrane around the fragment, which will be possible to visualize as a micronucleus next to the main nucleus of the cell (Silva et al., 2018; Sommer, Buraczewska, & Kruszewski, 2020).

The justification for this study is because amphibians are considered excellent environmental indicators, as they are highly sensitive to the quality of the environment, such as water quality. The skin of amphibians is thin and rich in blood vessels, and it is through it that amphibians interact with the environment. The *Leptodactylus* genus of frogs is terrestrial and aquatic in habit, so these amphibians are sensitive to both pollutants in soil and water. Characterized as an excellent bioindicator of environmental quality, in addition to the fact that frogs of the genus *Leptodactylus* are widely distributed in Amazonian streams.

Given the relevance of the topic, the aimed of this study were to evaluate the physicochemical quality in freshwater samples collected at the monitored points and to analyze the genetic integrity of cell samples from the peripheral blood of amphibian specimens of the genus *Leptodactylus*, which occur at the monitored points through the Micronucleus test (Mn).

Material and methods

Study area and sampling site

The municipality of Ji-Paraná (Figure 1) is in Rondônia State, Brazil. This municipality has an area of 6,896.649 km^2 and 136,825 inhabitants, according to the 2022 population census (IBGE, 2022). Ji-Paraná is in the second most important hydrographic basin in Rondônia State, and its main freshwater course is the Machado River, this River cuts the urban area in half. Machado River is the main tributary of the Madeira River, which in turn is the main tributary of the Amazonas River. The extension of the Machado River is about 80 thousand km^2, a fact that makes it the largest hydrographic basin inserted entirely in Rondônia State and its location is in the eastern portion, bathing several municipalities and serving for purposes such as freshwater supply for the population, agricultural irrigation, tourism and leisure. The Machado River is formed by the junction of two Rivers, the Barão de Melgaço River and the Pimenta Bueno River, being the point of confluence of these two Rivers near the city of Pimenta Bueno, in Rondônia State, Brazil.

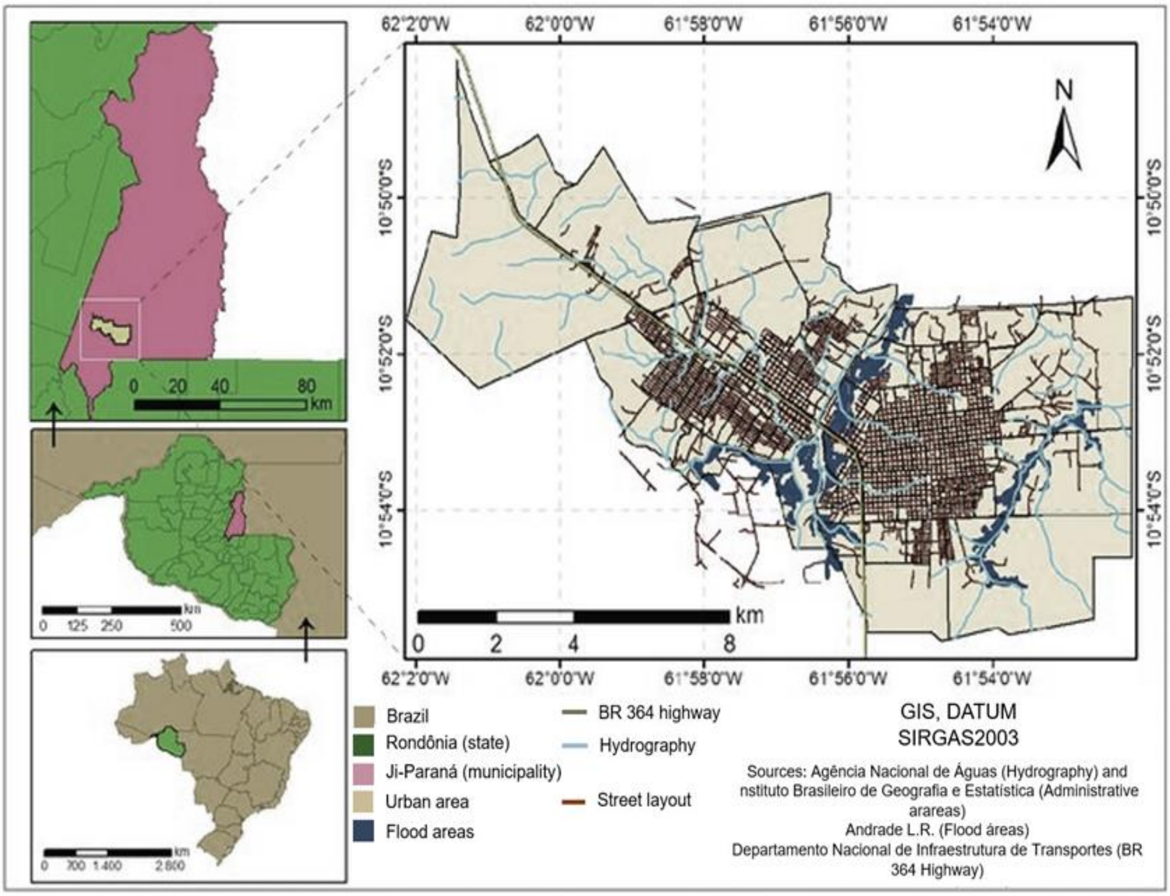

Figure 1. Geographic municipal and urban area location of the Ji-Paraná city, Rondônia State, Brazil. Source: Authors' archive.

The study area is the Pintado stream tributary located in the urban area and on the east site of the Machado River. The Pintado stream it is established in urban area of the Ji-Paraná city, it is 3.46 km long, its subbasin has an area of 3.95 km^2, and most of it has low vegetation. This subbasin has an elongated shape, close to rectangular, and this fact directly influences the possibility of less flooding, because the inclination and compaction of the soil facilitate the flow of water. There are lower points, where flooding can occur (Rogger et al., 2017).

The water samples for physicochemical analysis and the biological material (anurans of the genus *Leptodactylus* (Leptodactylidae) were collected at 3 points previously demarcated along the course of the Pintado stream, in urban area of the Ji-Paraná city (Figure 2), being Point 1 (10°52'41.5" S and 61°55'01.4" W) near the source, Point 2 (10°52'53.6" S and 61°55'36.5" W) intermediate region and Point 3 (10°52'35.2" S and 61°56'12.1" W) close to the point of confluence with the Machado River.

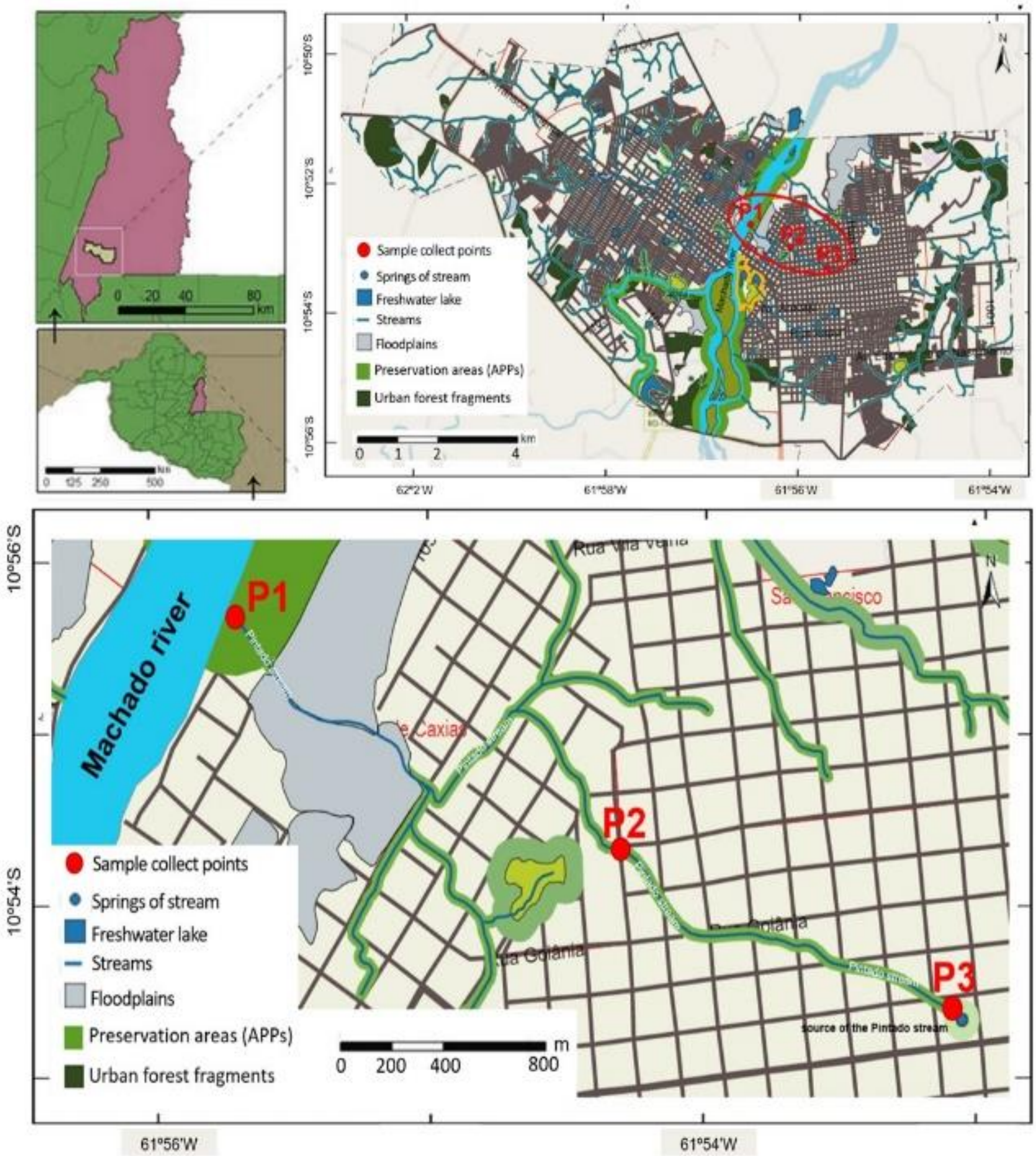

Figure 2. Location of sampling points in the Pintado stream belonging to urban area of the Ji-Paraná city, Rondônia State, Brazil. Source: Authors' archive.

Climatological informacion

The climate of the Rondônia State is classified in the Köppen system as predominantly Am - Tropical Rainy Climate (Alvares, Stape, Sentelhas, Gonçalves, & Sparovek, 2013). Being the climatological average of the air temperature, during the coldest month, superior to 18 °C (megathermal) and and a well-defined dry period, when there is a moderate water deficit with rainfall indexes below 50 mm per month. The average annual rainfall varies between 1,400 mm and 2,600 mm year^{-1}, while the monthly average air temperature varies between 24 and 26°C (CPTEC/INPE, 2022). The rainfall data during the development in the current study were obtained at the *Instituto Nacional de Pesquisas Espaciais* (INPE), *Centro para Previsão do Tempo e Estudos Climáticos* (CPTEC), in Ouro Preto do Oeste city, Rondônia State, Brazil.

Sample collections

After approval by the Commission for Ethics in Use with Animals (CEUA), at the *Universidade Federal de Rondônia* (UNIR), with Protocol No. 041-2022-A-CEUA-UNIR and permission from the Authorization and Information System on Biodiversity (SISBIO) registration No. 84820-1, and with authorization code No. 0848200120220914.

According to Tsukada et al. (2023), the sampling strategy was manual active search, with sampling effort of at least 3 and a maximum of 5 animals per sampling per night 6 to 8 pm. The collections of bioindicator samples were carried out dry season (performed 3 times) between the months of August and September of the year 2022, at night, according to the habits of amphibians of the genus *Leptodactylus*, at established collection points (Figure 3). Nine specimens were captured at the three different points monitored along the stream, totaling 27 amphibians captured manually. The amphibians used were of the species *Leptodactylus fuscus* and *L. petersii* (Leptodactylidae) (Figure 3).

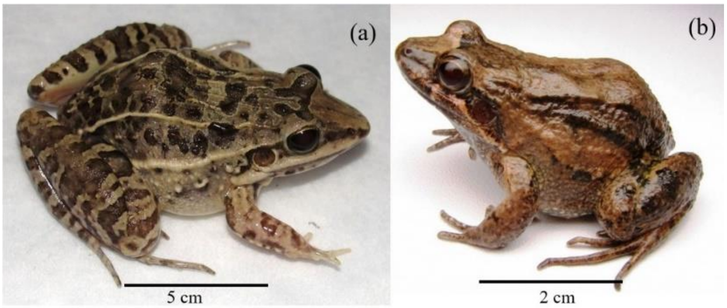

Figure 3. Adult amphibian species of *Leptodactylus fuscus* (a) and *L. petersii* (b). Source: Authors' archive.

With moistened hands and with nitrile gloves, immediately after capture, the animals were placed in a tray with water to measured and the collect procedure performed, with a puncture of 10 μL of peripheral blood in the femoral vein of the right hind limb. To perform this procedure, a 1 mL syringe with a 13 mm x 0.45 mm needle was used, one syringe for everyone. The blood smear of each animal was performed in the field, quickly after the collect in duplicate, for this, new and clean blades and extenders were used. Then the animal was immediately returned to its habitat. After the end of the analyzes, the results were computed in the Microsoft Office Excel software, then the data were transferred and analyzed in the OriginPro 2017 statistics program (OriginLab®).

Biological assessment

After drying the smears, the slides were immediately taken to the laboratory for staining of blood cells, using the Quick Panotic Kit. The kit consists of three components, namely: 0.1% triarylmethane, 0.1% xanthenes and 0.1% thiazine, which are used in that order, respectively (CONAMA, 2005). Each slide was dipped 10, 20 and 30 times in the dyes, following the order described above. At the end of this procedure, the slides were placed to dry at room temperature and the microscopic analysis procedure was performed. Microscopy consisted of counting 1000 erythrocyte cells per slide, totaling 2,000 cells per sample, using a 100X sub-immersion objective (Oliveira et al., 2022).

Physicochemical analysis

The following parameters were evaluated in the collected freshwater: total hardness, total alkalinity, dissolved oxygen, nitrate, nitrite, pH and conductivity using the physicochemical technical kit for freshwater provided by the company ALFAKIT® following the methodology proposed in the kit. To carry out the physicochemical analyses, 500 mL of freshwater were collected in the morning at the three collect points, in a colorless glass reagent bottle previously autoclaved and stored in a refrigerated thermal box and then immediately taken to the soil laboratory af the *Centro Universitário São Lucas*, Ji-Paraná city, for carrying out the analyses. Other extremely important parameters were also evaluated, namely: Aluminum (Al), Copper (Cu), Total Iron (Fe), Manganese Mn), Total Ammoniacal Nitrogen (NA), Sulfate and Zinc (Zn). Around 300 mL of freshwater were collected in sterilized containers, packed in a thermal box and sent to the Qualittá Environmental Analysis Laboratory, Ji-Paraná city, Rondônia State, Brazil.

The results of the samples were compared with the values determined through CONAMA Resolution No. 430/2011 (CONAMA, 2011) that amends and complements Resolution No. 357/2005 (CONAMA, 2005), established for freshwater conditions to be released from effluents, which is determined in Art. 4th the maximum value allowed that a certain pollutant can introduced into the water body, without affecting the quality of the water and its use, which is established through the classification classes.

Statistical analyzes

The data obtained were stored and organized in the Epi info™ software, version 3.5.3 - 2011 (OS: MS-Windows, C Sharp programming language). After being organized, the data were submitted to statistical analysis, using the arithmetic mean (μ), standard deviation (σ), and total amplitude, for a better visualization of the results. Then, the data were submitted to the Shapiro-Wilk test (α= 0.05) to verify if the samples came from a normal distribution (H_0). After verifying the normality and homoscedasticity of the data, average tests were applied. The averages of three monitored points were submitted to the Tukey's test, at 5% of significance.

A Pearson's correlation analysis correlation analysis was applied between the physicochemical data (the one above the limit) of the water with the abnormalities found in the blood cells.

All statistical analysis were performed using RStudio Development Core Team, version 3.5.3.

Results and discussion

The average annual rainfall varied between 1,400 mm and 2,600 mm year^{-1}, while the monthly average air temperature varied between 24 and 26 °C. Regarding meteorological data, the months of October 2021 (200 mm), December 2021 (189.50 mm), January 2022 (336.05 mm), and February 2022 (359.66 mm), showed the highest precipitation averages. While the months of August 2021 and September 2021 had the highest average temperatures, >35°C, respectively. There were no significant variations in the other months (Figure 4).

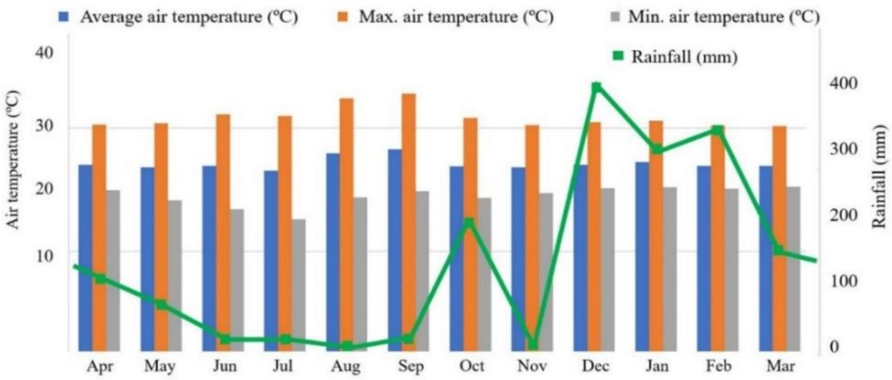

Figure 4. Monthly average rainfall (mm) and air temperature in Ji-Paraná city over the past few months.

Regarding relating the rainfall indices with the collection period in the months of August and September, it is possible to understand that the study was carried out in the most critical dry season of the last few months. Therefore, it is possible that material was collected during a period of higher concentration of nutrients and chemical residues in Pintado stream. The results of the physicochemical analysis in the water from Pintado stream showed that at sampling points 2 and 3, the total iron parameter is above the limit allowed by CONAMA Resolution No. 357/2005, with a difference between both (Table 1).

Although the element iron is of low toxicity, when it has a very high concentration it becomes a contaminant, as it affects the quality of water, both for consumption and for the ecosystem (Nelim, Santos, & Nascimento, 2019). There are several reasons why the presence of iron is undesirable in a body of water, some of which are: the reddish color when exposed to air (due to oxidation), it gives a metallic or bitter taste, favors bacterial growth that depends on iron, such as for example *Spyrophylum ferrugineum*, among others (Authman, Zaki, Khallaf, & Abbas, 2015).

Another parameter evaluated with a value above the established limit was the ammoniacal nitrogen, shown a high value in points 2 and 3 was 3.7 mg L^{-1}. Nitrogen can naturally present in shallow or underground waters, the concentration being very low and therefore not harmful to the water. This occurs because it is a compound easily adsorbed, either by the soil or by oxidation to nitrate and nitrite. However, high concentrations are associated with sources of pollution (Khatri, Tyagi, & Rawtani, 2017). It is important to point out that total ammoniacal nitrogen varies according to pH according to legislation, the following ratio: 3.7 mg L^{-1} for pH ≤ 7.5; 2.0 mg L^{-1} for pH 7.5 - 8.0; 1.0 mg L^{-1} for pH 8.0 - 8.5.

Table 1. Physicochemical characterization in water samples collected from the Pintado stream located in Ji-Paraná city, Rondônia State, Brazil.

Parameters	Sampling coletcs			
	Point 1	Point 2	Point 3	MVP
Total hardness (mg L^{-1})	160.0	199.0	165.0	500.0[b]
Total alkalinity (mg L^{-1})	20.0	91.0	120.0	-
Salinity (ppm)	29.0	82.0	135.0	>30.0[a]
Dissolved oxygen (mg L^{-1})	9.25	8.45	19.0	>5.0[a]
pH	6.0	8.0	8.0	6-9[a]
Nitrite (mg L^{-1})	0.1	0.5	0.5	1.0[a]
Nitrate (mg L^{-1})	1.0	1.0	0.7	10.0[a]
Conductivity	210.0*	300.0*	290*	100.0[c]
(μs cm^{-1})	0.0	0.0	0.0	≤0.1[a]
Aluminum (mg L^{-1})	0.0	0.0	0.0	≤0.009[a]
Copper (mg L^{-1})	0.19	1.38*	3.05*	≤0.3[a]
Total iron (mg L^{-1})	0.0	0.0	0.0	≤0.1[a]
Manganese (mg L^{-1})	0.94	11.34*	12.47*	≤3.7; 2.0; 1.0[a]
Total ammoniacal nitrogen (mg L^{-1})	3.1	15.0	10.5	≤250.0[a]
Sulfate (mg L^{-1})	0.0	0.0	0.0	≤0.18[a]

Reference values: CONAMA No. 357/2005. MPV: Maximum Permissible Value. *values above the established limit. [a]Values established by CONAMA Resolution No. 357. [b]Values established by the Ministry of Health, Ordinance No. 2,914/2011. [c]Values established by the Environmental Company of the São Paulo State - CETESB 2012.

The main source of hardness is the dissolution of minerals in water bodies, such as limestone and industrial effluents. In water bodies where the hardness is low, the biota becomes more sensitive to toxic products such as lead, zinc, copper and others (Moloantoa, Khetsha, Heerden, Castillo, & Cason, 2022). The Ministry of Health, through Ordinance No. 2914/2011 (Khatri et al., 2017), establishes that the maximum value allowed for the total hardness parameter is 500 mg L^{-1}, although it is necessary that the result is reliable since it can vary according to some factors, such as equipment used and analysis standard.

Alkalinity is a very relevant parameter, as it indicates how much acidity a solution can absorb without changing its pH, that is, alkalinity indicates the buffering capacity of a solution such as water (Piratoba, Ribeiro, Morales, & Gonçalves, 2017; Moloantoa et al., 2022). Thus, solutions with low alkalinity have a lower buffering capacity and consequently their pH can change precipitately when an acidic substance is added. On the other hand, when a solution has high alkalinity, its buffering capacity becomes greater and as a result it is less affected when an acidic substance is added, requiring a large amount of acid to change the pH that would occur in a sample with low alkalinity (Brasil, 2011).

According to Piratoba et al. (2017), the alkalinity of water does not have a health significance, except when it occurs due to hydroxides or when it contributes to the quality of total solids, which is composed of three main constituents such as hydroxides (OH^{1-}), bicarbonate (HCO_3-) and carbonates (CO_3^{2-}). The differentiation between the three forms of alkalinity in water varies according to pH: pH > 9.4 for hydroxides and carbonates; pH between 8.3 and 9.4 for carbonates and bicarbonates and pH between 4.4 and 8.3 for bicarbonates only. According to studies, in most aquatic environments, alkalinity is caused by the presence of bicarbonate. Normally, increased values of alkalinity are caused by the decomposition of organic matter and the release of carbon dioxide into the water, caused by the high respiratory rate of microorganisms. Most natural waters present alkalinity between 30 and 500 mg L^{-1} of $CaCO_3$ (Gaspar et al., 2018).

The consumption of oxygen present in water is given by the oxidation of organic matter and by the respiration of the organisms present. In natural waters, oxygen is essential for the survival of species, as most do not resist if there are concentrations below 4.0 mg L^{-1}(Shmeis, 2018). During the period when the organic matter present in the water is stabilizing, the bacteria use oxygen in the respiratory process, reducing the concentration of dissolved oxygen in water bodies or sewage (Pinto, Oliveira, & Pereira, 2010). According to Moloantoa et al. (2017) and Ribeiro, Sandro, and Boêno (2013), the high concentration of ammonium ion can influence the presence of dissolved oxygen in the environment, which can trigger ecological complications, because if there are high concentrations (above the limit established in the resolution) it can become toxic for some species.

Conductivity showed altered values at points 1, 2, and 3. According to CETESB (2012), conductivity values above 100 µS cm_{-1} are considered high, having an impact on water quality. CONAMA Resolution No. 357/2005 does not establish a limit value for this parameter. Conductivity measures the potential of water to conduct electric current and is altered by the number of ions present. It is a parameter that allows the actual verification of pollution or the possible source of local pollution, although it does not identify which chemical compound is responsible for such alteration. Increased electrical conductivity values can be associated with the presence of several compounds improperly dispensed into the water body, such as some types of detergents and soap (Ribeiro et al., 2013).

Pearson's correlation analysis showed that the abnormalities of binucleated undergoing cells apoptosis and spicules on the membrane, showed a high correlation ($r > 0.80$; $p < 0.05$) with Cu and Mn although low correlation ($r < 0.50$; $p < 0.05$) with the ammoniacal nitrogen and electrical conductivity variables. This information confirms that the mutations occurred due to pollution caused by agricultural and domestic effluents dumped into the Pintado stream (Figure 5).

(*r*)	Mn	Cu	NA	EC
Binucleated	0.88	0.73	0.40	0.22
Apoptosis	0.91	0.79	0.44	0.20
Spicules	0.94	0.83	0.30	0.11

Levels of Manganese (Mn) and Copper (Cu), ammoniacal nitrogen (NA), and electrical conductivity (EC).

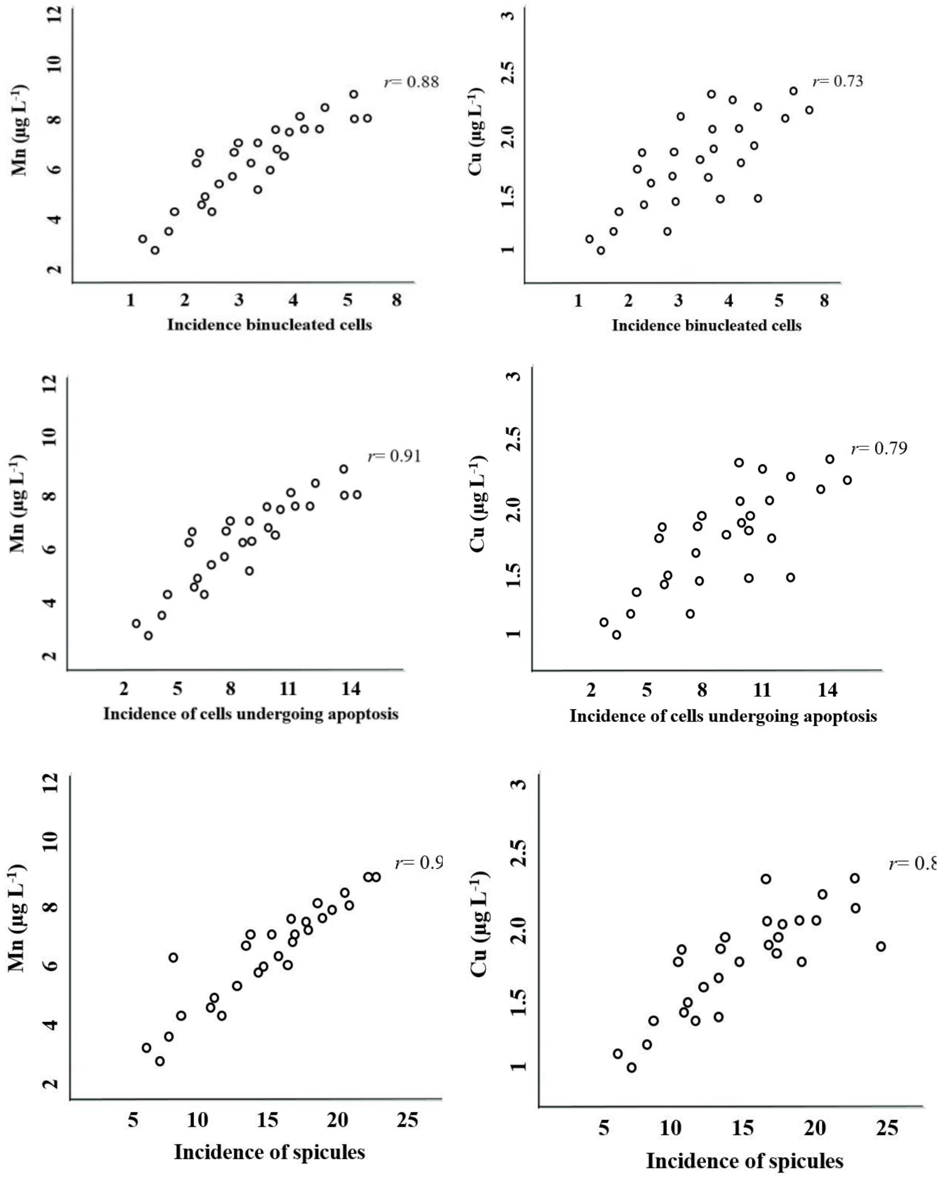

Figure 5. Pearson's correlation analysis between the physicochemical data of the Pintado stream water with the abnormalities found in the blood cells.

Alves et al. (2014) conducted an environmental monitoring study to determine the contents of 13 trace elements (As, Be, Cd, Cr, Cu, Pb, Mn, Hg, Ni, Tl, Sn, V, and Zn) in samples of surface water and sediments from Pardo River, São Paulo State, Brazil. A value of up to 43.01 μg L^{-1} of Mn was found. As in the current study, Mn showed the highest average concentrations both in the water and in the sediments, evidencing the incidence of agricultural activity and the geological characteristics of the hydrographic sub-basin.

Palacio et al. (2005) carried out a biomonitoring of water quality in Toledo River, Paraná State, Brazil. It was carried out using common onion (*Allium cepa* L.) as a bioindicator of heavy metal toxicity in water. For test liquids containing less than 30 μg L^{-1} of dissolved Cu, root growth was reduced by 40%. The results revealed high toxicity above 10^3 μg L^{-1}, with critical levels from 6 x 10^3 μg L^{-1} of Copper. While values of 7.3 x 10^3 μg L^{-1} of Cu were found in the Toledo River, confirming the high industrial contamination.

Concerning the biological analysis performed using the Mn to assess the presence of mutagenicity in cells, it demonstrated some types of abnormalities, namely: presence of micronucleus, binucleated cell, apoptosis and spicules in the cell membrane, as shown in Figure 6.

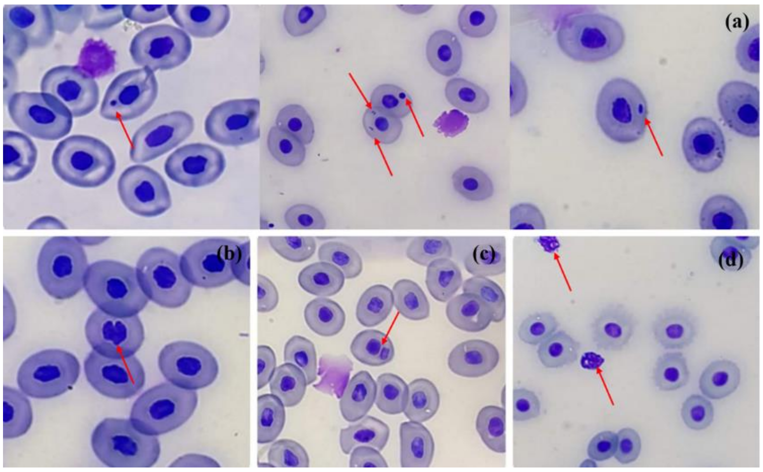

Figure 6. Micronucleus and anomalies observed in *Leptodactylus* erythrocytes. (a) cell with micronucleus; (b) cell in the process of division; (c) binucleated cell; (d) presence of spicules on the membrane.

According to the Table 2 above, the monitored points with the highest frequency of micronucleus in erythrocytes were points 2 and 3. The significant increase in relation to point 1 may be related to the presence of accumulated domestic and agricultural effluents in these places, coming from the entire course of the Pintado stream, these may be contaminated with toxic chemicals that lead to alteration of the local ecosystem. It is worth noting that the source of the stream is in uninhabited land and, although there are houses in the surroundings, its source is covered by secondary forest that protects the source, the flow of water from the source does not allow stagnant water to accumulate in the area. Although the water body seems protected and apparently there is little anthropogenic influence (small number of houses), chemical contamination and pollution can spread over long distances through rain, wind and groundwater.

Table 2. Frequency of micronucleus (Mn) occurrence in polychromatic erythrocytes (EPC) of *Leptodactylus* (for every 2000 erythrocytes analyzed), captured at the monitored points of the Pintado stream in Ji-Paraná city. Point 1 (source of the stream in the urban perimeter), 2, and 3 (within the urban perimeter).

Points	Mean ± SD
1	4.8 ± 2.6
2	35.8 ± 4.8*
3	71.7 ± 14.8**

N = 6 animals per point. The animals were captured and released in the same place immediately after blood collection. Mn: micronucleus, EPC: polychromatic erythrocytes. *$p < 0.05$: significant difference compared to point 1 (stream source in the urban perimeter) (ANOVA test, Tukey). Subtitle: The author, using Tukey's ANOVA test.

The chemical compounds present in effluents have an affinity for the genetic material of organisms, which can trigger changes in the DNA (Silva et al., 2018). Some compounds present in these effluents are metals, pesticides, hydrocarbons, among others, and have toxic and mutagenic potential. This type of substance that is released into the water may not have acute effects on the organisms that are exposed to it, although studies shown that it can cause tissue and genetic damage, drastically reducing the survival time of the species (Lima

& Santos, 2012). The continuous exposure of these animals to the chemical compounds presents in the aquatic environment in which they live, may be triggering bioaccumulation in the tissues, causing cellular damage, inhibiting the action of repair enzymes, modifying the DNA and negatively interfering in the physiology of the exposed organism (Galindo, Silva, & Rosário, 2012).

The normal amount of micronucleus found in erythrocytes varies according to species and is related to the repair capacity of the cell. Exposure to xenobiotics affects this repair capacity, therefore erythrocytes that have Micronucleus (Mn) reflect the genotoxic effects to which the organism is exposed (Fett-Conte & Sales, 2002).

Silva et al. (2018) carried out an ecotoxicological analysis of the water from the Ouro Preto stream in Ji-Paraná city. The authors performed the Mn test, nuclear morphological changes in peripheral erythrocytes, and concluded that there was a significant increase in the frequency of anomalies in the dry season, indicating the genotoxic and mutagenic potential of water. Nilin et al. (2019) developed studies with several ecotoxicological evaluation methods in different lentic and lotic aquatic environments, and concluded that in the dry season the volume of pollutants carried into aquatic environments is highly concentrated in the dry season, enhancing the ecotoxicological risk and chances of mutagenicity in native amphibians.

According to Lajmanovich et al. (2014), some studies usually observe anomalies found in cells, and not just the presence of micronucleus, as these anomalies may be related to processes involving cytotoxicity, mutagenicity and genotoxicity. Some organisms show these anomalies after being exposed to chemicals and pollutants.

The presence of morphological nuclear abnormalities, including binucleated cells, indicates possible exposure to xenobiotics, and consequently are indicators of mutagenicity (Brahan, Blazer, Shaw, & Mazik, 2017; Mehra & Chadha, 2020). Studies have shown that these alterations in cell morphology are induced by substances that have the potential to cause damage and DNA breaks, which may trigger mutations and subsequently carcinogenic processes, since damage to DNA can cause loss of genetic material and irreparable lesions (Silva et al., 2018). As shown in Figure 7a, there was no significant difference in the incidence of binucleated cells, compared to Point 1 (source of the Pintado stream).

According to AnvariFar et al. (2016), cells undergoing apoptosis (cell death) and binucleated cells are a type of self-destruction of cells that aims to maintain the body's homeostasis, occurring in a programmed manner. Cells undergoing apoptosis showed no significant difference ($p > 0.05$) for the monitored points. However, a higher binucleated cells value was observed at point 2 and a lower value at point 1 ($p < 0.05$) (Figure 7a and b). It is a fast process, with high energy demand, occurs in different stages and can stimulated by pathological processes in the organism. The incidence of cells undergoing apoptosis did not shown a significant difference between the points that were analyzed.

Another anomaly visualized during erythrocyte counting was spiculated cells (Figure 7c), although for this anomaly there was no significant difference ($p > 0.05$) between the monitored points. According to Silva et al. (2018) erythrocytes that have spicules on the membrane undergo de-characterization, by formation of bubbles or total lysis, making them similar to erythrocytes morphologically called acanthocytes.

A recent study was carried out by Silva et al. (2018) in the municipality of Ouro Preto do Oeste city, Rondônia State, Brazil, with the same genus *Leptodactylus* as a bioindicator species and obtained similar results regarding the presence of micronucleus in erythrocytes. Another study carried out by Hayashi et al. (2016) in the municipality of Ji-Paraná city, using freshwater fish (*Astyanax* sp.) as a bioindicator species, obtained significant changes in one of the monitored points, which is attributed to the large flow of vehicles, homes and commercial areas in the vicinity of the Pintado stream. Therefore, areas with human manipulation present a greater extent of damage to the ecosystem, not favoring the quality of life of the species. Therefore, it can be said that there are reflections of man's action on the ecosystem, as well as on the organisms that inhabit water bodies.

The meteorological data highlight significant rainfall in the months of January and February 2022, which correlates with periods of higher water runoff and potential nutrient dilution. Conversely, the dry season during August and September 2021 coincided with extreme temperatures (>35°C) and reduced water flow. This period likely contributed to the concentration of contaminants in the Pintado stream, as evidenced by elevated levels of total iron, ammoniacal nitrogen, and conductivity at sampling points 2 and 3. These values surpass the permissible limits outlined by CONAMA Resolution No. 357/2005, suggesting pollution linked to agricultural and domestic effluents.

The implications of these findings for local environmental management are significant. High pollutant concentrations during the dry season can exacerbate the ecological impacts on aquatic organisms, particularly amphibians used as bioindicators in this study. Elevated copper and manganese levels correlated with cellular abnormalities in *Leptodactylus* erythrocytes, further supporting the genotoxicity induced by pollutants. Local authorities should prioritize the implementation of sustainable urban and agricultural waste management systems to mitigate water contamination. Furthermore, environmental monitoring should focus on critical dry periods, when pollutants are more concentrated and pose heightened risks to the ecosystem.

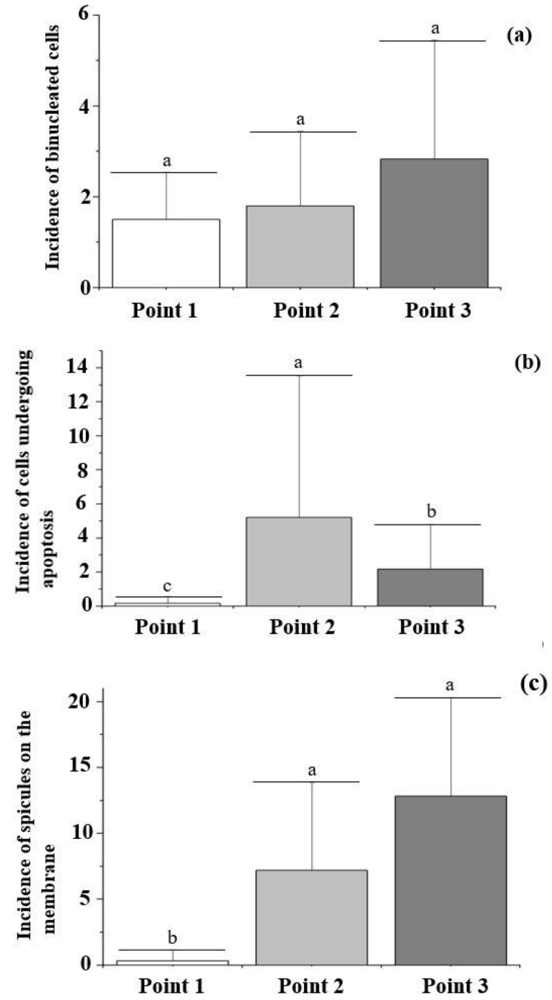

Figure 7. Incidence of binucleated cells (a), Incidence of cells undergoing apoptosis (b) and Incidence of spicules on the membrane (c), followed by the average and standard deviation found in *Leptodactylus* erythrocytes, captured at the monitored points of the Pintado steam. (N = 6), $p > 0.05$, shown no significant difference compared to Point 1 (stream source in the urban perimeter) (ANOVA test, Tukey).

The results underscore the need for urgent action in environmental conservation and management. Elevated pollutant levels, particularly during dry periods, present significant risks to aquatic ecosystems and bioindicator species like *Leptodactylus* and *Astyanax*. These findings highlight the critical need for improved waste management strategies to reduce the input of agricultural and domestic effluents into water bodies. Local authorities should prioritize the installation of sustainable sewage treatment systems, promote eco-friendly agricultural practices, and enforce regulations that limit pollutants in waterways. Additionally, continuous environmental monitoring should be intensified, especially during dry seasons when pollutant concentrations are more pronounced. Effective policies addressing these issues are essential to safeguard biodiversity, reduce genotoxic impacts, and maintain ecosystem integrity. Public awareness campaigns could further engage local communities in reducing waste and pollution, fostering a collaborative approach to environmental protection.

Conclusion

The physicochemical analysis revealed high levels of ammoniacal nitrogen, dissolved oxygen, electrical conductivity, manganese, and copper at various monitored points. Biological analysis showed significant increases in micronucleus frequency in *Leptodactylus* erythrocytes at points 2 and 3, while other anomalies like binucleated cells and apoptosis showed no significant changes. The increased micronucleus frequency indicates cellular alterations in the bioindicator species due to exposure to toxic xenobiotics. Overall, the findings suggest that environmental contamination from human activities is negatively impacting the ecosystem and contributing to ecological imbalance at the study site.

Acknowledgements

The research was supported by *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) through by *Fundação Rondônia de Amparo ao Desenvolvimento das Ações Científicas e Tecnológicas e à Pesquisa do Estado de Rondônia* (FAPERO), awarded a postdoctoral scholarship to Jerônimo Vieira Dantas Filho [167879/2022-7].

References

Alvares, C. A., Stape, J. L., Sentelhas, P. C., Gonçalves, J. L. M., & Sparovek, G. (2013). Koppen´s climate classification map for Brazil. *Meteorologische Zeitschrisft*, *22*(6), 711-728. DOI: https://doi.org/10.1127/0941-2948/2013/0507

Alves, R. I. S., Sampaio, C. F., Nadal, M., Schuhmacher, M., Domingo, J. L., & Segura-Muñoz, S. I. (2014). Metal concentrations in surface water and sediments from Pardo River, Brazil: Human health risks. *Environmental Research*, *133*, 149-155. DOI: https://doi.org/10.1016/j.envres.2014.05.012

AnvariFar, H., Amirkolaie, A. K., Miandare, H. K., Ouraji, H., Jalali, M. A., & Üçüncü, S. İ. (2016). Apoptosis in fish: environmental factors and programmed cell death. *Cell and Tissue Research*, *368*, 425-439. DOI: https://doi.org/10.1007/s00441-016-2548-x

Authman, M. M. N., Zaki, M. S., Khallaf, E. A., & Abbas, H. H. (2015). Use of fish as bio-indicator of the effects of heavy metals pollution. *Journal of Aquaculture Research & Development*, *6*(4), 1-13. DOI: https://doi.org/10.4172/2155-9546.1000328

Braham, R. P., Blazer, V. S., Shaw, C. H., & Mazik, P. M. (2017). Micronucleus and other erythrocyte nuclear abnormalities in fishes from the Great Lakes Basin, USA. *Environmental and Molecular Mutagenesis*, *58*(8), 570-581. DOI: https://doi.org/10.1002/em.22123

Brasil. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente [CONAMA]. (2011). *Resolução No. 430/2011*. Dispõe sobre as condições e padrões de lançamento de efluentes, complementa e altera a Resolução nº 357, de 17 de março de 2005, do Conselho Nacional do Meio Ambiente. Brasília, DF: CONAMA. Retrieved on Mar. 10, 2024 from http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=646

Brasil. Ministério do Meio Ambiente. Conselho Nacional do Meio Ambiente [CONAMA]. (2005). *Resulução No. 357/2005*. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. Brasília, DF: CONAMA. Retrieved on Mar. 10, 2024 from https://sicae.sisicmbio.icmbio.gov.br/usuario-externo/logout

Brasil. Ministério da Saúde. (2011). *Portaria No. 2914, de 12 de dezembro de 2011*. Dispõe sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. Brasília, DF: MS. Retrieved on Mar. 10, 2024 from https://bvsms.saude.gov.br/bvs/saudelegis/gm/2011/prt2914_12_12_2011.html

Brasil. Companhia Ambiental do Estado de São Paulo. [CETESB]. (2012). *Portaria No. 2914, de 12 de dezembro de 2011*. Dispõe sobre os procedimentos de controle e de vigilância da qualidade da água para consumo humano e seu padrão de potabilidade. São Paulo, SP: CETESB. Retrieved on Mar. 10, 2024 from https://cetesb.sp.gov.br/aguas-interiores/wp-content/uploads/sites/12/2018/01/PORTARIA-N%C2%BA-2.914-DE-12-DE-DEZEMBRO-DE-2011.pdf

Cristo, N. P., Silva, B. R., Moreira, E. G., & Silva, F. C. (2017). Ecotoxicological evaluation of dois de abril stream water using lambari (*Astyanax* sp.) as a bioindicator organism. *South American Journal of Basic Education, Technical and Technological*, *4*, 51-60. Retrieved on November 27, 2004 from https://periodicos.ufac.br/index.php/SAJEBTT/article/download/1267/942/0

Ferreira, D. C., Graziele, I., Marques, R. C., & Gonçalves, J. (2021). Investment in drinking water and sanitation infrastructure and its impact on waterborne diseases dissemination: The Brazilian case. *Science of The Total Environment*, *779*, 1-16. DOI: https://doi.org/10.1016/j.scitotenv.2021.146279

Fett-Conte, A. C., & Salles, A. B. C. F. (2002). The importance of the p53 gene in human carcinogenesis. *Brazilian Journal of Hematology and Hemotherapy*, *24*(2), 85-89. DOI: https://doi.org/10.1590/S1516-84842002000200004

Hayashi, M. (2016). The micronucleus tes - most widely used in vivo genotoxicity test. *Genes and Environment*, *38*(18), 1-6. DOI: https://doi.org/10.1186/s41021-016-0044-x

Galindo, T., Silva, E., & Rosário I. (2012). Micronucleus induction and toxicity by domestic efluente in two populations of *Bathygobius soporator* (Valenciennes, 1837) (Teleostei, Gobbidae) on the Coast of Salvador (BA), Brazil. *Brazilian Journal of Aquatic Science and Technology*, *16(1)*, 1-7. DOI: https://doi.org/10.14210/bjast.v16n1.p1-7

Gaspar, F. L., Pinheiro, B. R., Noriega, C. E. D., Araujo, M., Lefèvre, N., & Montes, M. J. F. (2018). Alkalinity, inorganic carbon and CO_2 flux variability during extreme rainfall years (2010-2011) in two polluted tropical estuaries NE Brazil. *Brazilian Journal of Oceanography*, *66*(1), 115-130. DOI: https://doi.org/10.1590/S1679-87592018149406601

Instituto Brasileiro de Geografia e Estatística [IBGE]. (2022). *Prévia da população calculada com base nos resultados do Censo Demográfico 2022 até 25 de dezembro de 2022*. Brasília, DF: IBGE. Retrieved on Mar. 10, 2024 from https://ftp.ibge.gov.br/Censos/Censo_Demografico_2022/Previa_da_Populacao/POP2022_Municipios.pdf

Instituto Brasileiro de Geografia e Estatística. Síntese de indicadores sociais [IBGE]. (2012). *Uma análise das condições de vida da população brasileira*. Rio de Janeiro, RJ: IBGE. Retrieved on Mar. 10, 2024 from https://www.ibge.gov.br/biblioteca/visualizacao/livros/liv95011.pdf

Instituto Nacional de Pesquisas Espaciais [INPE]. (2022). *Centro de previsão de tempo e estudos climáticos (CPTEC)*. Estação meteorológica de Ouro Preto do Oeste – RO. São Paulo, SP: CPTEC.

Khatri, N., Tyagi, S., & Rawtani, D. (2017). Recent strategies for the removal of iron from water: A review. *Journal of Water Process Engineering*, *19*, 291-304. *Brazilian Journal of Aquatic Science and Technology*, *19*, 291-304. DOI: https://doi.org/10.1016/j.jwpe.2017.08.015

Lajmanovich, R. C., Cabagna-Zenklusen, M. C., Attademo, A. M., Junges, C. M., Peltzer, P. M., Bassó, A., & Lorenzatti, E. (2014). Induction of micronuclei and nuclear abnormalities in tadpoles of the common toad (*Rhinella arenarum*) treated with the herbicides Liberty® and glufosinate-ammonium. *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*, *769*, 7-12. DOI: https://doi.org/10.1016/j.mrgentox.2014.04.009

Lima, A. M., & Santos, F. F. (2012). Analysis of physical-chemical properties and potentially toxic metals in water of the rio Claro, next to Jataí City - GO. *Brazilian Journal of Exact and Natural Sciences*, *14*(2), 239-255. DOI: https://doi.org/10.5935/RECEN.2012.02.05

Mehra, S., & Chadha, P. (2020). Naphthalene-2-sulfonate induced toxicity in blood cells of freshwater fish *Channa punctatus* using comet assay, micronucleus assay and ATIR-FTIR approach. *Chemosphere*, *265*, 129147. DOI: https://doi.org/10.1016/j.chemosphere.2020.129147

Mello, K., Taniwaki, R. H., Paula, F. R., Valente, R. A., Randhir, T. O., Macedo, D. R., ... Hughes, R. M. (2020). Multiscale land use impacts on water quality: Assessment, planning, and future perspectives in Brazil. *Journal of Environmental Management*, *270*, 110879. DOI: https://doi.org/10.1016/j.jenvman.2020.110879

Moloantoa, K. M., Khetsha, Z. P., Heerden, E. V., Castillo, J. C., & Cason, E. D. (2022). Nitrate water contamination from industrial activities and complete denitrification as a remediation option. *Water*, *14*(5), 1-31. DOI: https://doi.org/10.3390/w14050799

Nelin, J., Santos, A. A. O., & Nascimento, M. K. S. (2019). Ecotoxicology assay for the evalua-tion of environmental water quality in a tropical urban estuary. *Anais da Academia Brasileira de Ciências*, *91*(1), 1-10. DOI: https://doi.org/10.1590/0001-3765201820180232

Oliveira, C. A. C. R., Souto, P. S. S., & Conceição-Palheta, D. C., Bahia, M. O., Cunha, L. A., Santos, M. L. S., ... Bentes, B (2022). Genotoxicity assessment in two Amazonian estuaries using the *Plagioscion squamosissimus* as a biomonitor. *Environmental Science and Pollution Research*, *29,* 41344-41356. DOI: https://doi.org/10.1007/s11356-022-18767-1

Palacio, S. M., Espinoza-Quiñones, F. R., Galante, R. M., Zenatti, D. C., Seolatto, A. A., Lorenz, E. K., ... Tabacniks, M. H. (2005). Correlation between heavy metal ions (copper, zinc, lead) concentrations and root length of *Allium cepa* L. in polluted river water. *Brazilian Archives of Biology and Technology*, *48*(Spe.), 191-196. DOI: https://doi.org/10.1590/s1516-89132005000400024

Parmar, T. K., Rawtani, D., & Agrawal, Y. K. (2016). Bioindicators: the natural indicator of environmental pollution. *Frontiers in Life Science*, *9*(2), 110-118. DOI: https://doi.org/10.1080/21553769.2016.1162753

Pinto, A. L., Oliveira, G. H., & Pereira, G. A. (2010). Evaluation of efficiency of use of dissolved oxygen as a key indicator of quality of surface water basin stream Bom Jardim, Brasilândia/MS. *Revista GEOMAE*, *1*(1), 69-82.

Piratoba, A. R. A., Ribeiro, H. M. C., Morales, G. P., & Gonçalves W. G. (2017). Characterization of water quality parameters in the port area of Barcarena, PA, Brazil. *Ambiente e Água - An Interdisciplinary Journal of Applied Science*, *12*(3), 435-456. DOI: https://doi.org/10.4136/ambi-agua.1910

Ribeiro, E. A., Sandro, D., & Boêno, J. A. (2013). Water quality of stream due to release of effluent from cattle slaughter. *Brazilian Journal of Agricultural and Environmental Engineering*, *17*(4), 425-433. DOI: https://doi.org/10.1590/S1415-43662013000400011

Rogger, M., Agnoletti, M., Alaoui, A., Bathurst, J. C., Bodner, G., Borga, M., ... Blöschl, G. (2017). Land use change impacts on floods at the catchment scale: Challenges and opportunities for future research. *Water Resources Research*, *53*(7), 5209-5219. DOI: https://doi.org/10.1002/2017wr020723

Shmeis, A. R. M. (2018). Water Chemistry and Microbiology. *Comprehensive Analytical Chemistry, 81*, 1-56. DOI: https://doi.org/10.1016/bs.coac.2018.02.001

Silva, D. S., Silva, C. L., Portes, R. G. R., Hoffman, E., Oliveira, R. S., Martins, M., ... Silva, F. C. (2018). Ecotoxicological evaluation of water from the Ouro Preto stream using the bioindicator *Leptodactylus petersii*. *South American Journal of Basic Education, Technical and Technological*, *5*, 69-87. Retrieved on November 27, 2024 from https://periodicos.ufac.br/index.php/SAJEBTT/article/view/1709/1201

Sommer, S., Buraczewska, I., & Kruszewski, M. (2020). Micronucleus assay: The state of art, and future directions. *International Journal of Molecular Sciences*, *21*(4), 1-19. DOI: https://doi.org/10.3390/ijms21041534

Tsukada, E., Rodrigues, C. C., Jacinto, J. C. J., Franco-Belussi, L., Jones-Costa, M., Abdalla, F. C., ... Salla, R. F. (2023). The amphibian's spleen as a source of biomarkers for ecotoxicity assessment: Historical review and trends. *Science of the Total Environment*, *901*, 165915. DOI: https://doi.org/10.1016/j.scitotenv.2023.165915