Revista Brasileira de Ciências Ambientais
Brazilian Journal of Environmental Sciences

Assessment of genotoxicity biomarkers in neotropical fish species from streams of the Ivinhema River basin located in sugarcane cultivation areas

Avaliação de biomarcadores de genotoxicidade em espécies de peixes neotropicais de riachos da bacia do Rio Ivinhema localizados em áreas de cultivo da cana-de-açúcar

Vandressa Raquel Lucas Poloni Meira[1], *Lucilene Finoto Viana*[2], *Bruno do Amaral Crispim*[3,4], *Yzel Rondon Súarez*[1], *Alexeia Barufatti*[3], *Fábio Kummrow*[5], *Júlio César Jut Solórzano*[1], *Alessandra Paim Berti*[1]

ABSTRACT

The Ivinhema River basin has experienced the greatest expansion of sugarcane cultivation in the state of Mato Grosso do Sul, Brazil. The assessment of water quality through physical and chemical parameters and ecotoxicological studies, employing both bioindicators collected *in situ* and laboratory tests, provides more robust results for this region. In this context, the objective of this study was to analyze the composition and structure of the landscape around the sampling sites located in three streams (Vitória, Rosário, and Piravevê) belonging to the Ivinhema River basin to evaluate the impacts of sugarcane cultivation expansion on water quality and genotoxicity biomarkers *in situ* and *ex situ* using native fish species. In the sampling sites in the Rosário and Vitória streams, sugarcane is the main land use, while in the Piravevê stream, pasture areas represent the main land use. The acidic pH observed in the Vitória and Rosário streams seems to be related to the application of fertilizers and the fertigation in the sugarcane crop. Two species of detritivorous and five of omnivorous fishes were collected. A higher frequency of genotoxic damage was identified in detritivorous fish species. In the genotoxicity tests with *Astyanax lacustris*, the most frequent nuclear alteration induced by the water samples from all sampling sites was nuclear invagination. Our results indicated that the expansion of

RESUMO

A bacia do rio Ivinhema tem experimentado a maior expansão do cultivo de cana-de-açúcar no Estado de Mato Grosso do Sul, Brasil. A avaliação da qualidade da água por meio de parâmetros físicos e químicos e estudos ecotoxicológicos, empregando tanto bioindicadores coletados *in situ* quanto testes laboratoriais, fornecem resultados mais robustos para essa região. Nesse contexto, o objetivo deste estudo foi analisar a composição e a estrutura da paisagem ao redor dos pontos de amostragem localizados em três córregos (Vitória, Rosário e Piravevê) pertencentes à bacia do Rio Ivinhema, para avaliar os impactos da expansão do cultivo de cana-de-açúcar na qualidade da água e avaliar biomarcadores de genotoxicidade tanto *in situ* quanto *ex situ*, utilizando espécies de peixes nativos. Nos pontos de amostragem localizados nos córregos Rosário e Vitória, a cana-de-açúcar é o principal uso da terra, enquanto no córrego Piravevê, as áreas de pastagem representam o principal uso da terra. O pH ácido observado nos córregos Vitória e Rosário parece estar relacionado à aplicação de fertilizantes e à fertirrigação na cultura da cana-de-açúcar. Duas espécies de peixes detritívoros e cinco de peixes onívoros foram coletadas. Foi identificada uma maior frequência de danos genotóxicos nas espécies de peixes detritívoros. Nos testes de genotoxicidade com *Astyanax lacustris*, a alteração nuclear mais frequente induzida pelas

[1]State University of Mato Grosso do Sul – Dourados (MS), Brazil.

[2]Federal University of Mato Grosso do Sul – Dourados (MS), Brazil.

[3]Federal University of Grande Dourados – Dourados (MS), Brazil.

[4]State University of Tocantins – Augustinópolis (TO), Brazil.

[5]Federal University of São Paulo – Diadema (SP), Brazil.

Corresponding author: Bruno do Amaral Crispim – University of Tocantins – Rua Planalto, 601, Centro – CEP: 77960-000 – Augustinópolis (TO), Brazil. E-mail: bruno.ac@unitins.br

Conflicts of interest: the authors declare no conflicts of interest.

Funding: Viana, L.F. is supported by Doctoral grants (23/200.452/2014) from the Foundation for Support to the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT, *Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul*); Crispim, B.A. is supported by Doctoral grants from CAPES (1390960); Súarez, Y.R (305485/2021-0). and Barufatti (306607/2022-0), A. are supported by productivity grants from CNPq.

Received on: 01/31/2024. Accepted on: 09/10/2024.

https://doi.org/10.5327/Z2176-94781971

the sugarcane ridge in the Ivinhema River basin may cause negative impacts on the aquatic environment and native biota. These results contribute to the generation of data and information that can be used for public hearing requests that aim to discuss and review many aspects of legislation regarding agricultural activities around streams, as well as the need for restoration and management programs in these areas in order to conserve biodiversity.

Keywords: micronuclei; nuclear alterations; cytoplasmic alterations; *Astyanax lacustris*.

amostras de água de todos os pontos de amostragem foi a invaginação nuclear. Nossos resultados indicam que a expansão da cultura de cana-de-açúcar na bacia do Rio Ivinhema pode causar impactos negativos no ambiente aquático e na biota nativa. Esses resultados contribuem para a geração de dados e informações que podem ser usadas em pedidos de audiências públicas que visam discutir e revisar vários aspectos da legislação sobre atividades agrícolas ao redor dos córregos, bem como a necessidade de programas de restauração e manejo nessas áreas para conservar a biodiversidade.

Palavras-chave: micronúcleos; alterações nucleares; alterações citoplasmáticas; *Astyanax lacustris*.

Introduction

Agriculture is the main driver of the economy in many countries, such as Brazil, but the expansion of cultivated areas has caused widespread fragmentation and destruction of natural habitats, resulting in biodiversity loss (Gonino et al., 2019). This expansion has significantly reduced riparian vegetation cover, which is the main cause of silting and facilitates the runoff of inorganic and organic pollutants to water bodies, since riparian vegetation acts as a natural barrier/filter (Ribeiro Junior et al., 2018; Gonçalves et al., 2019; Riveros et al., 2021). Moreover, Barreto et al. (2020) reported that Brazil is among the largest consumers of pesticides intended to agricultural production, causing soil, water, and air pollution, as well as negative effects on non-target organisms in both terrestrial and aquatic environments. The presence of pesticides, both in groundwater and surface water, can also cause serious problems to human populations (Zeni et al., 2019). Considering all these factors, the agriculture is one of the main anthropogenic activities that negatively impact water quality worldwide (Martíni et al., 2021).

Sugarcane cultivation was established in Brazil approximately 500 years ago but the greatest expansion of the cultivated area and productivity occurred in the last 20 years (Filoso et al., 2015), making Brazil the largest producer of sugarcane and one of the leading producers/exporters of sugar and ethanol in the world (Cursi et al., 2022). Currently, around 90% of sugarcane production is concentrated in the Central-West region of the country, and due to favorable land prices and less restrictive environmental legislation, farmers and investors have expanded sugarcane production in the states of Mato Grosso, Mato Grosso do Sul, and Goiás (Tomei et al., 2020). Particularly in Mato Grosso do Sul, the Ivinhema River basin is one of the regions with the greatest expansion of cultivated areas occupied by sugarcane and with the installation of new industrial plants for the production of sugar, ethanol, and bioenergy (Ferreira and Silva, 2016). This expansion occurs predominantly through the conversion of agricultural areas (mainly those occupied by soybeans) and pastures into sugarcane plantation areas (Ferreira and Silva, 2016). It is important to highlight that the large-scale cultivation of sugarcane causes numerous environmental problems, including: 1. Reduction of biodiversity caused by deforestation and implementation of monoculture; 2. Contamination of soil and surface water by excessive use of agrochemicals (e.g., mineral fertilizers and pesticides); 3. Silting up of water bodies by soil erosion; 4. Release of ash and greenhouse gases during fires carried out before sugarcane harvest; and 5. Excessive use and dispersion of vinasse applied in the fertigation (Christofoletti et al., 2013).

Concerning pesticide use in Brazil, soybean (54%), corn (15%), cotton (9%), pastures (5%), and sugarcane (4%) crops represent 87% of the total agricultural area treated mainly with herbicides, insecticides, and fungicides (Gaboardi et al., 2023). Herbicides were the most commercialized class of pesticides throughout the country during 2020, and glyphosate and its salts, 2,4-D, and atrazine were the main representatives of this class. Among the most widely sold insecticides are acephate, malathion, imidacloprid, and chlorpyrifos, while among the most widely sold fungicides are mancozeb and chlorothalonil (Gaboardi et al., 2023). In the period between 2013 and 2018, the most commonly purchased pesticides in the Mato Grosso do Sul were glyphosate, 2,4-D, atrazine, acephate, mancozeb, and paraquat dichloride (García-Medina et al., 2020). Particularly in sugarcane cultivation, the herbicides 2,4-D, clomazone, ametrine, hexazinone+diuron, and sodium hydrogen methylarsonate and the insecticides fipronil, ethephon, and imidacloprid are the most used pesticides (Christofoletti et al., 2017).

Therefore, large-scale sugarcane production in the Ivinhema River basin can contribute to polluting the streams with a complex mixture of organic chemicals, such as herbicides (e.g., ametrine, atrazine, clomazone, and diuron) widely used in this crop (Acayaba et al., 2021), and inorganic chemicals present naturally in the soils, and as contaminants (e.g., chromium, cadmium, nickel, and mercury) in mineral fertilizers (Rashmi et al., 2020), some of which are genotoxic. The genotoxic damages that have often been correlated with impaired development, growth, and reproduction of aquatic biota cause injuries to the integrity of the genome, including mutations, nuclear alterations, and interference in mitosis in aquatic organisms (Whitehead et al., 2004; Silva et al., 2020). Due to their ecological relevance and wide applications, assessments employing biomarkers of genotoxicity in aquatic organisms related to pollutants from agricultural activities may be of particular interest to environmental managers and regulators, especially if these responses can be demonstrated in different organisms and multiple test systems, both in the laboratory and in the field (Whitehead et al., 2004; Cherednichenko et al., 2022).

In biomonitoring studies conducted under real field conditions, fishes have been used as bioindicators for their modulated and integrated responses to pollutants; they occupy different trophic levels, bioaccumulate toxic substances, and react to low concentrations of pollutants, allowing the assessment of DNA changes resulting from genotoxic damage, among other effects (Gutiérrez et al., 2015; Batista et al., 2016; Cherednichenko et al., 2022).

The greatest difficulty in biomonitoring under field conditions is the establishment of reference sites where fishes of the same species can be collected to be used as controls. An alternative to dealing with this problem is to collect water samples from the same sites where the fishes are caught and carry out genotoxicity tests to evaluate the same biomarkers in fishes kept under controlled conditions. For the genotoxicity laboratory tests, we chose the species *Astyanax lacustris*, which is widely found in the Ivinhema River basin. This genus is recommended by the Brazilian Council for the Control of Animal Experimentation (CONCEA, *Conselho Nacional de Controle de Experimentação Animal*) and is often used as a bioindicator/test-organism in biomonitoring studies and ecotoxicological tests due to its abundance, resistance, omnivorous feeding behavior, ease of capture, and size that makes it suitable for laboratory conditions. In addition, *A. lacustris* is considered a sentinel organism in environmental genotoxicity studies (Ghisi and Oliveira, 2019).

Considering that the water quality assessment through physicochemical parameters and ecotoxicological studies using field bioindicators and laboratory tests provide more robust results (Peluso et al., 2021), the objective of this study was to analyze the composition and structure of the landscape around the sampling sites located in three streams (Vitória, Rosário, and Piravevê) belonging to the Ivinhema River basin and assess genotoxicity biomarkers *in situ* and *ex situ* using native fish species in response to the impacts of sugarcane cultivation on water quality.

Material and Methods

Study area

The Ivinhema River basin is located on the right bank of the Paraná River, in Mato Grosso do Sul, with an area of 45,000 km^2 (Súarez et al., 2011). The Rosário stream is approximately 1.5 m wide and 0.75 m deep, with water velocity close to 0.69 m s^{-1}. In this stream, the riparian vegetation is scarce with a predominance of sugarcane cultivation areas along its banks. The Vitória stream has an average width of 5.5 m, with average depth of 0.9 m, and water velocity of 0.84 m s^{-1}. On its banks, sugarcane monoculture is also predominant. In the surroundings of the Rosário and Vitória streams, an increase in sugarcane cultivation areas was observed in the last five years, mainly replacing the areas occupied by livestock activities. The Piravevê stream is a more voluminous waterbody, with a width ranging from 7 to 15 m, an average depth of 1.4 m, and water velocity of 0.3 m s^{-1}. On its banks pasture areas are predominant (Dos Santos et al., 2020; Silva et al., 2023). The water, fishes, and physical and chemical parameters were collected in rainy season in March 2017. The project was approved by the Ethics Committee on the Use of Animals from the UEMS (State University of Mato Grosso do Sul; 011/2014, registered on fl. 08 of book 01). All sampling sites are located in the lower portion of the Ivinhema River basin (Figure 1).

Land use and cover assessment

For the assessment of land use and cover around each sampling site, we used aerial images with a resolution of 1 m (Digital Globe) generated via Google Earth Pro® software (2014 and 2015), and to perform the land use and cover analyses, buffers with a radius of 1.5 km were defined. The types of land use were classified according to Brazilian recommendations (IBGE, 2013) as sugarcane, pasture, water bodies, wet areas, exposed soil, fragmented vegetation, and human occupation. For the other analyses, we adopted the protocol described by Riveros et al. (2021) and Viana et al. (2021a).

Water quality physicochemical parameters

At each sampling site, the physical and chemical parameters were determined by a pre-calibrated Horiba multiparametric probe. The parameters analyzed were dissolved oxygen (mg L^{-1}), hydrogen potential (pH), water electrical conductivity (μS cm^{-1}), and temperature (°C).

Genotoxicity evaluation

Fish sampling and genotoxicity in situ *biomarkers analysis*

For fish sample collection in streams, a rectangular metal frame sieve measuring 0.8 m × 1.2 m with a 2-mm mesh was used. For each fish captured, the standard length (mm) was obtained using an ichthyometer, and the total weight (g), using a field scale. The taxonomic identification of the fish species was performed based on specialized literature (Graça and Pavanelli, 2007). The blood samples from the fish species were obtained with a cut on the caudal peduncle after specimens were immersed in cold water to reduce activity. Two blood smear slides were made for each fish sample, air-dried for 15 minutes, and used absolute alcohol for 15 minutes to fix them (De Jesus et al., 2016; De Souza et al., 2019; Viana et al., 2021b). Then, the slides were hydrolyzed for 10 minutes at 60°C with hydrochloric acid (HCl 1 N), washed in distilled water, the Schiff reactive to stained the slides overnight, and counter-stained with Fast green.

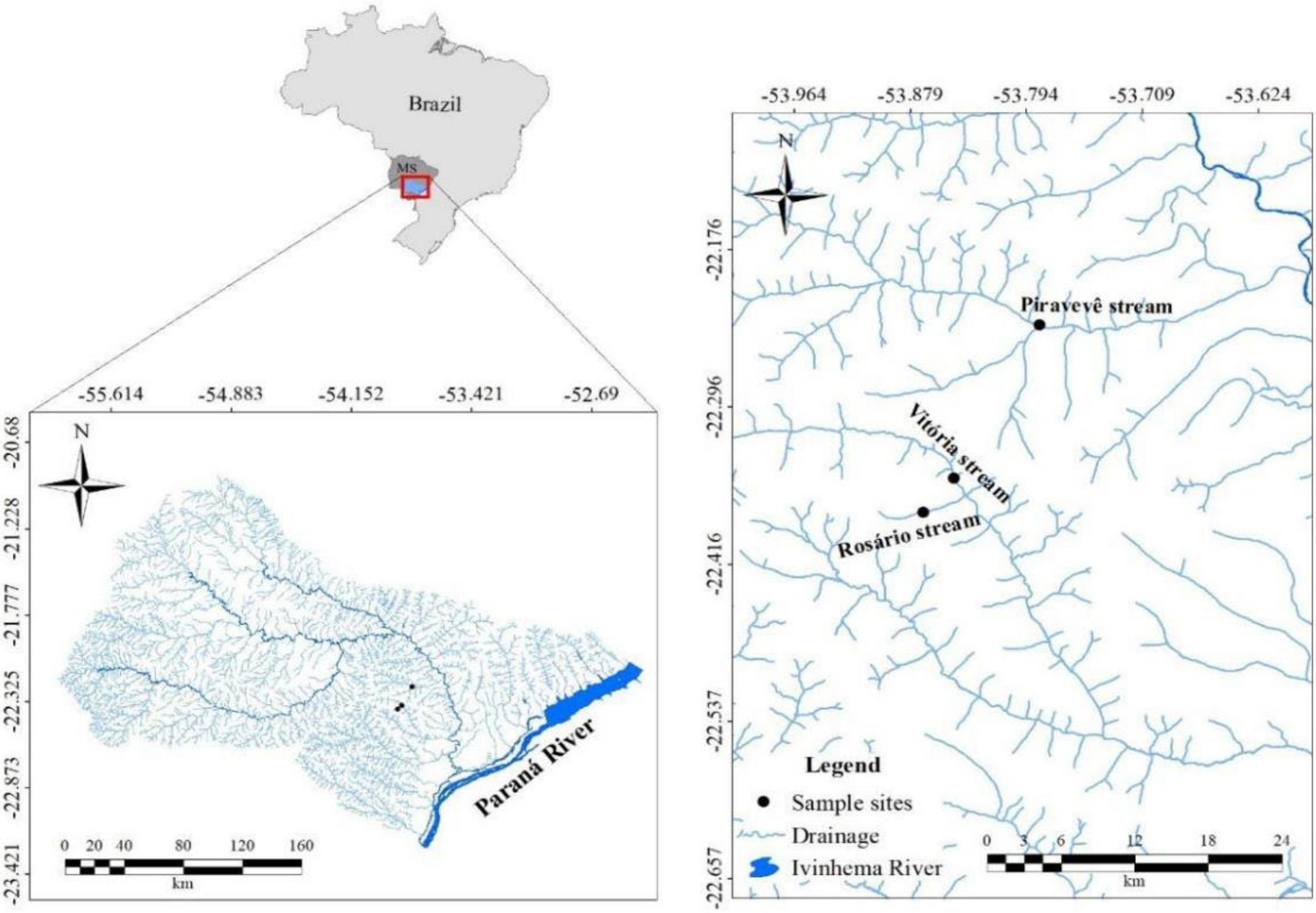

Figure 1 – Sampling sites located in Vitória, Rosário, and Piravevê streams, Ivinhema River basin, Mato Grosso do Sul, Brazil.

A Nikon optical microscope (Eclipse, E200) with a magnification of 1,000X was used for counting micronuclei and nuclear and cytoplasmic alterations in 2,000 erythrocytes per slide, totalizing 4,000 erythrocytes per fish sample. All nuclear alterations identified in the study were grouped and the ratio calculated between the total number of altered cells and the total number of cells observed was used to calculate the genotoxicity index. We followed the protocol described by De Souza et al. (2019) and Viana et al. (2021b) for both the micronucleus test and nuclear alterations analysis.

Astyanax lacustris *genotoxicity laboratory tests*

Polyethylene containers of 20 L previously decontaminated were used to collect superficial water samples at each sampling site. Afterward, the samples were taken to the laboratory and transferred to glass aquariums where genotoxicity tests were performed according to the Brazilian Association of Technical Standards [Associação Brasileira de Normas Técnicas (ABNT, 2022)] – Brazilian Regulatory Standards [Normas Brasileiras Regulamentadoras (NBR)] 15088 and the Organisation for Economic Co-operation and Development [OECD 203] (OECD, 2019) guidelines, with modifications. Dechlorinated water was used as the negative control (NC). Individuals of *A. lacustris* with length of 68.75 mm, standard deviation (±) 6.22 mm and weight of 72.00±32.03 g were used. Groups of ten fish supplied by a commercial fish farming company (Fazenda Douradense) were previously subjected to a period of quarantine and acclimatization under laboratory conditions and then added to the aquariums. The fish were not fed, and the temperature, pH, and dissolved oxygen levels were kept within the limits recommended by ABNT-NBR 15088 (ABNT, 2022) and OECD 203 (OECD, 2019) guidelines during the exposure period. The blood samples were obtained from the fish by caudal puncture with heparinized syringes after a 96-hour exposure period. For each specimen, two thin layer slides were prepared using a drop of blood. Subsequently, the same steps described above for genotoxicity were performed on fish *in situ*.

Statistical analysis

Water's physical and chemical parameters were presented as means ± standard deviations. The normality and homoscedasticity of these data were verified by the Shapiro-Wilk and Levene tests, respectively. Kruskal-Wallis nonparametric test was applied with Dunn's *post hoc* test (α=0.05) to compare the frequency of nuclear and cytoplasmic alterations in the erythrocytes and the genotoxicity index observed in fish collected *in situ*. A color map was generated using the "gplots" package with the average values of each genotoxic alteration observed in the fish species sampled in the Rosário, Vitória, and Piravevê streams. The more intense the red colors, the greater the proportions of alterations observed in each species. The color scale represents average values where red squares indicate values above average for a given change and blue squares indicate values below average. When the data met the assumptions, the parametric analysis test was performed, analysis of variance (ANOVA), followed by Tukey *post hoc* test; otherwise, we applied the non-parametric Kruskal-Wallis test, followed by Dunn's *post hoc* test. We considered a significance level of $p<0.05$ in both cases. The ANOVA and Tukey's *post hoc* test were used to compare the nuclear alterations and genotoxicity indexes obtained from the *A. lacustris* test. All analyses were performed using the R platform (R Development Core Team, 2021).

Results and Discussion

Land use and cover assessment

Agriculture and livestock are the predominant land uses around the three sampling sites. In the sampling sites located in the Rosário and Vitória streams, sugarcane is the main land use, while in the Piravevê stream, pasture areas represent the predominant land use. Fragmented vegetation has a similar contribution to soil cover in the three sampling sites, ranging from 21.00% (Rosário stream) to 26.93% (Piravevê stream). In general, human occupations have little representation in the use of land around all sampling sites, varying between 0.07% in the Rosário stream and 1.75% in the Vitória stream (Figure 2).

The replacement of native vegetation cover around aquatic environments by agricultural areas is a major environmental problem, mainly because water bodies are unprotected from chemical pollutants' ingress (Gonçalves et al., 2019). Dense vegetation cover acts as a barrier, dampening the runoff of sediments and pollutants into streams mainly during periods of increased rainfall, thus avoiding silting and imbalance in the structure and functioning of the ecosystem (Dos Santos et al., 2020). In addition, dense vegetation provides a reduction in eutrophication, promotes the conservation of fauna and flora, and facilitates the infiltration of rainwater into the soil (Tundisi and Tundisi, 2010). In this sense, the fragility of the riparian vegetation around streams leaves the environment more exposed and vulnerable to pollutants, compromising aquatic biota health (Ribeiro Junior et al., 2018; Viana et al., 2018; Gonçalves et al., 2019).

The replacement of native vegetation cover occurs due to the expansion of sugarcane cultivation areas in Brazil and is considered the main driver of the deforestation of native vegetation and fragmentation of natural habitats (Gonino et al., 2019). In Brazil, during the 2019–2020 harvest, 642.7 million tons of sugarcane were produced in a harvest area of 8.44 million hectares, with the Central-West region of Brazil responsible for 92% of national production (589 million tons), and the North and Northeast regions responsible for only 8% of national production (52.8 million tons) (Cursi et al., 2022). To meet domestic demand for ethanol in 2021, it was estimated that Brazil would need to expand the area cultivated with sugarcane by 6.4 million hectares, with this additional area coming mainly from the conversion of pasture areas (Cherubin et al., 2016).

Water quality physicochemical parameters

The Brazilian legislation – National Council for the Environment in Ordinance (CONAMA, *Conselho Nacional do Meio Ambiente*) – Resolution 357/2005 (Brasil, 2005) on fresh surface water quality classifies water bodies and establishes sets of concomitant uses for each of the classes. Class 2 freshwater bodies must guarantee water of sufficient quality for human consumption after conventional treatment, protection of aquatic communities, primary contact recreation (e.g., swimming, water skiing, and diving), irrigation, aquaculture, and fishing activities. Legislated standards are established for the most restrictive use (Brasil, 2005). The dissolved oxygen presented values in accordance with the standard established for Class 2 freshwater bodies at all sampling sites. As for pH, the values observed in the sampling sites located on Vitória and Rosário streams are in disagreement with the Brazilian standard. In both streams, the main land use is sugarcane cultivation areas. Brazilian legislation does not establish standards for water temperature and electrical conductivity parameters. The water temperature showed similar values at all sampling sites. However, the electrical conductivity indicated differences among the sampling sites with the highest value observed at the site located on Piravevê stream (Table 1).

One of the main factors affecting the quality of aquatic environments is the pH, and changes in its levels can affect fish growth, energy balance, metabolism, and reproductive physiology (Mukherjee et al., 2019). Thus, the low pH values observed in the sampling sites located on Vitória and Rosário streams can cause damage to the aquatic biota and can be attributed, at least in part, to the predominance of sugarcane cultivation (Christofoletti et al., 2013) in their surroundings (Figure 2). According to Nhiwatiwa et al. (2017), the low pH in the waters of rivers and streams located in sugarcane areas can be attributed to the extensive application of fertilizers, in addition to the fertigation process. Particularly, the use of vinasse in the fertigation process and the improper disposal of this waste generated by sugar and ethanol production can cause acidification and eutrophication in water bodies (Fuess et al., 2017). The acidification of water caused by vinasse is mainly associated with high concentrations of potassium, calcium, magnesium, organic solids, and other minerals (Ogura et al., 2022). Botelho et al. (2012) observed its lethal effects fish resulting from the drop in water pH.

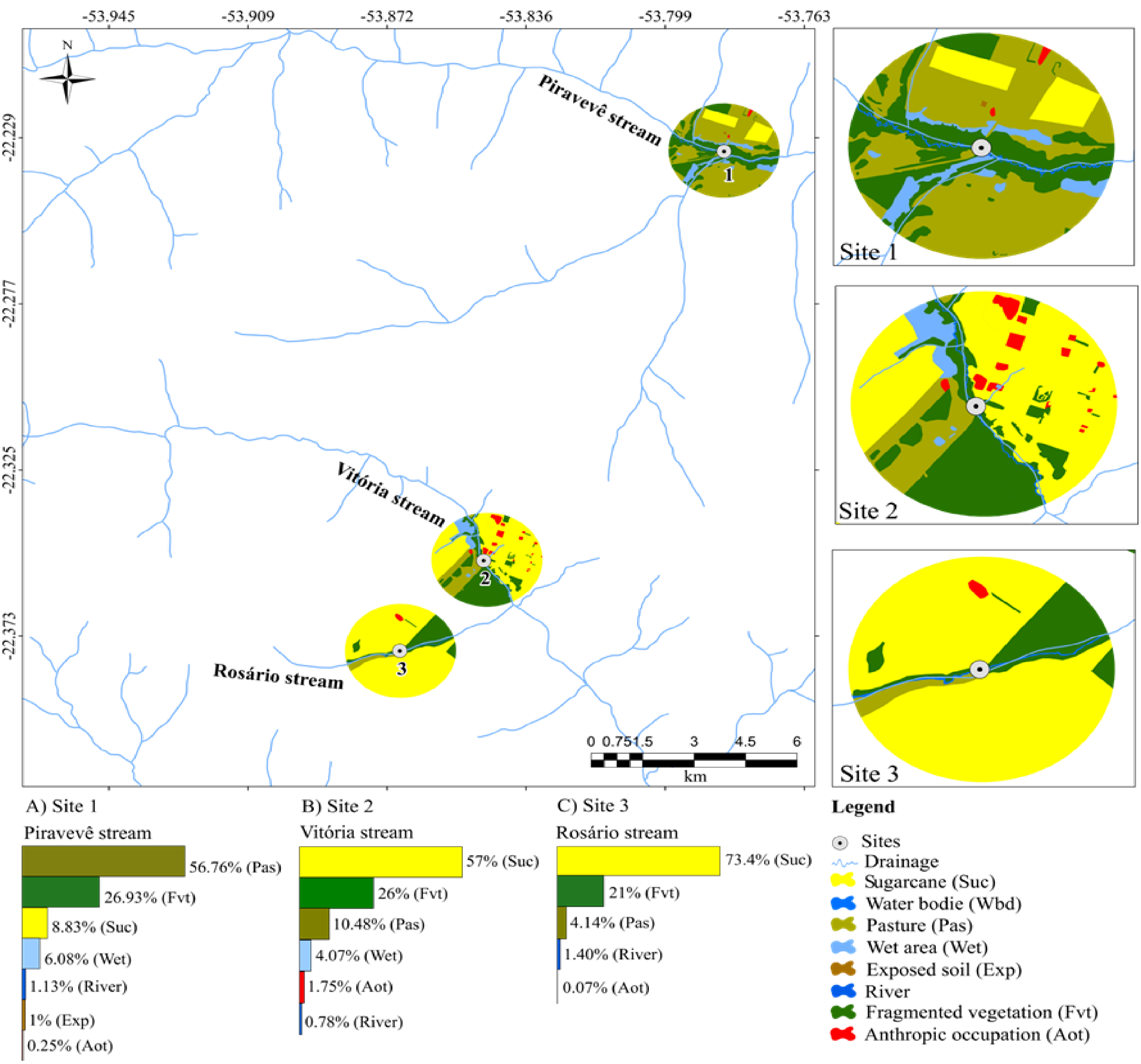

Figure 2 – Land use and cover around sampling sites located in Piravevê, Vitória, and Rosário streams, Ivinhema River basin, Mato Grosso do Sul, Brazil.

The expansion of agricultural areas, those occupied by sugarcane cultivation, is associated with changes in the physical and chemical parameters of the waters from the streams present in these areas. The excessive use of agrochemicals in areas located in the surrounding of water bodies is one of the main drivers of aquatic environment contamination (Li et al., 2020). For example, in sugarcane cultivation, several pesticides are used concomitantly, generating complex mixtures of pollutants that are potentially toxic to aquatic life (Li et al., 2020; Ogura et al., 2022).

Genotoxicity biomarkers evaluation in fish collected *in situ*

For the evaluation of genotoxicity biomarkers in fish samples, we were able to collect seven different native fish species, six of them belonging to the order Characiformes and only one belonging to the order Siluriformes (Table 2). The cytogenetic characteristics of fish peripheral blood cells have been widely used to evaluate genotoxic damage in agricultural areas impacted by pesticides. Fish erythrocytes generally have an oval shape with a centrally located oval or rounded nucleus, stained with an intense purple color. Therefore, cytogenetic analyses make it possible to identify different types of alterations in the structure of the cells that differ from the normal morphology of erythrocytes from each fish species. Thus, the micronuclei and other nuclear alterations are biomarkers for genotoxic pollutants (Cherednichenko et al., 2022).

We observed four types of nuclear alteration and one of cytoplasmic alteration in the collected fish samples. Among all fish species collected *in situ*, *Cyphocharax modestus* and *Hypostomus ancistroides*, both detritivorous species, showed the highest frequencies of genotoxic damage (Figures 3 and 4). The nuclear and cytoplasmatic alterations observed in *C. modestus* erythrocytes decreased in the following order: nuclear budding=cytoplasmic invagination>lobulated nucleus. In *H. ancistroides* decreased in the following order: nuclear invagination>lobulated nucleus>nuclear budding=cytoplasmic invagination (Figure 3). No significant difference was observed in the genotoxicity biomarkers frequency (p=0.386) between both detritivorous species – *C. modestus* and *H. ancistroides*, but both detritivores fish species differed from the omnivorous fish species (p=0.001) (Figure 4). *A. lacustris* showed a higher frequency of micronuclei. *Moenkhausia forestii* showed a lower frequency of genotoxicity biomarkers (Figure 3).

Table 1 – Physicochemical parameters (means ± standard deviation), dissolved oxygen, hydrogen ionic potential, conductivity, and temperature, for the sampled sites in Piravevê, Vitória, and Rosário streams, belonging to the Ivinhema River basin, in Mato Grosso do Sul, Brazil.

Sampling site	Physicochemical parameters			
	DO (mg L^{-1})	Conductivity (µS cm^{-1})	pH	Temperature (°C)
Piravevê	6.09±0.05	44.00±0.00	6.00±0.51	26.14±0.34
Vitória	8.00±1.66	18.50±10.61	**5.08±0.72**	27.11±0.47
Rosário	8.00±0.47	24.00±33.22	**5.00±0.47**	27.00±0.28
MVA*	>5	Not established	6 to 9	Not established

*Maximum values allowed for Brazilian legislation CONAMA Resolution 357/2005 for Class 2 freshwater bodies (Brasil, 2005). DO: dissolved oxygen; pH: hydrogen ionic potential. Values in bold in disagreement with current legislation.

The presence of micronuclei in cells can be considered a universal indicator of pollution that can be used to quickly and accurately determine the clastogenic action of different chemicals. When analyzing the presence of micronuclei in fish erythrocytes, several morphological types of nuclear and cytoplasmic anomalies were found; however, it is important to highlight that classification and complete information about their origins are lacking. Despite the absence of this knowledge, there is evidence that nuclear and cytoplasmic abnormalities occurring in fish erythrocytes indicate the development of degenerative processes (Cherednichenko et al., 2022). This damage to genetic material is related to the manifestation of various cancers in addition to other effects such as disturbances in fertility, longevity, and growth of affected organisms, which can also have consequences at the population level (Dalzochio et al., 2018).

Modern ecotoxicology is increasingly focused on determining biomarkers in native species of bioindicator organisms (e.g., fish), as these determinations allow for comprehensive assessments of the toxic effects of pollutants present in a given environment at both the individual and population levels. The use of fish as bioindicator organisms makes it possible to obtain a rapid response to a complex biological change, in an ecosystem or population, based on the degree of influence of various anthropic factors (Cherednichenko et al., 2022). The high frequency of genotoxicity biomarkers observed in *C. modestus* and *H. ancistroides* was probably related to their detritivores feeding behaviors. Viana et al. (2018) reported that *H. ancistroides* collected from streams near urban and rural areas presented a greater frequency of genotoxic alterations than other species with no detritivores behaviors. This fact can be explained, at least in part, by the environmental fate of many nonpolar genotoxic pollutants that remain suspended in the water column bounded to the suspended particulate matter during a certain period but tend to be deposited in the bottom sediments of rivers and streams. Thus, fish foraging bottom sediments from polluted aquatic environments end up biomagnifying higher amounts of these pollutants (Labarrère et al., 2012). Therefore, fish species with benthopelagic behavior may be more exposed to genotoxic pollutants and present higher frequencies of genotoxicity biomarkers (Labarrère et al., 2012).

Table 2 – Fish species collected from the three sampling sites located in Piravevê, Vitória, and Rosário streams, belonging to the Ivinhema River basin, in Mato Grosso do Sul, Brazil.

Species	n	Piravevê	Vitória	Rosário	Length (mm)	Weight (g)	Feeding behaviors
Astyanax fasciatus	23	0	14	9	56.53±7.90	52.00±24.95	Omnivorous
Astyanax lacustris	23	3	18	2	63.83±9.37	103.28±52.83	Omnivorous
Cyphocharax modestus	8	8	0	0	38.47±9.17	19.44±14.32	Detritivores
Hypostomus ancistroides	6	2	0	4	72.14±16.40	109.63±67.00	Detritivores
Moenkhausia forestii	37	25	0	12	37.60±6.31	20.31±13.81	Omnivorous
Piabina argentea	20	0	0	20	55.20±7.61	34.85±16.68	Omnivorous
Pseullogrammus kennedyi	6	6	0	0	37.44±2.80	15.41±3.07	Omnivorous

N: total number of individuals.

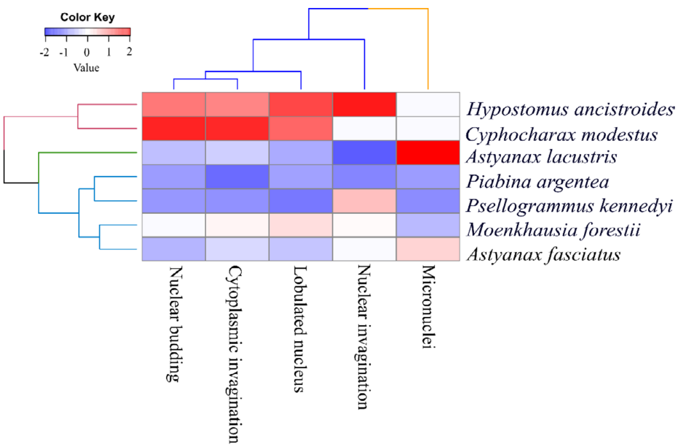

Figure 3 – Hierarchical clustering of the means of genotoxicity biomarkers observed in erythrocytes of fish species samples collected *in situ* in Piravevê, Vitória, and Rosário streams, Ivinhema River basin, in Mato Grosso do Sul, Brazil.
In color scale: white: mean; red: standard deviation above mean;blue: standard deviation below means.

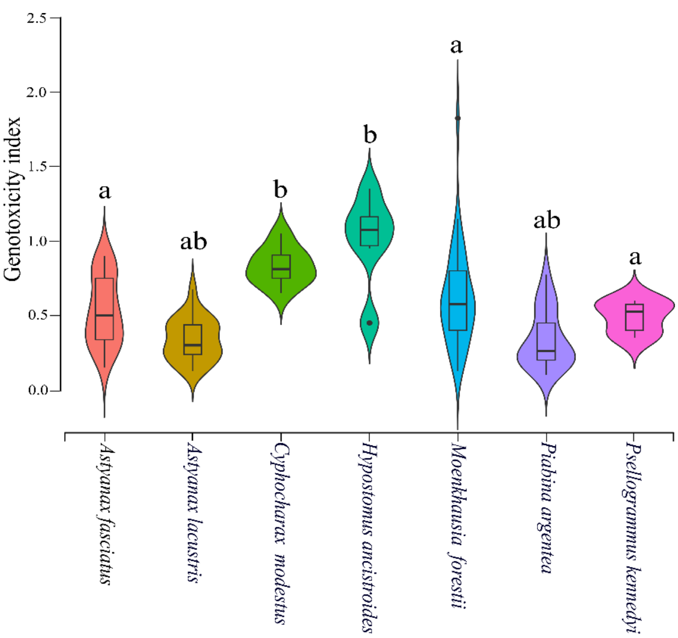

Figure 4 – Genotoxicity index (median and interquartile deviation) obtained from fish species collected *in situ* in Piravevê, Vitória, and Rosário streams, Ivinhema River basin, Mato Grosso do Sul, Brazil.
Different letters represent significant difference (p<0.05).

Hussain et al. (2018) described different damages, in addition to mortality, in fish and other aquatic organisms associated with the release of sugarcane crop residues into water bodies. These damages can contribute to the decline of native populations of aquatic organisms in these areas (Gonino et al., 2019). Particularly, nuclear alterations in fish erythrocytes are evidence of the presence of genotoxic pollutants in aquatic environments. These pollutants can interfere with the cell division process, causing damage to the genetic material and, eventually, interfering with the biological cycle of organisms (García-Mediana et al., 2020; Riveros et al., 2021; Viana et al., 2021a). Thus, the evaluation of genotoxicity biomarkers using different fish species collected *in situ* generates more consistent results owing to different sensitivity to aquatic environment stressors (Silva et al., 2015). According to Zhang et al. (2019), evaluating biomarkers using native fish species collected *in situ* not only reflects toxic impacts from chemical pollutants but also reveals their ability to adapt to environmental unfavorable conditions. Therefore, genotoxic biomarkers in fish erythrocytes are considered alerts of the environmental conditions to which individuals are subjected in natural aquatic ecosystems (Hussain et al., 2018; Kassa, 2021).

Genotoxicity laboratory tests with *Astyanax lacustris*

For the genotoxicity biomarkers, nuclear budding, binucleated cells, micronuclei, and cytoplasmic invagination, no significant differences were found between NC and water samples from the three sampling sites ($p>0.05$) (Table 3). The most frequent nuclear alteration in *A. lacustris* erythrocytes was nuclear invagination, presenting a significant difference between NC and water samples from Vitória and Rosário streams ($p=0.001$) (Table 3). Lobulated nuclei showed a significant difference between NC and water sample from Rosário stream ($p=0.015$) (Table 3). For both nuclear invagination and lobulated nuclei, water samples from Vitória and Rosario streams did not differ from each other ($p=0.064$) (Table 3). These results differed from the evaluation of genotoxicity biomarkers observed in *A. lacustris* collected *in situ* at the three sampling sites where micronuclei were the most frequent genotoxicity biomarkers.

In the test with *A. lacustris*, nuclear invagination was the alteration that presented the highest frequency, especially in Vitória and Rosário streams, places with greater proportions of agricultural areas, particularly sugarcane cultivation. In general, the impacts of genotoxic events on erythrocytes before cell division can generate some nuclear changes in addition to triggering micronuclei or even cell death (Garcia et al., 2017). In our study, most genotoxic damage in fish exposed to water samples from Vitória and Rosário streams may be associated with contaminants derived from the cultivation of sugarcane. Our results corroborate those previously reported by Viana et al. (2017) who observed that fish exposed to polluted waters from rural areas caused chromosomal alterations and DNA damage in *A. lacustris*.

For the genotoxicity index in *A. lacustris*, the water samples from Vitória and Rosário streams showed a significant difference related to the NC ($p=0.001$) (Figure 5). The water sample from Piravevê stream also showed a significant difference in genotoxicity index in compared to the Rosário stream ($p=0.036$). The water sample from Rosário stream did not differ significantly ($p=0.260$) from Vitória stream.

Table 3 – Frequencies of nuclear and cytoplasmic alterations (median and interquartile shift) obtained from water samples from the Piravevê, Vitória, and Rosário streams, belonging to the Ivinhema River basin, in Mato Grosso do Sul, Brazil, using the genotoxicity test with *Astyanax lacustris*.

Alterations	Negative Control	Water sampling sites		
		Piravevê	Vitória	Rosário
Nuclear invagination	0.0250|0.0250a	0.1500|0.0563ab	0.1750|0.0375b	0.2125|0.0625b
Nuclear budding	0.0250|0.0250a	0.0000|0.0250a	0.0250|0.0188a	0.0250|0.0250a
Binucleated cell	0.0250|0.0250a	0.0625|0.0250a	0.0375|0.0625a	0.0375|0.0313a
Lobulated nucleus	0.0250|0.0250a	0.0125|0.0250a	0.0250|0.0188b	0.0625|0.1063ab
Micronuclei	0.0000|0.0000a	0.0125|0.0250a	0.0000|0.0188a	0.0250|0.0313a
Cytoplasmic invagination	0.0250|0.0250a	0.0125|0.0250a	0.0250|0.0188a	0.0625|0.1063a

Different letters represent significant difference (p<0.05).

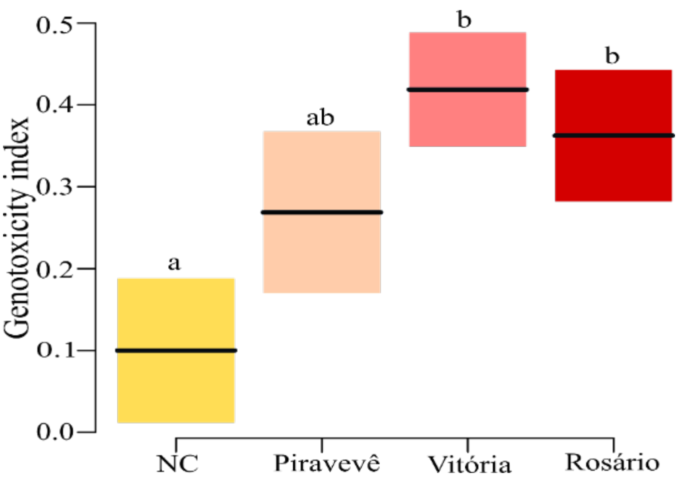

Figure 5 – Genotoxicity index (mean and confidence interval) of the test with *Astyanax lacustris* exposed to water samples from Piravevê, Vitória, and Rosário streams, Ivinhema River basin, Mato Grosso do Sul, Brazil. NC: negative control. Different letters represent significant difference (p<0.05).

Genotoxicity indices for *A. lacustris* between Vitória and Rosário streams were significantly different from NC, confirming greater genetic damage from the waters of these two streams. The micronucleus incidence of raw vinasse was significantly higher when compared to the NC for all dilutions tested. For the comet assay, the scores obtained for samples of raw vinasse and chemically treated vinasse (neutral pH) were similar to the results of untreated vinasse. The incidence of nuclear abnormalities was significantly higher than the NC for the vinasse raw samples. Therefore, soil application and/or improper disposal of vinasse near water bodies can pose serious risks to aquatic life.

Conclusion

In the surroundings of the three streams, reduced native vegetation cover was observed due to the expansion of agricultural areas, mainly intended for sugarcane cultivation, which is contributing to the deterioration of water quality and consequently inducing damage to the genetic material of native fish. Vitória and Rosário streams presented lower pH, which may be related to the fertigation with vinasse. Higher frequency of genotoxic damage was identified in fish species with detritivorous feeding habits. In the genotoxicity tests with *A. lacustris*, the most frequent nuclear alteration induced by the water samples from all sampling sites was nuclear invagination. The water samples collected from Vitória and Rosário streams, both areas under strong influence of sugarcane crop, were the most genotoxic. Together, our results demonstrate the negative impacts on the aquatic environment and native biota related to the expansion of the sugarcane ridge in the Ivinhema River basin.

Acknowledgements

We thank the Council for Scientific and Technological Development (CNPq Universal, *Conselho Nacional de Desenvolvimento Científico e Tecnológico*) and the Coordination for the Improvement of Higher Education Personnel (CAPES, *Coordenação de Aperfeiçoamento de Pessoal de Nível Superior*) for financial assistance. Viana, L. F. is supported by Doctoral grants from the Foundation for Support to the Development of Education, Science and Technology of the State of Mato Grosso do Sul (FUNDECT, *Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul*); Crispim, B. A. is supported by Doctoral grants from CAPES; and Súarez, Y. R. is supported by productivity grants from CNPq.

Authors' Contribution

Meira, V.R.L.P.: data curation; investigation; writing – original draft. **Viana**, L.F.: conceptualization; data curation; formal analysis; writing – original draft. **Crispim**, B.A.: conceptualization; data curation; methodology; investigation; writing – review & editing. **Súarez**, Y.R.: data curation; writing – review & editing. **Barufatti,** A.: writing – review & editing. Kummrow, F.: writing – review & editing. **Berti**, A.P.: writing – review & editing. **Solórzano**, J.C.J.: methodology; writing – original draft.

References

Acayaba, R.D.; Albuquerque, A.F.; Ribessi, R.L.; Umbuzeiro, G.A.; Montagner, C.C., 2021. Occurrence of pesticides in waters from the largest sugar cane plantation region in the world. Environmental Science and Pollution Research, v. 28, 9824-9835. https://doi.org/10.1007/s11356-020-11428-1.

Associação Brasileira de Normas Técnicas (ABNT), 2022. NBR 15088. Ecotoxicologia aquática - toxicidade aguda: teste com peixes. ABNT, Rio de Janeiro, 31 p.

Barreto, M.L.P.; Netto, A.M.; Silva, J.P.S.; Amaral, A.; Borges, E.; França, E.J.; Vale, R.L., 2020. Gray water footprint assessment for pesticide mixtures applied to a sugarcane crop in Brazil: a comparison between two models. Journal of Cleaner Production, v. 276, 124-254. https://doi.org/10.1016/j.jclepro.2020.124254.

Batista, N.J.C.; Cavalcante, A.A.C.M.; De Olieira, M.G.; Medeirso, E.C.N.; Machado, J.L.; Evangelista, S.R. Dias, J.F.; Santos, C.E.I.; Duarte, A.; Silva, F.R.; Silva, J., 2016. Genotoxic and mutagenic evaluation of water samples from a river under the influence of different anthropogenic activities. Chemosphere, v. 164, 134-141. https://doi.org/10.1016/j.chemosphere.2016.08.091.

Botelho, R.G.; Tornisielo, V.L.; Olinda, R.A.; Maranho, L.A.; Machado-Neto, L., 2012. Acute toxicity of sugarcane vinasse to aquatic organisms before and after pH adjustment. Toxicological and Environmental Chemistry, v. 94 (10), 2035-2045. https://doi.org/10.1080/02772248.2012.738516.

Brasil, 2005. Conselho Nacional do Meio Ambiente. Resolução CONAMA n. 357, de 17 de março de 2005. Diário Oficial da União, Brasília.

Cherednichenko, O.; Pilyugina, A.; Nuraliev, S., 2022. Cytogenetical bioindication of pesticidal contamination. In: Singh, P.; Singh, S.; Sillanpää, M. (Eds.), Pesticides in the natural environment: sources, health risks, and remediation. Amsterdam, Elsevier, pp. 227-260. https://doi.org/10.1016/B978-0-323-90489-6.00010-0.

Cherubin, M.R.; Franco, A.L.C.; Cerri, C.E.P.; Karlen, D.L.; Pavinato, P.S.; Rodrigues, M.; Davies, C.A.; Cerri, C.C., 2016. Phosphorus pools responses to land-use change for sugarcane expansion in weathered Brazilian soils. Geoderma, v. 265, 27-38. https://doi.org/10.1016/j.geoderma.2015.11.017.

Christofoletti, C.A.; Escher, J.P.; Correia, J.E.; Marinho, J.F.U.; Fontanetti, C.S, 2013. Sugarcane vinasse: environmental implications of its use. Waste Management, v. 33 (12), 2752-2761. https://doi.org/10.1016/j.wasman.2013.09.005.

Christofoletti, C.A.; Souza, C.P.; Guedes, T.A.; Ansoar-Rodríguez, Y., 2017. O emprego de agrotóxicos na cultura de cana-de-açúcar, In: Fontanetti, C.S.; Bueno, O.C. (Eds), Cana-de-açúcar e seus impactos: uma visão acadêmica. Bauru, Canal6, pp. 51-61.

Cursi, D.E.; Hoffmann, H.P.; Barbosa, G.V.S.; Bressiani, J.A.; Gazaffi, R.; Chapola, R.G.; Fernandes Junior, A.R.; Balsalobre, T.W.A.; Diniz, C.A.; Santos, J.M.; Carneiro, M.S., 2022. History and current status of sugarcane breeding, germplasm development and molecular genetics in Brazil. Sugar Tech, v. 24, 112-133. https://doi.org/10.1007/s12355-021-00951-1.

Dalzochio, T.; Rodrigues, G.Z.P.; Simões, L.A.R.; Souza, M.S.; Petry, I.E.; Andriguetti, N.B.; Silva, G.J.H; Silva, L.B.; Gehlen, G., 2018. *In situ* monitoring of the Sinos River, southern Brazil: water quality parameters, biomarkers, and metal bioaccumulation in fish. Environmental Science and Pollution Research, v. 25, 9485-9500. https://doi.org/10.1007/s11356-018-1244-7.

De Jesus, I.S.; Cestari, M.M.; Bezerra, M.A.; Affonso, P.R., 2016. Genotoxicity effects in freshwater fish from a Brazilian impacted river. Bulletin of Environmental Contamination and Toxicology, v. 96 (4), 490-495.https://doi.org/10.1007/s00128-016-1755-1.

De Souza, J.P.; Sposito, J.C.V.; Crispim, B.A.; Silva, F.G.; Oliveira, K.M.P.; Kummrow, F., Nascimento, V.A.; MontagneR, C.C.; Viana, L.V.; Solórzano, J.C.J.; Barufatti, A., 2019. From collection to discharge: physical, chemical, and biological analyses for fish farm water quality monitoring. Ecotoxicology, v. 28, 13-25. https://doi.org/10.1007/s10646-018-1991-8.

Dos Santos, S.L.; Viana, L.F.; Merey, F.M.; Crispim, B.A.; Solorzanoa, J.C.; Barufatti, A.; Cardoso, C.A.L.; Lima-Junior, S.E., 2020. Evaluation of the water quality in a conservation unit in Central-West Brazil: Metals concentrations and genotoxicity *in situ*. Chemosphere, v. 251, 126365. https://doi.org/10.1016/j.chemosphere.2020.126365.

Ferreira, P.S.; Silva, C.A., 2016. A expansão da cana-de-açúcar na bacia hidrográfica do Rio Brilhante, Mato Grosso do Sul: o uso da técnica de NDVI como instrumento para evidenciar dinâmicas territoriais. Geografares, v. 22, 66-81. https://doi.org/10.7147/GEO23.12340.

Filoso, S.; Carmoa, J.B.; Mardegan, S.F.; Lins, S.R.M.; Gomes, T.F.; Martilelli, L.A., 2015. Reassessing the environmental impacts of sugarcane ethanol production in Brazil to help meet sustainability goals. Renewable and Sustainable Energy Reviews, v. 52, 1847-1856. https://doi.org/10.1016/j.rser.2015.08.012.

Fuess, L.T.; Rodrigues, I.J.; Garcia, M.L., 2017. Fertirrigation with sugarcane vinasse: Foreseeing potential impacts on soil and water resources through vinasse characterization. Journal of Environmental Science and Health, v. 52 (11), 1063-1072. http://dx.doi.org/10.1080/10934529.2017.1338892.

Gaboardi, S.C.; Candiotto, L.Z.P.; Panis, C., 2023. Agribusiness in Brazil and its dependence on the use of pesticides. Hygiene and Environmental Health Advances, v. 8, 100080. https://doi.org/10.1016/j.heha.2023.100080.

Garcia, A.L.H.; Matzenbacher, C.A.; Santos, M.S.; Prado, L.; Picada, J.N.; Premoli, S.M.; Corrêa, D.S.; Niekraszewicz, L.; Dias, J.F.; Grivicich, I.; Silva, J., 2017. Genotoxicity induced by water and sediment samples from a river under the influence of brewery effluent. Chemosphere, v. 169, 239-248. https://doi.org/10.1016/j.chemosphere.2016.11.081.

García-Medina, S.; Galar-Martínez, M.; Gómez-Oliván, L.M.; Torres-Bezaury, R.M.D.C.; Islas-Flores, H.; Gasca-Perez, E., 2020.The relationship between cyto-genotoxic damage and oxidative stress produced by emerging pollutants on a bioindicator organism (*Allium cepa*): The carbamazepine case. Chemosphere, v. 253, 126675. https://doi.org/10.1016/j.chemosphere.2020.126675

Ghisi, N.C.: Oliveira, E.C., 2019. Biomonitoring of Diffuse Contamination in the Subtropical Region of Brazil: Multibiomarker Assessment in Neotropical Freshwater Fishes. In: Gómez-Oliván, L. (Ed.), Pollution of Water Bodies in Latin America. Cham, Springer, pp. 255-273. https://doi.org/10.1007/978-3-030-27296-8_14.

Gonçalves, C.S.; Holt, R.D.; Christaman, M.C.; Casatti, L., 2019. Environmental and spatial effects on coastal stream fishes in the Atlantic rain forest. Biotropica, v. 52 (1), 139-150. https://doi.org/10.1111/btp.12746.

Gonino, G.M.R.; Figueiredo, B.R.S.; Manetta, G.I.; Alves, G.H.Z.; Benedito, E., 2019. Fire increases the productivity of sugarcane, but it also generates ashes that negatively affect native fish species in aquatic systems. Science of the Total Environment, v. 664, 215-221. https://doi.org/10.1016/j.scitotenv.2019.02.022.

Graça, W.J.; Pavanelli, C.S., 2007. Peixes da planície de inundação do alto rio Paraná e áreas adjacentes. Editora da Universidade Estadual de Maringá, Maringá, 241 p.

Gutiérrez, J.M.; Villar, S.; Plavan, A.A., 2015. Micronucleus test in fishes as indicators of environmental quality in subestuaries of the Río de la Plata (Uruguay). Marine Pollution Bulletin, v. 91 (2), 518-523. https://doi.org/10.1016/j.marpolbul.2014.10.027.

Hussain, B.; Sultana, T.; Sultana, S.; Masoud, M.S.; Ahmed, Z.; Mahboob, S., 2018. Fish eco-genotoxicology: comet and micronucleus assay in fish erythrocytes as *in situ* biomarker of freshwater pollution. Saudi Journal of Biological Sciences, v. 25 (2), 393-398. https://doi.org/10.1016/j.sjbs.2017.11.048.

Instituto Brasileiro de Geografia e Estatística (IBGE), 2013. Bases cartográficas contínuas - Brasil. (Accessed: November 22, 2020) at:. https:// downloads.ibge.gov.br/downloads_geociencias.htm.

Kassa, B.A., 2021. Cytotoxicity and genotoxicity evaluation of municipal wastewater discharged into the head of Blue Nile River using the *Allium cepa* test. Scientific African, v. 13, e00911. https://doi.org/10.1016/j.sciaf.2021.e00911.

Labarrère, C.R.; Menezes, B.D.; Melo, M., 2012. Avaliação dos teores de zinco em brânquias, carcaça, fígado e musculatura de diferentes espécies de peixes capturados no Rio São Francisco (MG, Brasil). Revista Geonomos, v. 20 (1), 86-91. https://doi.org/10.18285/geonomos.v20i1.31.

Li, Y.; Abegunrin, T.P.; Guo, H.; Huang, Z.; Are, K.S.; Wang, H.; Gu, M.; Wei, L., 2020. Variation of dissolved nutrient exports by surface runoff from sugarcane watershed is controlled by fertilizer application and ground cover. Agriculture, Ecosystems & Environment, v. 303, 107121. https://doi.org/10.1016/j.agee.2020.107121.

Martíni, A.F.; Favaretto, N.; De Bona, F.D.; Durães, M.F.; Souza, L.C.P.; Goularte, G.D., 2021. Impacts of soil use and management on water quality in agricultural watersheds in Southern Brazil. Land Degradation and Development, v. 32 (7), 1-18. https://doi.org/10.1002/ldr.3777.

Mukherjee, A.; Bhowmick, A.R.; Mukherjee, J.; Moniruzzaman, M., 2019. Physiological response of fish under variable acidic conditions: a molecular approach through the assessment of an eco-physiological marker in the brain. Environmental Science and Pollution Research, v. 26, 23442-23452. https://doi.org/10.1007/s11356-019-05602-3.

Nhiwatiwa, T.; Dalu, T.; Brendonck, L., 2017. Impact of irrigation based sugarcane cultivation on the Chiredzi and Runde Rivers quality, Zimbabwe. Science of The Total Environment, v. 587-588 (1), 316-325. https://doi.org/10.1016/j.scitotenv.2017.02.155.

Ogura, A.P.; Moreira, R.A.; Silva, L.C.M.; Negro, G.S.; Freitas, J.S.; Pinto, T.J.S.; Lopes, L.F.P.; Yoshii, M.P.C.; Goulart, B.V.; Montagner, C.C.; Espíndola, E.L.G., 2022. Irrigation with water contaminated by sugarcane pesticides and vinasse can inhibit seed germination and crops initial growth. Archives of Environmental Contamination and Toxicology, v. 82 (3), 330-340. https://doi.org/10.1007/s00244-022-00914-x.

Organisation for Economic Co-operation and Development (OECD), 2019. Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines. Test. OECD, Paris, 24 p.

Peluso, J.; Coll, C.S.P.; Cristos, D.; Rojas, D.E.; Aronzon, C.M., 2021. Comprehensive assessment of water quality through different approaches: Physicochemical and ecotoxicological parameters. Science of The Total Environment, v. 800, 149510. https://doi.org/10.1016/j.scitotenv.2021.149510.

R Development Core Team, 2021. R: a language and environment for statistical computing. R Development Core Team, Vienna.

Rashmi, I.; Roy, T.; Kartika, K.S.; Pal, R.; Coumar, V.; Kala, S., Shinoji, K.C., 2020. Organic and inorganic fertilizer contaminants in agriculture: impact on soil and water resources. In: Naeem, M.; Ansari, A.; Gill, S. (Eds.), Contaminants in agriculture. Cham, Springer, pp. 3-41. https://doi.org/10.1007/978-3-030-41552-5_1.

Ribeiro Junior, J.W.; Siqueira, T.; Brejão, G.L.; Zipkin, E.F., 2018. Effects of agriculture and topography on tropical amphibian species and communities. Ecological Applications, v. 28 (6), 1554-1564. https://doi.org/10.1002/eap.1741.

Riveros, A.F.; Solórzano, J.C.J.; Monaco, I.A.; Cardoso, C.A.L., Súarez, Y.R.; Viana, L.F., 2021. Toxicogenetic effects on fish species in two sub-basins of the upper Paraguay river, Southern Pantanal-Brazil. Chemosphere, v. 264 (1), 128383. https://doi.org/10.1016/j.chemosphere.2020.128383.

Silva, E.M.S.; Viana, L.F.; do Amaral Crispim, B.; Veiga Francisco, L.F.; Súarez, Y.R.; Kummrow, F.; Barufatti, A.; Berti, A.P., 2023. Evaluation of water quality in streams under strong influence of sugarcane cultivation using the Allium cepa Test. Ecotoxicology and Environmental Contamination, v. 18 (1), 97-103. https://doi.org/10.5132/eec.2023.01.11.

Silva, E.P.; Benvindo-Souza, M.; Cotrim, C.F.C.; Motta, A.G.C.; Lucena, M.M.; Aontoniosi Filho, N.R.; Pereira, J.; Formiga, K.T.M.; Silva, D.M., 2020. Genotoxic effect of heavy metals on *Astyanax lacustris* in an urban stream. Heliyon, v. 6 (9), e05034. https://doi.org/10.1016/j.heliyon.2020.e05034.

Silva, S.V.S.; Dias, A.H.C.; Dutra, E.S.; Pavanin, A.L.; Morelli, S.; Pereira, B.B., 2015. The impact of water pollution on fish species in southeast region of Goiás, Brazil. Journal of Toxicology and Environmental Health, Part A, v. 79 (1), 8-16. https://doi.org/10.1080/15287394.2015.1099484.

Súarez, Y.R.; De Souza, M.M.; Ferreira, F.S.; Pereira, M.J.; Da Silva, E.A.; Ximenes, L.Q.L.; Azevedo, L.G.; Martins, O.C.; Lima-Júnior, S.E., 2011. Patterns of species richness and composition of fish assemblages in streams of the Ivinhema River basin, Upper Paraná River. Acta Limnologica Brasiliensia, v. 23 (2), 177-188. https://doi.org/10.1590/S2179-975X2011000200008.

Tomei, J.; Oliveira, L.L.; Ribeiro, C.O.; Ho, L.L.; Montoya, L.G., 2020. Assessing the relationship between sugarcane expansion and human development at the municipal level: a case study of Mato Grosso do Sul, Brazil. Biomass and Bioenergy, v. 141, 105700. https://doi.org/10.1016/j.biombioe.2020.105700.

Tundisi, J.G.; Tundisi, T.M., 2010. Impactos potenciais das alterações do Código Florestal nos recursos hídricos. Biota Neotropica, v. 10 (4), 67-75. https://doi.org/10.1590/S1676-06032010000400010.

Viana, L.F.; Crispim, B.A.; Sposito, J.C.V.; Melo, M.P.; Francisco, L.F.V.; Nascimento, V.A. Barufatti, A., 2021a. High iron content in river waters: environmental risks for aquatic biota and human health. Revista Ambiente & Água, v. 16, e2751. https://doi.org/10.4136/ambi-agua.2751.

Viana, L.F.; Kummrow, F.; Cardoso, C.A.L.; Lima, N.A.; Solórzano, J.C.J.; Crispim, B.A.; Barufatti, A.; Florentino, A.C., 2021b. High concentrations of metals in the waters from Araguari River lower section (Amazon biome): Relationship with land use and cover, ecotoxicological effects and risks to aquatic biota. Chemosphere, v. 285, 131451. https://doi.org/10.1016/j.chemosphere.2021.131451.

Viana, L.F.; Súarez, Y.R.; Cardoso, C.A.L.; Crispim, B.A.; Cavalcante, D.N.C.; Grisolia, A.B.; Lima-Junior, S.E., 2018. The Response of neotropical fish species (Brazil) on the water pollution: metal bioaccumulation and genotoxicity. Archives Environmental Contamination and Toxicology, v. 75 (3), 476-485. https://doi.org/10.1007/s00244-018-0551-9.

Viana, L.F.; Súarez, Y.R.; Cardoso, C.A.L.; Crispim, B.A.; Grisolia, A.B.; Lima-Junior, S.E., 2017. Mutagenic and genotoxic effects, and metal contaminations in fish of the Amambai River, Upper Paraná River, Brazil. Environmental Science and Pollution Research, v. 24 (35), 27104-27112. https://doi.org/10.1007/s11356-017-0276-8.

Whitehead, A.; Kuivila, K.; Orlando, J.L.; Kotelevtsev, S.; Anderson, S.L., 2004. Genotoxicity in native fish associated with agricultural runoff events. Environmental Toxicology and Chemistry, v. 23 (12), 2868-2877. https://doi.org/10.1897/03-649.1.

Zeni, J.O.; Pérez-Mayorga, M.A.; Roa-Fuentes, C.A.; Brejão, G.L.; Casatti, L., 2019. How deforestation drives stream habitat changes and the functional structure of fish assemblages in different tropical regions. Aquatic Conservation, v. 29 (8), 1238-1252. https://doi.org/10.1002/aqc.3128.

Zhang, W.; Xie, H.Q.; Zou, X.; Li, J.; Xu, L.; LI, Y. Zhou, Z.; Jin, T.; Ma, D.; Zhao, B., 2019. The toxic effects of *in situ* exposure of a native fish species (*Anabas testudineus*) to electronic waste pollution. Science of the Total Environment, v. 690, 1170-1177. https://doi.org/10.1016/j.scitotenv.2019.06.479.