Environmental diagnosis of water quality *in situ* and *ex situ* in streams in Upper Paraná River Basin, Brazil

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Abstract

The aim of this study was to (i) evaluate water quality using limnological parameters in three streams belonging to the Ivinhema River basin, (ii) investigate the composition and structure of the landscape around the locations sampled and their possible contribution to changes in water quality, (iii) investigate genotoxicity in fish *in situ* as environmental bioindicators, and (iv) evaluate the cytotoxic and genotoxic potential of water through bioassays in animal and plant models. A total of seven fish species were sampled *in situ* as bioindicators of environmental toxicogenetic damage: Astyanax fasciatus, Astyanax lacustris, *Cyphocharax modestus*, *Moenkhausia forestii, Piabina argentea*, Psellogrammus kennedyi, and Hypostomus ancistroides. Four types of nuclear and one cytoplasmic alteration were observed, with nuclear invagination being the most frequent. Animal bioassays showed five types of nuclear and one cytoplasmic alteration. In the plant bioassay, a reduction in germination and cell division was observed, indicating the cytotoxic and genotoxic effects of the water for *Allium cepa* cells. Thus, genotoxic alterations in fish and plant bioassays may reflect the different types of land use and cover around streams and the conversion of forest fragments into agricultural areas, mainly due to the expansion of sugarcane crops. The evaluation of the toxicogenetic status of the three streams is essential to emphasize the need to create projects to recover the native vegetation cover, which would improve the quality of the habitat and ensure the permanence of native species.

Introduction

In aquatic ecosystems, anthropic activities cause changes in the structure of local vegetation, compromising the quality of water resources and the collapse of the aquatic biota (Carminatto et al. 2020). The reduction of riparian vegetation cover directly affects water quality, because, among several functions, riparian vegetation acts as a natural filter that dampens the transport of pollutants through the river and regulates limnological parameters (Ribeiro Junior et al. 2018). The changes in the landscape around rivers and streams lead to the formation of homogeneous and unhealthy vegetation, which directly affects and reduces local biodiversity that depend on good environmental quality for its maintenance (Roa-Fuenteset al. 2019; Zeni et al. 2019). Being that, habitat fragmentation is considered one of the main threats to biodiversity (Zeni et al. 2019; Gonçalves et al. 2019).

In Brazil, to increase agricultural productivity, a wide variety of pesticides, herbicides, and fertilizers are used in soil preparation and crop maintenance. However, these agrochemicals can be released gradually into both the water table and water bodies, where they are conducted through the course of rivers and transported through hydrological periods, causing severe damage to the health of aquatic biota, especially the fish (Zeni et al. 2019). Thus, aquatic environments are the initial or final destination of contaminants (Dusman et al. 2014).

In this sense, research related to the toxicological status of aquatic environments is increasingly relevant to understand the biological conditions of organisms that survive in these areas and the genetic damage associated with the presence of contaminants in the water (dos Santos et al. 2020; Riveros et al. 2021).

Therefore, the use of environmental bioindicators organisms has been an important diagnostic and monitoring tool for environmental impacts in aquatic ecosystems (Bianchi et al. 2019). Because organisms respond to the effects of certain environmental impacts in the form of macrolesions on the DNA molecule resulting in genotoxic damage (Batista et al. 2016). The genotoxic effects are changes in the nucleus or in the cellular nuclear morphology resulting from potentially toxic contaminants in the aquatic environment, which is interfering in the cell division process, reflecting in damages in the genetic material of the aquatic organisms (Pereira da Silva et al. 2020).

Considering the ecological and environmental importance of the streams of the Ivinhema River basin and the lack of information on its environmental health and toxicogenetic status, an environmental monitoring study was carried out to evaluate the impact of agricultural activities in the area. Therefore, the aim of this study was to (i) evaluate water quality using limnological parameters in three streams belonging to the Ivinhema River basin, (ii) investigate the composition and structure of the landscape around the locations sampled and their possible contribution to changes in water quality, (iii) investigate genotoxicity in fish *in situ* as environmental bioindicators, and (iv) evaluate the cytotoxic and genotoxic potential of water through bioassays in animal and plant models.

Methodology

Study area

Three sampling sites were selected at Vitória and Piravevê small basins, lower portion of Ivinhema River Basin, Upper Paraná basin (Fig. 1). The Ivinhema River basin is located on the right bank of the Paraná River in Mato Grosso do Sul, Brazil and is about 600 km long with an area of 45,000 km² (Súarez et al. 2011).

Rosário stream are a first order stream, with approximately 1.5m width and 0.75m depth and water velocity near to 0.69 $m.s^{-1}$. Riparian vegetation are scarce with predominance of grasslands and highly anthropized forest and sugarcane at stream border. Vitória Stream are a fourth order stream with mean width of 5.5m, depth varying from 0.9m and water velocity 0.84 $m.s^{-1}$, riparian cover are also grasslands and pasture in sampled portion of the basin. Piravevê stream are more voluminous site (fifth order), with width varying from 7 to 15m and mean depth of 1.4m and water velocity of 0.3$m.s^{-1}$, they present secondary vegetation and pasture predominance at riparian vegetation. Considering these sites, Rosário and Vitória streams are located in area that recently (last five years) present higher increase of sugarcane cultivation, replacing livestock and Piravevê stream still present native (highly anthropized) vegetation and pasture. All portions are located to near 5km long to urban areas and did not receive urban sewage, however can be indirectly affected by improper disposal of domestic sewage from small farms surrounding streams.

Sampling

Water and fish were collected in November 2016 and March 2017 using rectangular sieve (0.8x1.2m) and dragging nets (1.5x5m) in non standardized sampling effort in a portion of approximately 100 long in all streams, near to highways that facilitated access to the river

Data collection and analysis

Limnological parameters: To characterize the limnological conditions of the studied areas, the following parameters were obtained *in situ* using a previously calibrated Horiba multi-parameter probe: dissolved oxygen ($mg \cdot L^{-1}$), pH, electrical conductivity of water ($\mu S \cdot cm^{-1}$), and temperature (°C).

Land use

The map of land use of the Piravevê, Vitória, and Rosário streams was made from high-resolution aerial images obtained from Google Earth Pro® software (years 2014 and 2015), with a resolution of 1 m (Digital Globe). For study limits, buffers with a radius of 1.5 km were generated around each sampling point and the categories of land use were classified as agriculture, fragmented vegetation, wet areas, water bodies, or anthropic occupation (IBGE, 2013). For image interpretation, an unsupervised classification (Clustering) was used, using the tools from the ArcGIS® program, Trial version 10.3 (ESRI 2015), calculating the areas and percentages of each category of land occupation based on buffer areas.

Collection and genotoxicity of fish in situ: For the collection of fish species in the streams, a rectangular metal frame sieve measuring 0.8 m ×1.2 m with a 2-mm mesh was used. After catching the fish were identified according to Graça and Pavanelli (2007). Then, larger-sized species above of standard length (30.00 mm), were selected, being (A. fasciatus, A. lacustris, *Cyphocharax modestus*, *Moenkhausia forestii, Piabina argentea*, Psellogrammus kennedyi, and Hypostomus ancistroides), to obtain blood samples. Shortly after, biometric data were measured: standard length (mm) and total weight (g). Then, the fish were immersed in cold water *in situ* to reduce their activity to obtain blood samples through a cut in the caudal peduncle. For each fish sample, two blood smear slides were made that were air dried for 15 min and fixed in absolute alcohol for another 15 min (Schmid 1975; Jesus et al. 2016; De Souza et al. 2019). Then, the slides were hydrolyzed in hydrochloric acid (HCl; 1N) for 10 min at 60°C, washed in distilled water, stained with reactive Schiff overnight, and counter-stained with Fast green. After preparation, 2000 cells per slide were analyzed, resulting in a total of 4000 erythrocytes per individual, using a Nikon optical microscope (Eclipse, E200), with a magnification of 1000× for micronuclei counting and identification of nuclear and cytoplasmic alterations. To calculate the genotoxicity index, all nuclear alterations identified in the study were grouped, the ratio between the total number of altered cells and the total number of cells observed calculated, and this ratio multiplied by 100. For the micronucleus test and nuclear alterations, we followed the protocol described by Schmid (1975) and Souza et al. (2019).

Genotoxicity bioassay with Astyanax lacustris: Water samples collected of each sites, was stored in polyethylene containers of 20 L, taken to the laboratory, and placed in independent glass aquariums (40 cm × 30 cm × 20 cm) (ABNT 2016). For the negative control (NC), groundwater was placed in other aquarium. We selected the fish species *A. lacustris*, as it is widely used as a model organism, for

performing the toxicogenic effects tests (Viana et al. 2018; Nascimento et al. 2020). Ten *A. lacustris* were placed in each aquarium, provided by a commercial fish farm (Douradense farm), with standard length (mean ± standard deviation: 68.75 ± 6.22 mm) and weight (mean ± standard deviation: 72.00 ± 32.03 g). The fish were well fed throughout the exposure period, and the temperature (26°C), pH (7.15), and dissolved oxygen (7.5 mg/L) were kept within the appropriate limits for the animals. After a period of 72 h, blood samples were obtained from the fish by caudal puncture with heparinized syringes. For each specimen, two thin layer slides were prepared using a drop of blood. Subsequently, the same steps described in the genotoxicity item of fish *in situ* were performed.

Allium cepa test: Seeds of *A. cepa* were sown in plates with approximately 3 mL of water sampled, previously collected in sterile plastic containers, from the investigated streams. These seeds were left to germinate for a period of 96 h at a temperature of 23 ± 3°C, according to the procedure adapted from Leme and Marin-Morales (2009). After germination, the seeds were counted manually and their roots measured with digital calipers (Digmess). Later, the roots were fixed in Carnoy's solution (3:1 Absolute ethanol:glacial acetic acid), subjected to hydrolysis with 1 $mol \cdot L^{-1}$ HCl, washed with distilled water, and stained with Schiff's reagent. For each treatment, ten slides of the root meristem were made and a total of 500 cells per slide were analyzed under a Nikon microscope (Eclipse, E200) at a magnification of 400×. Positive control (PC) seeds were exposed to the herbicide trifluralin (0.84 ppm), and distilled water was used as a NC. Cytotoxicity, genotoxicity, and mutagenicity parameters, mitotic index (MI), chromosomal alteration index (CAI), mutagenicity index (MTI), and cell death rate (CDR), were calculated according to Leme and Marin-Morales (2009) and Francisco et al. (2018).

Statistical analysis

Before the analyses, the data were subjected to the Shapiro Wilk test to verify their normality. Analysis of variance (ANOVA) ($\alpha = 0.05$) was performed to evaluate the genotoxicity indices in fish. To compare the frequency of nuclear and cytoplasmic alterations in the erythrocytes of fish species both *in situ* and in the bioassay analyzed in the different streams, the Kruskal Wallis nonparametric test was applied with Dunn's *posteriori* ($\alpha = 0.05$). To evaluate the cytotoxic damage in a plant model, ANOVA ($\alpha = 0.05$) was performed. The tests were conducted using the R platform (R Core Team 2019).

Results

Limnological parameters

Among the physicochemical parameters, dissolved oxygen in all sampled streams was in accordance with the Brazilian legislation CONAMA (357/2005) for freshwater class 2 (Table 1). The pH in the Vitória and Rosário streams was lower than that established according to the legislation (Table 1). For water temperature and electrical conductivity, there is no maximum limit established by Brazilian legislation.

Table 1
Physicochemical parameters (mean ± S.D), dissolved oxygen (DO), hydrogen ionic potential (pH), electrical conductivity (Cond.), and temperature (Temp.), for the sampled sites in Piravevê, Vitória and Rosário streams, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

Stream	Physicochemical parameters			
	DO (mg L^{-1})	Cond. (µS.cm^{-1})	pH	Temp. (ºC)
Piravevê	6.085 ± 0.050	44.00 ± 0.000	6.00 ± 0.509	26.14 ± 0.339
Vitória	8.00 ± 1.655	18.5 ± 10.607	**5.08 ± 0.721**	27.11 ± 0.474
Rosário	8.00 ± 0.467	24.00 ± 33.220	**5.00 ± 0.474**	27.00 ± 0.276
CONAMA (357/05)	> 5	Not established	6 to 9	Not established
Maximum allowed value by Brazilian legislation (Resolution 357/2005) for Class 2 of the National Environment Council (CONAMA 2005).				

Land Use

Regarding land use and cover in the three streams, we observed the predominance of anthropic activities. Piravevê had 56.76% of its area represented by the expansion of pasture, and Vitória and Rosário had a predominance of agricultural areas with 57% and 73.4%, respectively. In these agricultural areas, monoculture sugarcane was observed, and consequently attributed to the reduction of vegetation cover around the three streams (Fig. 2).

Genotoxicity of fish in situ

For the evaluation of environmental quality, seven species of native fish with different eating habits were sampled *in situ* belonging to the order Characiformes, A. fasciatus (Cuvier 1819), A. lacustris (Lütken 1875), *Cyphocharax modestus* (Fernández-Yépez 1948), *Moenkhausia forestii* (Benine and Oliveira 2009), *Piabina argentea* (Reinhardt 1867), and Psellogrammus kennedyi (Eigenmann 1903), and one belonging to the order Siluriformes, Hypostomus ancistroides (Ihering 1911) (Table 2).

Table 2
Fish species identified in three streams, Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

Species (N)	Piravevê	Vitória	Rosário	Length (mm)	Weight (g)	Feeding habits
A. fasciatus (23)	0	14	9	56.53 ± 7.90	52.00 ± 24.95	Omnivorous
A. lacustris (23)	3	18	2	63.83 ± 9.37	103.28 ± 52.83	Omnivorous
C. modestus (8)	8	0	0	38.47 ± 9.17	19.44 ± 14.32	Detritivorous
H. ancistroides (6)	2	0	4	72.14 ± 16.40	109.63 ± 67.00	Detritivorous
M. forestii (37)	25	0	12	37.60 ± 6.31	20.31 ± 13.81	Omnivorous
P. argentea (20)	0	0	20	55.20 ± 7.61	34.85 ± 16.68	Omnivorous
P. kennedyi (6)	6	0	0	37.44 ± 2.80	15.41 ± 3.07	Omnivorous
(N) = total number of individuals.						

For genotoxic alterations in the fish species, we found four types of nuclear and one cytoplasmic alteration, the most frequent alteration being nuclear invagination for all species (Table 3).

Table 3
Frequencies of nuclear and cytoplasmic alterations (median and interquartile deviation), of fish species in situ in the streams Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

Species	Alterations				
	Nuclear invagination	Nuclear budding	Lobulated nucleus	Micronuclei	Cytoplasmic invagination
A. fasciatus	0.325|0.250a	0.000|0.0125a	0.050|0.100a	0.000|0.062a	0.025|0.062a
A. lacustris	0.225|0.125a	0.000|0.025a	0.025|0.037a	0.000|0.137a	0.025|0.05a
C. modestus	0.326|0.118a	0.187|0.087b	0.175|0.137b	0.012|0.031b	0.05|0.125a
H. ancistroides	0.562|0.231b	0.137|0.200b	0.237|0.231b	0.000|0.018a	0.062|0.093a
M. forestii	0.325|0.225a	0.025|0.075a	0.050|0.175a	0.000|0.025a	0.025|0.100a
P. argentea	0.412|0.131b	0.000|0.000a	0.000|0.0188a	0.000|0.000a	0.012|0.062a
P. kennedyi	0.212|0.243a	0.000|0.000a	0.025|0.056a	0.000|0.000a	0.025|0.0383a
Different letters represent significant differences ($p < 0.05$).					

Nuclear invagination in *H. ancistroides* and *P. argentea* did not differ from each other ($p > 0.05$) but differed from other species. Nuclear budding and lobulated nucleus in *C. modestus* and *H. ancistroides* did not differ but showed differences in relation to the other species. Regarding micronuclei, only *C. modestus* showed more genotoxic damage. There was no significant difference between species regarding cytoplasmic invagination ($p > 0.05$) (Table 3).

Considering the *in situ* genotoxicity index, there was a significant difference between the species. *C. modestus* and *H. ancistroides* were the ones with the highest frequencies of genotoxic damage compared to the others. *A. fasciatus* differed from *C. modestus* and *H. ancistroides* ($p < 0.05$). *A. lacustris* showed differences between *C. modestus, H. ancistroides*, and *M. forestii* ($p < 0.05$). *C. modestus* differed from *P. argentea* ($p < 0.05$). *H. ancistroides* differed from *M. forestii*, *P. argentea*, and *P. kennedyi* ($p < 0.05$). Lastly, *M. forestii* differed from *P. argentea* ($p < 0.05$) (Fig. 3).

Allium cepa bioassay

Regarding the bioassay using *A. cepa*, it was observed that the water samples from the three streams significantly inhibited seed germination compared to the NC ($p < 0.05$) (Table 4).

Table 4
Cytotoxicity and genotoxicity in A. cepa cells exposed to water samples from the streams Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

Alterations	Endpoint	NC	PC	Piravevê	Vitória	Rosário
Citotoxicity	% GR	100.00 ± 0.00a	68.45 ± 5.15	50.60 ± 11.34b*	58.93 ± 3.09b*	77.38 ± 7.43c*
	RRG	4.74 ± 1.44a	2.43 ± 0.92	3.63 ± 1.12a	6.56 ± 2.36ab	6.86 ± 1.70ab
	MI	80.09 ± 5.66a	17.90 ± 6.76	15.08 ± 4.44b*	11.17 ± 10.45b*	20.84 ± 3.03b*
	CDR	0.00 ± 0.02a	0.00 ± 0.35	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Genotoxicity	CAI	0.29 ± 0.29a	5.83 ± 2.02	2.73 ± 0.83ab	5.42 ± 5.65b*	2.23 ± 1.25ab
Mutagenicity	MTI	0.00 ± 0.00a	0.17 ± 0.00	0.14 ± 0.17a	0.34 ± 0.71ª	0.00 ± 0.00a

% GR: germination, RRG: relative root growth, MI: mitotic index, CDR: cell death rate, CAI: Chromosomal alteration index, MTI: mutagenicity index, NC: negative control, PC: positive control. Different letters represent significant difference.

*Statistically different from NC ($p < 0.05$).

The germination percentage (%GR) of the water samples from the Rosário stream differed significantly from Piravevê and Vitória, presenting a higher number of germinated seeds. When the relative growth (RRG) of the root was analyzed, no significant difference between the streams and the NC was observed, only between the streams Piravevê, Vitória, and Rosário. There was a significant difference in MI between the NC and the streams, demonstrating that the water from these streams caused a decrease in cell division in the meristem cells of *A. cepa* roots. No difference was found between the CDR of the streams, demonstrating a low frequency of cell death (Table 4). In terms of CAI, NC showed a significant difference only in relation to Vitória. However, no significant difference was observed ($p > 0.05$) in MTI (Table 4).

Bioassay using A. lacustris

Regarding the bioassay using *A. lacustris*, we observed five types of nuclear and one cytoplasmic alteration (Table 5).

Table 5
Frequencies of nuclear and cytoplasmic alterations (median and interquartile deviation) of the bioassay using A. lacustris exposed to water samples from the streams Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

Alterations	NC	Stream		
		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 ($p < 0.05$). NC: negative control.				

The most frequent nuclear alteration in the erythrocytes of *A. lacustris* was nuclear invagination, presenting a significant difference between the negative control and the Vitória and Rosário streams. Lobulated nuclei also showed a significant difference between the Vitória and Rosário streams (Table 5). Regarding nuclear budding, binucleated cells, micronuclei, and cytoplasmic invagination, no significant differences were found between NC and the streams ($p > 0.05$) (Table 5).

Regarding the genotoxicity index of the bioassay with *A. lacustris*, there was a difference between the NC and Vitória and Rosário streams ($p < 0.05$) (Fig. 4). Conversely, among the three streams, there were no significant differences ($p < 0.05$) (Fig. 4).

Discussion

The limnological parameters sampled in the three streams and dissolved oxygen was within the criteria established by CONAMA (357/2005). There are no values stipulated by the legislation regarding temperature. In the Vitória and Rosário streams, the pH presented values below those established by the legislation, which may be associated with the highest proportions of sugarcane monoculture around these streams (Fig. 2). According to Nhiwatiwa et al. (2017), the low pH in the waters of rivers and streams near sugarcane plantations can be attributed to the extensive application of fertilizers, in addition to the fertigation process. In addition, other chemicals are also used in agricultural activities, which can change the pH of water (Sposito et al. 2019). Another aggravating factor in environmental imbalance is the reduction of riparian forests and vegetation surrounding the aquatic environment, reducing the area of protection of these environments against contaminants (Viana et al. 2018). The low

pH in these streams indicates environmental imbalance, because acidic water promotes metabolic alterations in fish representing a threat to species conservation.

This pH value can also be a response to the reduction of vegetation cover, which causes an imbalance in the physicochemical parameters of water, such as rising temperature and drop in pH. In aquatic environments, the latter can inhibit the growth of detritivorous bacteria and alter the development of zooplankton and phytoplankton, causing an imbalance (Birungi et al. 2007). Furthermore, low pH is associated with increased organic matter and decreased dissolved oxygen concentration (Bueno et al. 2005). Algae proliferation, due to nutrient enrichment from urban effluents, without proper treatment in aquatic environments, can result in decomposition and also reduce water pH (Marotta et al. 2008).

The areas near the streams showed a predominance of agricultural areas and a reduction in vegetation cover. However, Piravevê had a lower proportion of agriculture and a higher proportion of pasture areas in relation to the other streams. The replacement of vegetation cover around aquatic environments by agricultural areas is a major environmental problem, mainly because water bodies are unprotected from pollutants (Gonçalves et al. 2019). Dense vegetation cover has a barrier function, dampening the transport of sediments and pollutants into the stream during periods of increased rainfall, thus avoiding silting and imbalance in the structure and functioning of the ecosystem (Castro and Souza 2013; dos Santos et al. 2020). In addition, such coverage 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 vegetation around the streams leaves the environment more exposed and vulnerable to contaminants, compromising the health of aquatic biota, especially the fish. Several studies have reported the serious consequences of reducing plant cover for the health of aquatic organisms, which may cause a reduction in species due to the degradation of environmental quality (Viana et al. 2018, 2019; Ribeiro Junior et al. 2018; Gonçalves et al. 2019).

When the genotoxic index was analyzed, *C. modestus* and *H. ancistroides* suffered greater damage to their genetic material in relation to the other species, which may be related to the feeding habits (detritivory) of these two species. Viana et al. (2018) reported that *H. ancistroides* presented a greater number of genotoxic alterations in streams near urban and rural areas, compared to other species that did not have a detritivorous diet. This is because many contaminants are suspended for a period in the water, but later settle with the sediments of rivers and streams. Thus, the fish foraging at the bottom of aquatic environments end up biomagnifying higher loads of contaminants (Cardone 2006; Labarrère et al. 2012). Another reason for the greater genetic damage in *H. ancistroides* is that because of their benthopelagic behavior, these fish accumulate more pollutants, which consequently reflect as genetic damage (Labarrère et al. 2012). In this context, the literature suggests that the evaluation of environmental genotoxicity in different species of fish *in situ* generates more consistent results due to their different sensitivities to the aquatic environment (Leung et al. 2014; Silva et al. 2016). Zhang et al. (2019) highlighted that evaluating native fish species *in situ* in aquatic environments not only reflects toxic impacts, but also reveal their ability to adapt to unfavorable conditions in the environment. Several

anthropic activities alter land use near aquatic environments, reducing the connection between riparian vegetation cover and the aquatic environment (Gonçalves et al. 2019).

The analysis of cytotoxicity, genotoxicity, and mutagenicity of the water samples from the streams resulting from the bioassay with *A. cepa* indicated that all samples significantly affected seed germination. However, considering relative root growth, no significant difference was observed between the NC and the samples from the streams, as presented in Table 4. According to the MI results, there was a reduction in cell division in all sites in relation to the NC. We also verified a significant increase in CAI in the Vitória stream compared to the NC, indicating that the waters of these three streams are cytotoxic and genotoxic to the cells of *A. cepa*. However, none of the water samples showed mutagenic effects on the cells.

In the present study, significant changes were observed in seed germination and cell division of *A. cepa* exposed to water samples from the evaluated streams in relation to the NC, as shown in Table 4. The inhibition of germination, as well as the decrease in MI values and increase in CAI, indicated the presence of substances in the water that promoted cytotoxicity and genotoxicity in *A. cepa* cells. The MI serves as an indicator for the analysis of the cytotoxic potential of various substances that may inhibit or increase cell proliferation (Leme and Marin-Morales 2009; Francisco et al. 2018; Sposito et al. 2019). On the other hand, the chromosomal alterations measured by CAI indicated the presence of clastogenic and aneugenic agents in the environment, which allowed defining the genotoxic effect (Athanásio et al. 2014).

Previous studies also reported a decrease in MI and an increase in CAI were also observed in *A. cepa* cells after exposure to water samples when compared to the NC in different periods and streams (Athanásio et al. 2014; Da Silva et al. 2018). These studies associated the alterations of these parameters with the agricultural products used around the collection sites. Based on this, the results of this study may have been influenced by the effect of the use of agricultural products around the sampling sites (Fig. 2).

The period of collection of water samples may also have influenced the cytotoxic and genotoxic effects verified, because during rainy periods there is an increase in the flow of water that transports the compounds derived from leaching and percolation from diffuse sources, which can have negative impacts on the environment. Similar results to those observed in our study were also verified by Salles et al. (2016), who evaluated the dry and rainy seasons and obtained lower MI values and higher CAI values for *A. cepa* cells exposed to surface water samples from the rainy season in relation to the dry season. Considering the MI and CAI, the cells of *A. cepa* tested with the water sample of Vitória stream were the ones that most demonstrated inhibition of cell division and induction of chromosomal alterations in relation to the other treatments. These effects may be related to the land use around this site, since, as observed in Fig. 2, the Vitória stream, in addition to presenting conversion to agriculture land, also presented higher anthropic occupations in its surroundings compared to the others. However, further studies in these streams are necessary to identify the nature of pollutants present in the water samples that cause cytotoxicity and genotoxicity.

In the bioassay with *A. lacustris*, nuclear invagination was the alteration that presented the highest frequency, especially in the Vitória and Rosário streams. 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). Nuclear invagination is closely related to cell repair, which, when detecting a defective chromosomal area, initiates a repair process, eliminating the affected area with imperfections occurring in the nuclear membrane (Shimizu et al. 1998). In this sense, literature reports that nuclear alterations are responses to exposure to genotoxic agents (Ergene et al. 2007; Riveros et al. 2021).

As observed, the greatest genotoxic damage in fish exposed to water from the Vitória and Rosário streams may be associated with some type of contaminant in the water body, derived from the cultivation of sugarcane, which causes genetic damage to erythrocytes. Furthermore, we can predict that the genotoxic damage observed in fish may be linked to low vegetation cover due to the expansion of agricultural areas near these streams. The results of the present study corroborate those of Viana et al. (2017) and Dourado et al. (2016), who reported that fish exposed to polluted waters in this region had chromosomal alterations and DNA damage. The genotoxicity indices of the bioassay with *A. lacustris* observed in the Vitória and Rosário streams were significantly different from the negative control, confirming a higher number of genotoxic damage from the waters of these two streams. In the evaluation of fish species *in situ*, nuclear invagination was the most evident alteration in all fish species; this being the first response of the organism when detecting chromosomal imperfections, initiating a process of repair and/or elimination of the affected area, resulting in imperfections in the membrane before finishing the process and characterized by nuclear anomalies (Thomé et al. 2016; Oliveira et al. 2018). Thus, different land uses associated with agricultural activities such as pastures and sugarcane cultivation may variably affect the environmental integrity of water bodies, and later the biota of streams. In this sense, even though the Piravevê stream had higher proportions associated with the pasture area, the results showed that the impacts were lower when compared to the Vitória and Rosário streams where the occupation in their surroundings is associated with agricultural areas, especially the monoculture of sugarcane.

In summary, the three streams presented low native vegetation cover due to the expansion of agricultural areas directly reflecting the loss of environmental quality. The Vitória and Rosário streams presented lower pH, which may be related to the higher proportion of agricultural areas around these sites. Regarding *in situ* fish sampling, more genotoxic damage was identified in fish species with detritivorous feeding habits. Therefore, the combination of different tests and tools applied in this study were fundamental to assess the toxicogenetic status of these sites and emphasize the need for the recovery of riparian vegetation cover to improve the quality of the habitat and exposed organisms. In the bioassay with *A. lacustris*, a greater number of genotoxic alterations were observed in fish erythrocytes in the Vitória and Rosário streams. In the bioassay with *A. cepa*, the water samples from all streams caused a decrease in cell division and induction of chromosomal alterations in the cells; however, these changes were more pronounced in the Vitória stream, where there were higher proportions of agriculture land and

little local vegetation coverage. It is worth mentioning, therefore, the need to create a reforestation project of native species throughout the study area to maintain the balance of this aquatic ecosystem.

Declarations

Data availability

The data is not stored in a database, but we accept to share.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest related to the publication of this manuscript.

Ethical approval This article does not contain any studies with human participants performed by any of the authors. The experiment was performed with approval from the Ethics Committee in Animal Research (Opinion No. 10/2015) of the Federal University of Grande Dourados/UFGD, and all procedures involving animals were performed in accordance with the ethical standards of the institution.

Author Contribution Vandressa Raquel Lucas Poloni Meira: Analysis, data collection and editing; Elicléia Maria de Souza Silva: Data collection, analysis and editing; Lucilene Finoto Viana: Conceptualization, data collection, analysis review and editing; Bruno do Amaral Crispim: Conceptualization, data collection, analysis and review; Luiza Flávia Veiga Francisco: Analysis, review and editing; Yzel Rondon Súarez: Data collection, analysis, review and editing; Alexeia Barufatti: Review and editing; Júlio César Jut Solórzano: Data collection and review, and Alessandra Paim Berti: Conceptualization and review.

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Figures

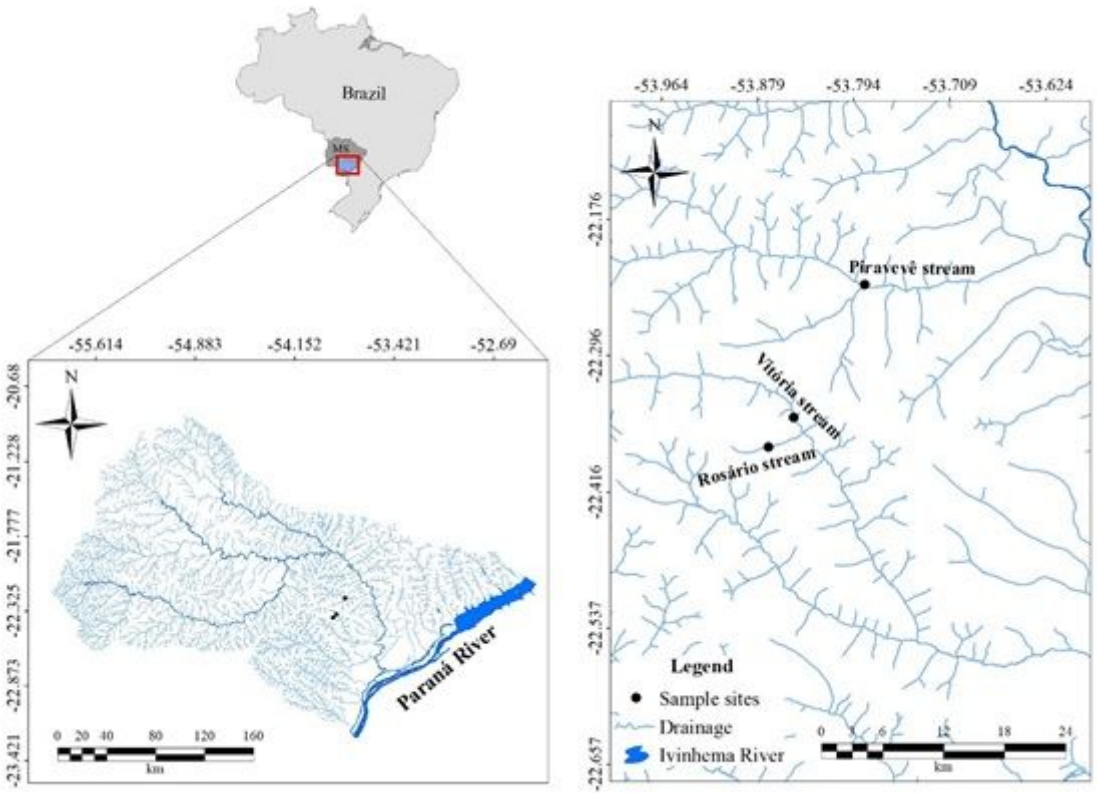

Figure 1

Location of the sampling sites in streams, Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, Upper Paraná River, Mato Grosso do Sul.

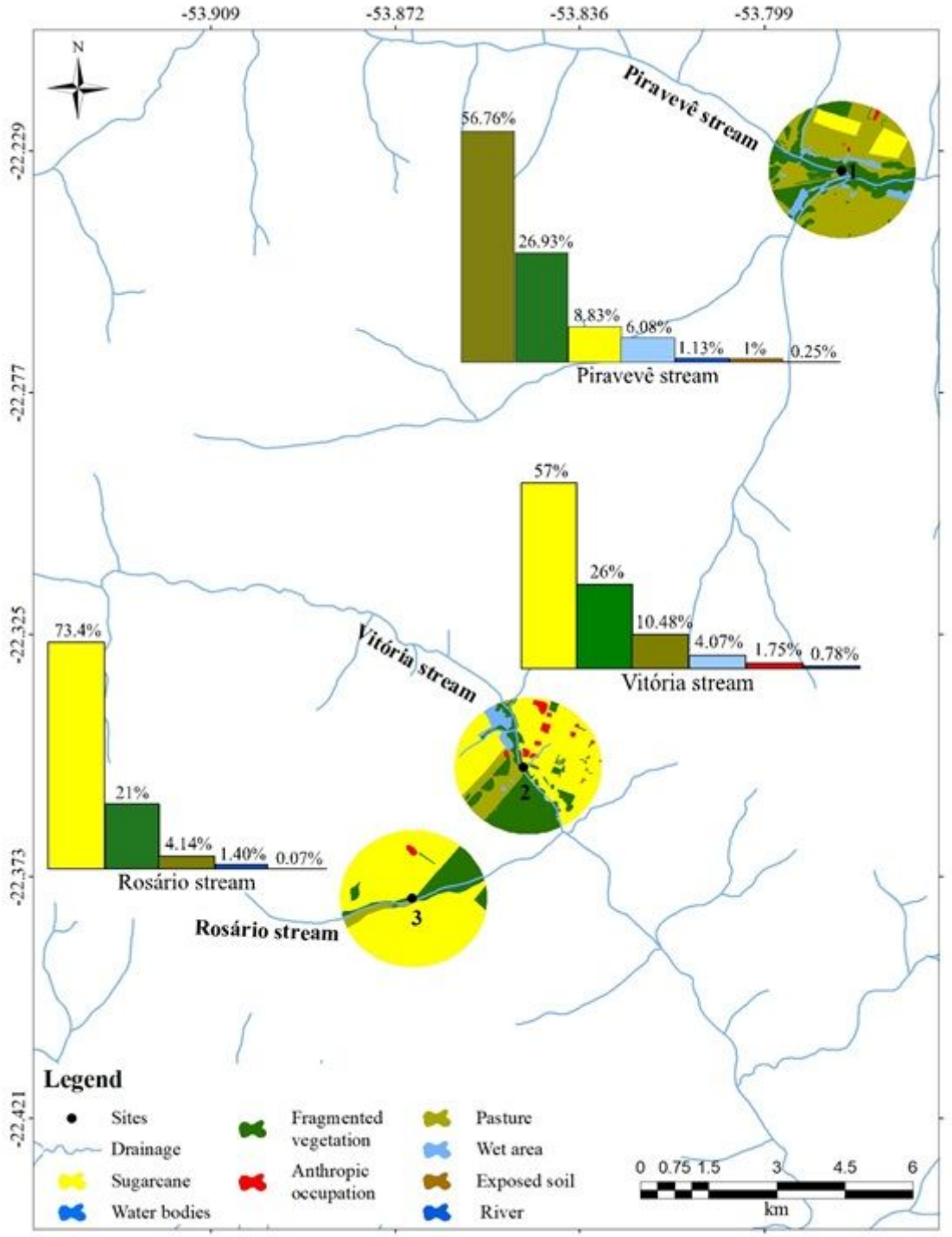

Figure 2

Land use and cover around three streams, Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

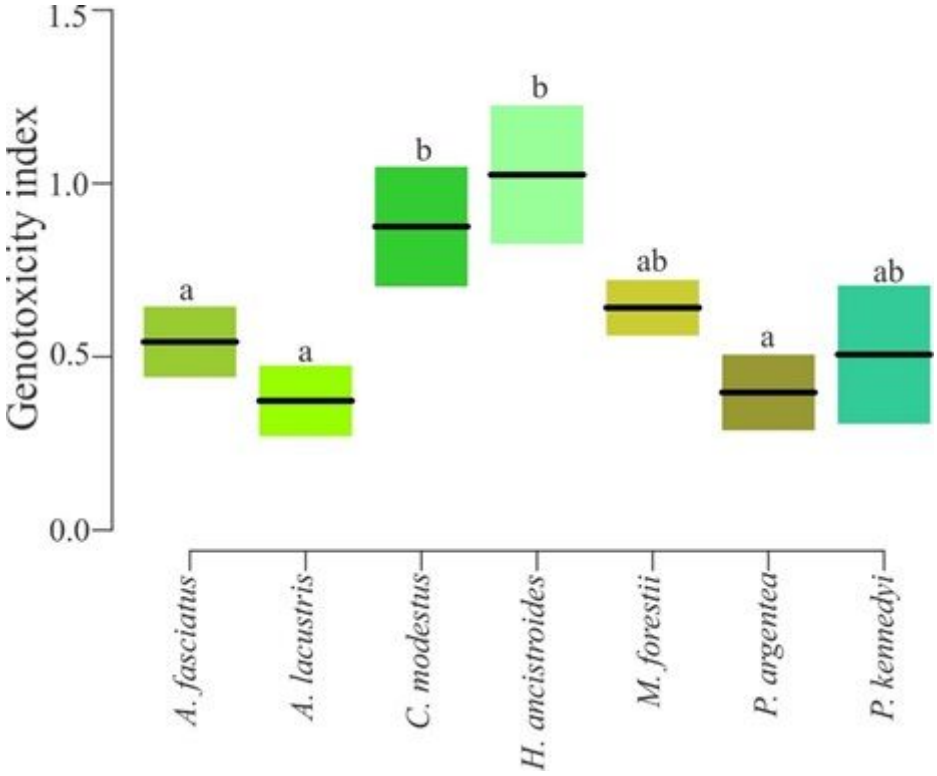

Figure 3

Genotoxicity index relation (mean and confidence interval), of fish species in situ in the Piravevê, Vitória and Rosário streams, belonging to the Ivinhema River basin, in Mato Grosso do Sul.

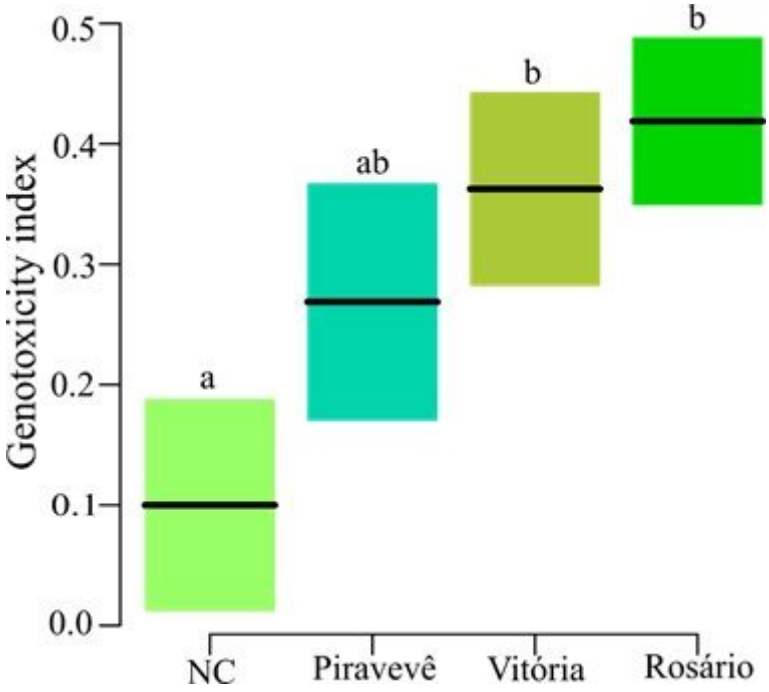

Figure 4

Genotoxicity index (mean and confidence interval), of the bioassay with A. lacustris exposed to water samples from the streams Piravevê, Vitória and Rosário, belonging to the Ivinhema River basin, in Mato

Grosso do Sul.