

Original Article

# Water Quality and Vegetation Health in Urban Conservation Areas: A Case Study of Paragem Municipal Natural Park

Marina Stefanos Schibichewski<sup>1</sup>, Bruno do Amaral Crispim<sup>1</sup>, Alexeia Barufatti<sup>1\*</sup>

<sup>1</sup>Laboratory of Ecotoxicology and Genotoxicity, Faculty of Biological and Environmental Sciences, Federal University of Grande Dourados (UFGD), Dourados, MS, Brazil

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## Abstract

The Paragem Municipal Natural Park (PMNP), an integral protection conservation unit, plays a crucial role in the conservation and recovery of ecosystems located in the city of Dourados/MS. Located in an urban area, it faces challenges related to human influence, which can affect its terrestrial and aquatic communities. This study aimed to analyze the vegetation index and diagnose the water quality of a tributary of the Paragem stream located within the PMNP. The research involved examining the Green Leaf Index, physical-chemical and microbiological parameters of the water, as well as ecotoxicological tests covering three trophic levels. In addition, the genotoxic effect of the water on a piscine model was investigated. The results revealed low vegetation cover and degradation of the park's water quality, evidenced by microbial contamination, toxicity to primary producers, and genetic damage to fish. Therefore, these results indicate the need for monitoring and environmental education measures, ecological restoration, and water resource recovery and conservation management in the PMNP to favor the recovery and conservation of its environmental resources.

**Keywords:** Anthropogenic impact; Ecotoxicology; Genotoxicity; Vegetation index; Water health.

## Resumo

O Parque Natural Municipal do Paragem (PMNP), uma unidade de conservação de proteção integral, desempenha um papel crucial na conservação e recuperação dos ecossistemas localizados na cidade de Dourados/MS. Localizado em uma área urbana enfrenta desafios relacionados à influência antrópica, que pode afetar suas comunidades terrestres e aquáticas. O presente estudo teve como objetivo analisar o índice de vegetação e diagnosticar a qualidade da água de um afluente do córrego Paragem situado dentro do PMNP. A pesquisa envolveu a análise de Índice Foliar Verde, parâmetros físico-químicos e microbiológicos da água, além de ensaios ecotoxicológicos abrangendo três níveis tróficos. Além destes, investigou-se o efeito genotóxico da água em um modelo pisce. Os resultados revelaram baixa cobertura vegetal e uma degradação da qualidade hídrica do parque, evidenciada pela presença de contaminação microbiana, toxicidade para produtores primários e danos genéticos em peixes. Portanto, esses resultados indicam a necessidade de medidas de monitoramento e educação ambiental, restauração ecológica e gestão do recurso hídrico recuperação e conservação no PMNP, a fim de favorecer a recuperação e conservação de seus recursos ambientais.

**Palavras-chave:** Impacto Antrópico; Ecotoxicologia; Genotoxicidade; Índice de vegetação; Saúde da água.

\*Corresponding author: alexeiabarufatti@ufgd.edu.br

## INTRODUCTION

The Municipal Nature Parks, conceived as fully protected conservation units, are dedicated to the conservation and recovery of ecosystems. In addition to being crucial for maintaining the urban climate balance, these areas are also ideal for carrying out scientific research and implementing environmental education programs (Brasil 2000, IMAD 2007). In particular, the Paragem Municipal Natural Park (PMNP) was created in the city of Dourados/MS to protect and restore the Paragem stream valley by Municipal Law No. 3.009/07. This area, part of the Atlantic Forest Semideciduous Seasonal Forest (IMAD 2007), faces challenges due to its proximity to inhabited areas, resulting in the inappropriate disposal of materials and solid waste in its territory. In addition, the PMNP suffers indirect impacts from a sewage treatment plant (STP) and irregular water and sewage connections found in the park area, which compromise its integrity and ecosystem (IMAD 2007).

Therefore, studies that assess the quality of environmental resources are essential to determine the level of conservation. A relevant factor in these analyses is the presence of vegetation, which improves the quality of water bodies and provides numerous ecosystem services, such as nutrient distribution, erosion, and temperature control (Saklaurs *et al.* 2022). In addition, it is essential to note that the PMNP is home to vital springs for the Paragem stream (IMAD 2007), which makes environmental diagnosis a valuable tool for examining the characteristics of surface waters and the effects of human activities on these ecosystems (Pessoa *et al.* 2018). The increase in contaminants in these bodies of water can be evidenced by changes in the physical-chemical parameters of the water, as well as the presence of microorganisms from the metabolism of endothermic animals, indicative of potential fecal contamination (Américo-Pinheiro *et al.* 2021). In addition, ecotoxicological and genotoxicity tests are emerging as fundamental tools in environmental diagnosis. Exposure of organisms to polluted environments can lead to pathologies that compromise their genetic, physiological, and behavioral functions and, in some cases, result in genetic anomalies passed on to subsequent generations (Hussain *et al.* 2020; Saha 2021; Viana *et al.* 2017). Species such as algae, crustaceans, and fish are recognized for their sensitivity to environmental pollution, functioning as efficient bioindicators in detecting contamination (Saha 2021; Sikorski 2021; Yadav and Pandey 2018). Primary producers, particularly microalgae such as *Raphidocelis subcapitata* (synonym *Pseudokirchneriella subcapitata*), are highly susceptible to contaminants. These aquatic organisms play a crucial role in the balance of aquatic ecosystems, providing the food base for subsequent trophic levels (Huarachi-Olivera *et al.* 2019). Given that crustaceans

of the genus *Daphnia* feed on these microalgae, their use as bioindicators is evident, since their mobility and reproductive capacity are affected in the presence of contamination (Sikorski 2021). In addition, chronic exposure of fish to polluted environments signals environmental damage and can result in significant genetic alterations, adversely affecting their quality of life and health (Hussain *et al.* 2020; Viana *et al.* 2017).

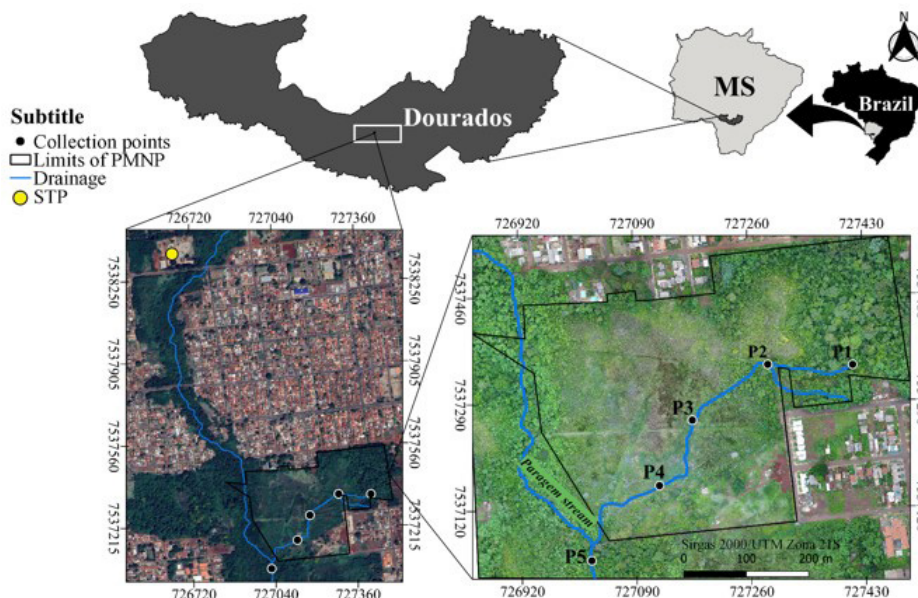
Environmental diagnostics are fundamental as they provide valuable information on biological, physical, chemical and microbiological variables. They can identify possible detrimental effects on these variables in aquatic communities, playing a key role in implementing preventive and corrective measures (CONAMA 2005; Pacheco *et al.* 2018). Since no similar work has been carried out in this area, this study aimed to analyze the vegetation index and examine water quality in a tributary of the Paragem stream located in the Paragem Municipal Natural Park, an area designated a complete protection conservation unit.

## MATERIAL AND METHODS

### Characterization of the study area

The study was conducted in the PMNP in Dourados, Mato Grosso do Sul, Brazil. Data collection occurred in March 2022, covering four strategic locations along a tributary of the Paragem stream (P1, P2, P3 and P4). This selection included points stretching from the stream's source to where it flows into the Paragem stream (P5)(Fig 1).

Located on the urban perimeter and surrounded by residential areas, the PMNP represents a fully protected conservation area that extends over 183,522.63 m<sup>2</sup>, as established by Bill No. 064/2020. The proximity of a STP imposes a direct human influence on the Paragem stream, highlighting the PMNP's imperative need to preserve the valley bottom ecosystem, a remnant of the biodiverse Semideciduous Seasonal Forest of the Atlantic Rainforest. For collecting environmental data, strategic points were selected within the park (P1, P2, P3, and P4) and the Paragem stream (P5). At each point, water samples were collected in plastic bottles and immediately cooled in cool boxes with ice to ensure they were preserved until they were analyzed at the Ecotoxicology and Genotoxicity Laboratory - Universidade Federal da Grande Dourados (UFGD).



**Figure 1.** Location map of the PMNP and the sewage treatment plant (STP) in Dourados, MS, Brazil. The collection points are indicated as P1 (22°15'16.1''S, 54°47'35.7''W) - Spring area; P2 (22°15'16.1''S, 54°47'40.1''W) - Meeting of springs; P3 (22°15'19.0''S, 54°47'44.0''W) - Wetland adjacent to the water main; P4 (22°15'22.4''S, 54°47'45.7''W) - Flooded area; P5 (22°15'26.3''S, 54°47'49.2''W) - Section of the Paragem stream.

Source: Authors

### Vegetation Index Analysis GLI (Green Leaf Index)

On April 12, 2022, images of the park area were collected using a Remotely Piloted Aircraft (RPA) with an autopilot and integrated RGB camera. DroneDeploy 2.160.0 software was used for flight planning, setting an altitude of 150 m. The captured images were processed to create a 2D orthomosaic, which made it possible to analyze the Green Leaf Index (GLI). The study area was delimited with a 10 m perimeter around each sampling point (P1, P2, P3, P4 and P5), and the entire park was included. The GLI was calculated using the equation that considers the green, red, and blue color bands (Louhaichi *et al.* 2001) using the QGIS 3.22 software. GLI values range from -1 to 0.5, where negative numbers indicate exposed soils or areas devoid of vegetation, and positive numbers denote the presence of vegetation, reaching up to 0.5 in areas of dense vegetation. Consequently, five categories were defined based on the GLI scale: healthy (0.3 to 0.5), moderate (0.2 to 0.3), low (0.1 to 0.2), very low (0 to 0.1), and absent (-1 to 0).

### Water quality diagnosis

#### Physico-chemical data

Physico-chemical parameters were measured using a previously calibrated HI9829 Hanna® multi-parameter probe. The equipment measured temperature (°C), dissolved oxygen (mg/L), electrical conductivity ( $\mu\text{S}/\text{cm}$ ), pH and total dissolved solids (TDS). In addition, the transparency and turbidity of the water were analyzed at the same collection points, using the Secchi disc and an AP 2000 WT - Policontrol turbidimeter for each parameter, respectively.

### Microbiological assessment of water

Total Coliforms and *Escherichia coli* were analyzed using the 3M™Petrifilm™ system, according to the manufacturer's instructions. The water samples, identified as P1, P2, P3, P4, and P5, were diluted in saline solution at 100 g/L, following a 1:10 ratio (i.e., 1 mL of water in 9 mL of saline solution), until a  $1 \times 10^{-4}$  dilution factor was reached. Subsequently, the samples were homogenized in a vortex. For the analysis, 1 mL of each dilution was placed in the center of the agar of the 3M™Petrifilm™ plates. These plates were incubated at  $37 \pm 1^\circ\text{C}$  in a New Lab NL 81/27 bacteriological oven. Identification and counting of total coliforms and *E. coli* were carried out after incubation periods of 24 h and 48 h, respectively. The assessment was made using dilutions starting at  $10^{-1}$  and the results were expressed in Colony Forming Units per 100 mL (CFU/100 mL). Total coliforms were indicated by a red color accompanied by gas bubbles, while *E. coli* CFUs were identified by a blue color and accompanied by gas bubbles.

### Ecotoxicological tests

#### Chronic toxicity test on *Raphidocelis subcapitata*

The ecotoxicological tests for chronic toxicity using the algae *R. subcapitata* were conducted by the ABNT-NBR 12648 (2023) and OECD 201 (2011) guidelines. To carry out the tests, the algal inoculum with a  $1 \times 10^5$  cells/mL density was exposed to 40 mL of the raw water sample collected at P1, P2, P3, P4 and P5. These samples were enriched with solutions from an Oligo culture medium. In addition, a negative control (NC) was prepared using Oligo medium and deionized water. The assays carried out in duplicate, were maintained under controlled

conditions for 72 h, including continuous stirring at 160 rpm, constant illumination with fluorescent light at 4500 lux, and a temperature of  $25\pm 2$  °C. After this period, the number of algal cells was counted using a Neubauer chamber and an XDS-3 Alltion inverted optical microscope at 250x magnification. The count was compared with that of the NC.

### **Acute toxicity test on *Daphnia similis***

The procedures for maintaining cultures and conducting toxicity tests with the organism *D. similis* followed the guidelines established by ABNT NBR 12713 (2022) and OECD 202 (2004). For these tests, 24-hour-old neonates were selected, all from the same culture. The neonates were exposed to the water samples for 48h at a controlled temperature ( $20\pm 2$ °C) and photoperiod (12:12 h light/dark). MS medium was used as a NC. For each water sample (P1, P2, P3, P4, and P5), 05 organisms were added, chosen at random, in four replicates with 10 mL in closed containers and without food. At the end of exposure, the number of immobile organisms in each concentration tested was recorded, allowing the percentage of immobility to be calculated. Validation of the tests was ensured by checking that the rate of immobile organisms in NC did not exceed 10% at the end of the test.

### **Acute toxicity test on *Astyanax lacustris***

In the acute toxicity test carried out with the *A. lacustris* species (provided by the Universidade Federal do Mato Grosso do Sul - Aquidauana Unit), the fish were initially acclimatized for 30 days. This acclimatization occurred in aquariums with a capacity of 450L, equipped with constant aeration systems. The environmental conditions were strictly controlled, keeping the temperature at  $26\pm 2$  °C and a photoperiod of 12h of light alternating with 12h of darkness. In addition, the fish were fed pelleted feed containing 32% crude protein (Douramix Animal Nutrition, Lot 100121).

The acute toxicity test was carried out on adults of *A. lacustris*, with an average weight of  $5.28\pm 1.09$  g, by ABNT NBR 15088 (2022) and OECD 203 (2019) guidelines. Ten fish were exposed to water collected from each point (P1, P2, P3, P4 and P5) and the NC (dechlorinated water) for the toxicity tests. The total test period was 96h, with a total water change after 48h. It is important to note that the fish were not fed throughout the test period. The water parameters of temperature ( $26\pm 2$ °C), pH ( $7.5\pm 0.2$ ), dissolved oxygen ( $7.5\pm 0.5$  mg/L) and conductivity ( $455 - 470\mu\text{S}/\text{cm}$ ) were kept constant. Project 08/2018 relating to this study was submitted and approved by the UFGD animal ethics committee.

### **Genotoxicity test on *A. lacustris***

The genotoxicity assessment of the water samples collected from P1, P2, P3, P4 and P5 was carried out on adults of *A. lacustris* subjected to the toxicity test. Five fish were selected for each sampling point and the blood was collected after 96h

of exposure. The micronucleus test and the analysis of nuclear morphological changes were carried out on blood samples taken from the fish's tail vein. This procedure followed the protocol of Schmid (1975) and Heddle *et al.* (1983) with modifications adapted to the study context. The blood cells were stained using Schiff and Fast Green dyes. Microscopic analysis was done using a Nikon Eclipse E200 optical microscope at 1000x magnification. One thousand erythrocytes were counted per slide for each fish. The genotoxicity (GI) and mutagenicity (MI) index were calculated based on the frequency of nuclear morphological changes observed, following the methodology proposed by Carrasco *et al.*, 1990. This approach allowed for a quantitative and qualitative assessment of the possible genotoxic and mutagenic effects of the water at the collection sites.

### **Statistical analysis**

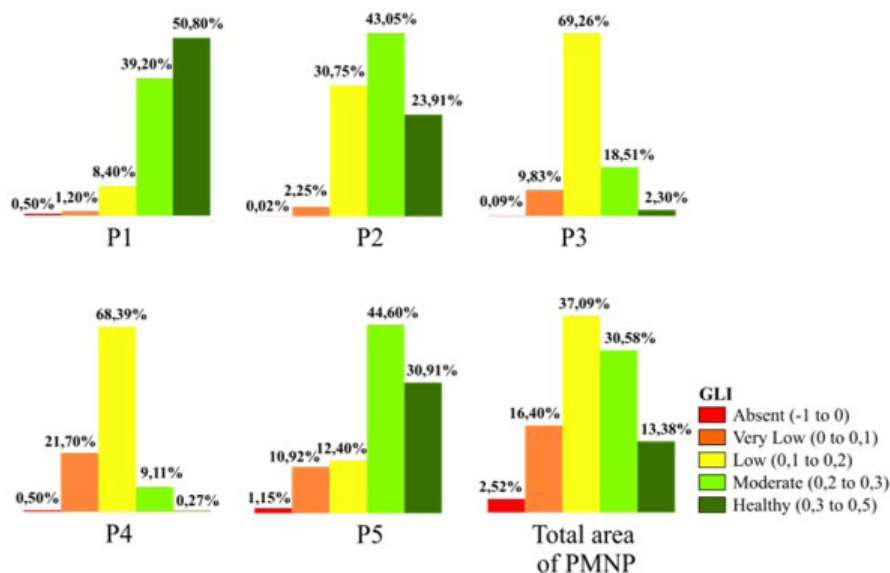
For the statistical analysis of the genotoxicity observed in *A. lacustris*, the R software version 2022.02.1 was used. Initially, the data collected was submitted to the Shapiro-Wilk test to verify the normality of the distribution. As the data did not show a normal distribution and was therefore considered non-parametric, the Kruskal-Wallis test was used. This test was used to compare the results obtained from the NC with those from the sampling points. For a more detailed analysis, the Dunn's posteriori test was applied for a more specific comparison between the groups. In both statistical tests - Kruskal-Wallis and Dunn - a significance level 0.05 was adopted. This statistical criterion was used to determine the relevance of the differences observed between the groups of data analyzed.

## **RESULTS**

### **Evaluation of the vegetation index (GLI)**

The GLI analysis within the boundaries of the PMNP showed considerable variation in the condition of the vegetation. When considering the park's total area, it was observed that 37.09% of the vegetation has a low density and 30.58% has a moderate density. Notably, only 13.38% of the vegetation in the area was classified as healthy, as illustrated in Fig. 2.

Different percentages of vegetation were observed around the points. In P1, there was 50.80% healthy vegetation and 8.40% low-density vegetation. At P2, healthy vegetation comprised 23.91%, while low-density vegetation accounted for 30.75%. At P3 and P4, the lowest percentages of healthy vegetation were recorded, with P3 showing 69.26% low-density vegetation and only 2.30% healthy, and P4 with 68.39% low-density and 0.27% healthy. On the other hand, P5 showed 23.91% healthy vegetation and 30.75% low level (Fig. 2).



**Figure 2.** Representative graphs of GLI (Green Leaf Index) for a 10 m radius around each of the sampling P1, P2, P3, P4, P5 and the total area of the PMNP. Source: Authors.

## Diagnosis of the water body

### *Physico-chemical and microbiological parameters*

The National Environment Council (CONAMA) resolution 357 (2005) establishes that springs and freshwater streams within fully protected conservation units should be classified as a particular class. Article 13 states, “In special class waters, the natural conditions of the body of water must be maintained”. Based on this framework, to assess the quality of the water in P1, P2, P3 and P4, the physical-chemical and microbiological parameters of the water were compared with the criteria established for class 1 fresh surface water, used because it is the most restrictive according to the regulations (CONAMA

2005). In contrast, P5 was classified as class 4, as defined by a previous study by the Mato Grosso do Sul Environment Institute (IMASUL 2018). According to the study that classified the Paragem stream, the concentrations of biochemical oxygen demand (BOD) and coliforms are only suitable for class 4 waters, which justifies its categorization in this class.

Regarding the results of the evaluation of the physical-chemical parameters of the water at the different collection points, it was possible to observe dissolved oxygen (DO) values that did not comply with the legislation at P3 (5.80 mg/L) and P4 (4.35 mg/L). All measured values pH parameters, total dissolved solids (TDS), and turbidity meet legal standards (Table 1).

**Table 1.** Physico-chemical and microbiological parameters of the water at the collection points of the tributaries (P1, P2, P3 and P4) and the Paragem stream (P5), compared to the criteria established by CONAMA for Class 1 and 4 surface water.

Points	DO	pH	Temp	Cond	TDS	Trans	Turb	Total coliforms	<i>E. coli</i>
P1	6.40	6.46	25.45	283	142	60	1.10	<b>2,000</b>	0
P2	6.92	6.92	24.1	264	132	28	9.28	<b>23,000</b>	<b>10,000</b>
P3	5.80	6.69	23.0	299	148	47	6.33	<b>14,000</b>	0
P4	4.35	6.84	23.4	262	131	26	1.37	<b>1,000</b>	0
P5*	3.82	7.05	25.4	503	251	30	7.03	<b>80,000</b>	<b>38,000</b>
<b>Fresh surface water classes (CONAMA 357)</b>									
Class 1	>6	6.0-9.0	-	-	<500	-	<40	<200	
Class 4*	>2	6.0-9.0	-	-	-	-	-	-	

DO: Dissolved Oxygen (mg/L); Temp: Temperature (°C); Cond: Electrical Conductivity (µS/cm); TDS: Total Dissolved Solids (mg/L); Trans: Transparency (cm); Turb: Turbidity (UNT); Total Coliforms (24 h) and *E. coli* (48 h) expressed in CFU/100 mL. Values in disagreement with CONAMA 357/2005 are in bold. (—) indicates the absence of data in this regulation.

Regarding total coliform values, all sites exceeding the 200 CFUs/100 mL limit for class 1 bodies. P1 and P2 had 2,000 CFUs/100 mL and 23,000 CFUs/100 mL, respectively, as did P3 (14,000) and P4 (1,000). The number of CFUs in P5 is alarming, with 80,000 CFUs/100 mL, and high concentrations of the enterobacterium *Escherichia coli* were also found in P2 and P5 (Table 1), indicating fecal contamination of the water.

#### Ecotoxicological evaluation on *R. subcapitata*, *D. similis* and *A. lacustris*

The chronic toxicity test on *R. subcapitata* showed an inhibition of algal biomass growth of more than 10% compared to the NC at all sampling points. The inhibitions observed were 29.8% in P1, 41.7% in P2, 44.2% in P3, 27.1% in P4 and 63% in P5. The highest inhibition value was recorded in P5 (63%) and the lowest in P4 (27.1%), indicating variations in

toxicity between the different points. However, at all points, there was no reduction in mobility in *D. similis* or mortality in *A. lacustris*, indicating no toxicity in these organisms.

#### Genotoxicity test on *A. lacustris*

The samples collected at the sites not induce the formation of micronuclei, so it was not possible to calculate the mutagenicity index (MI). However, different types of nuclear alterations were observed in the erythrocytes: nuclear invagination (NI), nuclear budding (NB), vacuolized nucleus (VN) and binucleated cell (BC). These alterations served as the basis for calculating the Genotoxicity Index (GI), although no statistically significant difference was observed for the VN and BC alterations compared to the control. P4 showed no statistically significant differences in relation to any of the alterations observed (Table 2).

**Table 2.** Median and interquartile deviation of nuclear alterations: Nuclear Invagination (NI), Nuclear Budding (NB) and Genotoxicity Index (GI) observed in *A. lacustris* erythrocytes.

	NI	NB	GI
NC	0.05 0.01	0.02 0.05	0.10 0.01
P1	0.20 0.15*	0.15 0.05*	0.45 0.30*
P2	0.25 0.05*	0.15 0.15*	0.45 0.15*
P3	0.20 0.10*	0.05 0.05	0.25 0.10
P4	0.10 0.05	0.05 0.05	0.10 0.00
P5	0.27 0.08*	0.05 0.02	0.32 0.10*

\*Statistically significant difference between the negative control (NC) and each of the different points ( $p < 0.05$ ).

In P1 and P2, the NI and NB changes and the GI were statistically different from the control. In P3 and P5, they showed significant values for NI; in P5, the control was also statistically different from GI (Table 2).

## DISCUSSION

Considering the GLI analysis, P1 and P5 stood out for having higher proportions of healthy vegetation, with 50.80% and 30.91%, respectively (Fig. 2). These areas are recognized for their contribution to protecting biodiversity and providing various ecosystem services (Metzger *et al.* 2019). However, the predominant presence of the invasive species *Leucaena leucocephala* (Leucena) (IMAD 2007) in these areas may contribute to an imbalance in the local ecosystem, possibly due to its accelerated reproduction and lack of natural predators, which prevents the growth of native species (Kato-Noguchi and Kurniadie 2022).

In addition, the urban influence in the park's vicinity contributes to the vegetation cover's fragmentation, as evidenced by the predominance of low-density vegetation. Research carried out by Matsumoto *et al.* (2012) and Lunas

and Ribas (2013) in urban parks in Dourados, such as the Antenor Martins Municipal Park, the Arnulpho Fioravante Municipal Park and the PMNP, also found similar effects, reinforcing the need to implement conservation plans in degraded urban areas.

The predominance of the Poaceae family at P3 and P4 was associated with the high percentage of vegetation classified as low-level, according to Morgan and Ebsary (2020). This scenario indicates a marked fragmentation of vegetation in these areas. It is worth emphasizing the importance of conserving riparian vegetation, especially in areas surrounding rivers and streams. Riparian vegetation plays a fundamental role in preserving the aquatic environment, acting as a natural filter that prevents large quantities of sediment and chemical substances from flowing into water bodies. In addition, as Fritzsos *et al.* (2005) and Viana *et al.* (2020) pointed out, riparian vegetation contributes to controlling sun exposure and regulating water temperature, vital for maintaining aquatic biodiversity and the ecological balance of freshwater ecosystems.

Regarding water quality, the low dissolved oxygen (DO) concentrations observed at some points (Table 1) suggest

a possible overload of organic matter, probably due to the dumping of domestic effluents, which can compromise aquatic biodiversity (De Araújo 2013; Vieira 2019). This parameter is an indicator of the health of the aquatic ecosystem, reflecting the capacity of the body of water to sustain aquatic life.

Although not specified in CONAMA resolution 357/2005, temperature, electrical conductivity, and transparency are essential for assessing water quality. The thermal stability of aquatic ecosystems, indicated by the limited temperature variation observed ( $\pm 2^{\circ}\text{C}$ ), is crucial for preserving aquatic life (ANA 2005; Bornette and Puijalón 2011).

Knowing that natural waters should have electrical conductivity levels between 10 and 100  $\mu\text{S}/\text{cm}$ , the values found (262 to 503  $\mu\text{S}/\text{cm}$ ) indicate that the environment is impacted, either by soil leaching or by the load of domestic or commercial sewage (Piratoba *et al.* 2017; Almeida 2019). The high electrical conductivity at all points suggests the presence of dissolved substances in the water, possibly due to anthropogenic influence. This increase in conductivity can have negative implications for the chemical composition of the water and, consequently, for aquatic organisms (CETESB 2016). It was possible to observe that the conductivity of the water increased as the total dissolved solids were added, as shown in Table 1.

Transparency is another relevant parameter for maintaining aquatic life. It indicates an ideal depth of 20 to 60 cm at which sunlight penetrates the water, allowing photosynthetic activity. Considering that the values obtained vary from 26 to 60 cm between the points, the photic zone is suitable for the development of organisms (CETESB 2016; Oliveira 2000).

Considering that P2 is a confluence of springs, the high coliform values at this point may be influenced by an area with direct contact with homes and a clandestine sewage connection near the site. The alarming number of CFUs in P5 (80,000 CFUs/100 mL) may be related to the presence of wastewater from the STP discharged into the Paragem stream. Coliforms can be present in waters with high organic content, such as industrial effluents, plant material, or soils in the process of decomposition, showing a continuous process of contamination by organic matter (ANA 2005; CETESB 2016).

However, high turbidity and the significant presence of total coliforms and *E. coli*, especially at P5, indicate contamination and possible risks to public health and the environment. These microbiological indicators suggest the need for immediate action in monitoring the management and treatment of effluents to ensure the prevention of the entry of contaminants. Given the ambition of the PMNP managers to make the park available for public visitation, it is crucial to emphasize that recreational use of local surface waters can pose a risk to human health. Specifically, there is a significant increase in the risk of gastrointestinal diseases associated with these waters. This danger is particularly pronounced in urban environments, where the microbiological quality of surface waters can be severely compromised, especially during rainy periods. On these occasions, surface runoff can lead to

the dispersion of wild animal waste and the transportation of sediment, exacerbating water contamination (Frias *et al.* 2020; Américo-Pinheiro *et al.* 2021).

In ecotoxicological tests, the inhibition of *R. subcapitata* growth may be due to the presence of contaminants from commercial herbicides widely used in the region. It is important to note that the municipality in which the park is located has an economy predominantly focused on agricultural activities. Recent research points to water contamination from using metolachlor, which is widely used to control Poaceae, it is possible that this contaminant is present in the water. (Groen 2020; Machado and Soares 2021).

In addition, chemical substances released by other algae and plants can act as a natural herbicide so that competition between species can occur (DellaGreca *et al.* 2010). These allelochemicals mainly include fatty acids such as chlorelin, released by *Chlorella vulgaris*, with known inhibitory activity for *R. subcapitata* (Fergola *et al.* 2007; DellaGreca *et al.* 2010; Tan *et al.* 2019; Chaïb *et al.* 2021). The bioaccumulation of toxic substances by primary producers can lead to chronic harmful effects at different trophic levels, highlighting the complexity and interconnectedness of ecotoxicological impacts on the aquatic ecosystem (Huarachi-Olivera *et al.* 2019).

Although the contamination of the water collected has not yet caused lethal effects, the results suggest the presence of genotoxic agents, signaling an environmental impact. Previous studies, such as that by De Sousa *et al.* (2020), have found nuclear alterations in aquatic organisms, even without resulting in mortality. In addition, Dos Santos *et al.* (2020) identified chemical contaminants in the Várzeas do Rio Ivinhema State Park (an integral protection unit), suggesting potential damage to fish genetic material. Research has also reported genotoxicity in fish induced by herbicides from different chemical groups (González *et al.* 2017; Gupta and Verna 2020).

Nuclear invagination was the nuclear alteration that showed the highest frequency, and the exact mechanism of formation of its nuclear erythrocyte abnormality is still not fully understood, knowing only that several cell types form during the proliferative phase of the cell cycle (Braham *et al.* 2017; Tasneem and Yasmeen 2018). Although the water samples collected in the park did not result in toxicity to microcrustaceans and fish, there are contaminants carried possibly by the lack of vegetation in the areas bordering the water bodies that may be contributing to the existence of contaminants that promoted genetic damage in *A. lacustris* erythrocytes. In addition, the discharge of domestic sewage and the use of herbicides, xenobiotics and other pollutants also can alter the structure of DNA. This scenario highlights the urgent need to implement preventive and corrective measures to protect and conserve the PMNP's environment. The lack of vegetation and the degradation of water bodies require immediate action, such as improving infrastructure and increasing enforcement, as well as environmental education projects to raise awareness of the park's conservation.

Updating the management plan is also essential to ensure the implementation of effective environmental protection strategies, contributing to the sustainability and preservation of this valuable ecosystem.

## CONCLUSION

It can be concluded that the PMNP is in an advanced state of environmental degradation, evidenced by its highly reduced vegetation cover, which comprises only 13.38% of healthy vegetation. In addition, the quality of the park's water is unsatisfactory, reflected by microbial contamination and changes in electrical conductivity. We detected toxicity to algae and the genetic damage observed in fish erythrocytes indicate significant negative impacts on the aquatic ecosystem.

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## AUTHOR CONTRIBUTIONS

MSS: Data curation; Formal analysis; Investigation; Methodology; Roles/Writing - original draft.

BAC: Formal analysis; Investigation; Methodology; Supervision; Validation; Visualization; Writing - review & editing.

AB: Formal analysis; Funding acquisition; Investigation; Supervision; Validation; Visualization; Writing - review & editing.

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