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Analysis of water quality, bioindicators, contaminants and their cytogenetic impacts in a Cerrado reservoir

Análise da qualidade da água, bioindicadores, contaminantes e seus impactos citogenéticos em um reservatório do Cerrado

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ABSTRACT

The fragmentation of Brazilian rivers due to the construction of hydroelectric dams has altered aquatic ecosystems, resulting in the accumulation of residues in sediments. This study evaluated the cytotoxicity and genotoxicity of water and sediments from the Cana Brava reservoir, located in the Tocantins-Araguaia basin in the Cerrado biome, using the *Allium cepa* test. Sampling points were situated in the Cana Brava reservoir near two tributaries, the Bonito River and the Varjão Stream, areas susceptible to contaminant input due to proximity to urban areas. Physicochemical parameters, metals through atomic absorption spectrometry, toxicity, and analyses of bioindicators such as microalgae and macrophytes were assessed. Near the Varjão Stream, algal richness was higher, with the presence of *Chara rusbyana*, which indicates good water quality. Near the Bonito River, pollution-indicating microalgae such as *Euglena sp*., *Nitzschia sp*., and *Oscillatoria sp*. were identified, along with a prevalence of *Salvinia auriculata*, favored by nutrient availability. Cytogenetic tests revealed that sediments, especially from the site near the Varjão Stream, caused chromosomal abnormalities and cytogenotoxic effects, with an increase observed after 72 hours. Principal components analysis showed that phosphorus and potassium in the sediment from the site near the Bonito River correlated with cellular alterations at 48 hours, while sediments near the Varjão Stream resulted in a higher frequency of cytogenetic alterations at 72 hours. These results highlight local impacts on water quality and biological diversity, recommending continuous monitoring to mitigate the adverse effects of pollution in the Cana Brava reservoir.

Keywords: *Allium cepa*; water; sediment; aquatic macrophytes; reservoir.

RESUMO

A fragmentação de rios brasileiros pela construção de barragens hidrelétricas alterou os ecossistemas aquáticos e resultou em acúmulo de resíduos nos sedimentos. Este estudo avaliou a citotoxicidade e a genotoxicidade da água e dos sedimentos do reservatório de Cana Brava, bacia do Tocantins-Araguaia, no Cerrado, utilizando o teste *Allium cepa*. Os pontos de amostragem foram localizados no reservatório de Cana Brava perto de dois tributários, o Rio Bonito e o Córrego Varjão, áreas suscetíveis à entrada de contaminantes devido à proximidade de áreas urbanas. Foram avaliados parâmetros físico-químicos, metais por espectrometria de absorção atômica, toxicidade, além de análises de bioindicadores, como microalgas e macrófitas. Próximo ao Córrego Varjão, a riqueza de algas foi maior, com a presença de *Chara rusbyana*, que indica boa qualidade da água. Próximo ao Rio Bonito, microalgas indicadoras de poluição, como *Euglena sp.*, *Nitzschia sp.* e *Oscillatoria sp.*, foram identificadas, juntamente com a regularidade de *Salvinia auriculata*, favorecida por nutrientes. Testes citogenéticos revelaram que sedimentos, especialmente do ponto próximo ao Córrego Varjão, causaram anormalidades cromossômicas e efeitos citogenotóxicos, com aumento após 72 horas. A análise de componentes principais mostrou que fósforo e potássio no sedimento do ponto próximo ao Rio Bonito se correlacionaram com alterações celulares em 48 horas, enquanto sedimentos do ponto próximo ao Córrego Varjão resultaram em maior frequência de alterações citogenéticas em 72 horas. Estes resultados destacam os impactos locais na qualidade da água e na diversidade biológica, recomendando-se o monitoramento contínuo para mitigar os efeitos adversos da poluição no reservatório de Cana Brava.

Palavras-chave: *Allium cepa*; água; sedimento; macrófitas aquáticas; reservatório.

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Introduction

Hydroelectric dams and their reservoirs have become widespread across many Brazilian rivers, leading to river fragmentation and the accumulation of various residues from human activities in their sediments, as highlighted by recent studies (Rambo et al., 2017; Nogueira et al., 2021; Canlı et al., 2023). These residues, including pesticides, trace metals, and metalloids, pose significant environmental risks (Peresin et al., 2023). Inadequate monitoring of human activities can compromise the quality of water sources, impacting aquatic organisms due to their heightened susceptibility to pollutants (Factori et al., 2014; Tretyak and Palianytsia, 2023). Studies have demonstrated the toxic effects of these pollutants on aquatic organisms, influenced by factors such as the type of contaminant, duration of exposure, and water quality (Omar et al., 2013; Soto et al., 2020; Akhtar et al., 2021; Essawy et al., 2021).

Sediments, comprising a mixture of organic and inorganic substances with diverse physical, chemical, and biological properties (Sarkar et al., 2004), act as the primary reservoir for insoluble or poorly soluble pollutants. They can accumulate these pollutants at concentrations much higher than those in the water column, posing a significant risk of diffuse contamination. Sedimentary pollutants can have widespread impacts on aquatic ecosystems and human health, particularly through water and fish consumption (Silva et al., 2020). Moreover, sediments not only store pollutants but also contribute to contaminant release into the water column through processes like desorption, remobilization, redox reactions, and decomposition. Metals and other pollutants present in sediments can profoundly affect water quality and persist as a long-term source of contamination for aquatic organisms. Unlike organic pollutants, metals are non-biodegradable and remain in the environment indefinitely. Even when sediments in aquatic environments contain metal concentrations within the limits permitted by environmental legislation, they can still harm aquatic communities. This is because some chemicals can interact synergistically, meaning their molecules can react together, resulting in more intense toxic effects than each substance would cause individually in the aquatic ecosystem (Garcia-Käufer et al., 2014; Mathur and Panwar, 2024).

Biomarkers are commonly used to assess the impact of pollutants by monitoring cytotoxicity and genotoxicity in aquatic environments (Corredor-Santamaría et al., 2016; Ek-Huchim et al., 2022). A variety of tests are available for this purpose (de Campos Ventura et al., 2008; Prieto et al., 2008; Factori et al., 2014; Botelho et al., 2015; Nascimento et al., 2020) aiding in the evaluation of both chemical and environmental substrate toxicity (Freire et al., 2023). Effective management and monitoring of reservoirs offer numerous benefits, safeguarding their functionality and water quality (Wagner and Erickson, 2017).

In this study, the biological model used was the Allium cepa test. This organism was chosen due to its applicability in different environments, as it can be exposed to various environmental matrices, such as water and sediments (Geras et al., 2011). Furthermore, it is highly sensitive in detecting cytogenetic alterations and toxicity in aquatic environments (Kassa, 2021).

This test offers an accessible, sensitive, and effective way to monitor the toxic impacts of pollutants, as the bulbs are economical, easy to cultivate, and do not require special treatment conditions (Pesnya et al., 2022). The meristematic cells of the roots are highly susceptible to toxic agents and exhibit rapid growth, allowing for the detection of alterations within a few days (Leme and Marin-Morales, 2009). The test also enables the observation of changes in different cell division processes, making it an important indicator of genotoxic damage (Ramos et al., 2020) and capable of assessing overall toxicity, such as root growth inhibition, which indicates cellular or systemic damage.

The present study aimed to evaluate the presence of contaminants and the cytogenotoxic potential of the water and sediments in the Cana Brava reservoir, located in the Tocantins-Araguaia basin within the Cerrado biome. Biological communities of microalgae were also analyzed as bioindicators of water quality in the reservoir, seeking an integrated understanding of the factors that may affect the ecological health of this environment. This reservoir follows the cascading formation of the Tocantins River and is considered a multipurpose reservoir, with the main activities being electricity generation, aquaculture, tourism, and leisure. Studying this ecosystem is crucial for understanding the interactions between environmental and human activities, especially given the increasing anthropogenic pressure on these reservoirs. This assessment is necessary because the tributaries that feed the reservoir are affected by anthropogenic impacts, including various types of contaminants and eutrophication factors, which can compromise the quality of water and sediments, harming the aquatic community and the potential uses of water.

Materials and Methods

Study area

The study investigates a reservoir located in the Tocantins-Araguaia basin, the largest watershed entirely within Brazil, which covers approximately 918,000 km^2, representing 11% of Brazil's total area. This basin spans three Brazilian regions: North, Northeast, and Midwest. The Tocantins-Araguaia basin has significant hydropower potential (Ferreira et al., 2022), with seven hydroelectric dams already installed along its main course. Additionally, the region serves as a crucial agricultural frontier, particularly for soybean production, and is rich in minerals such as asbestos, copper, nickel, bauxite, iron, manganese, gold, and silver (Ministério do Meio Ambiente, 2006). Mining activities in the Cerrado biome require the implementation of strict environmental controls to limit the discharge of industrial effluents into water bodies and mitigate deforestation. The adoption of sustainable practices not only protects the Cerrado's biodiversity but also ensures water quality and ecological balance (Marques et al., 2022).

The study focused on the Cana Brava Hydroelectric Plant reservoir, located at 13°24'6" S, 48°8'33" W, covering an area of 139 km^2 in the Northern part of the Brazilian state of Goiás (Figure 1). This reservoir plays a crucial role in the state's economy, supporting tourism, sport fishing, and aquaculture due to its proximity to urban centers. The state government has invested in local fish farming to stimulate employment and contribute to the economy, directly and indirectly, aiming to export the fish produced.

Samples of water, sediment, and microalgae communities were collected in August 2021 during the dry season. The sampling sites were positioned in the reservoir, near two tributaries that feed it: the Bonito River and the Varjão Stream. These sites were chosen because the tributaries are close to urban areas and flow into the reservoir, making them potential pollution sources. A single sampling campaign was conducted, during which both water and sediment samples were drawn in duplicate at each sampling point. The water samples were collected using a Van Dorn bottle and stored at 4°C. A total of 10 liters of water were obtained per point, with 5 liters for the sample and 5 liters for the duplicate. The sediment samples were collected using a Petersen grab, and 2 kg of sediment were extracted at each point, with 1 kg for the sample and 1 kg for the duplicate. All samples were kept refrigerated for 3 days, which was the time required to transport them to the laboratory where analyses were performed. Sediment processing involved creating 1:4 elutriates (sediment: ultrapure water), following the procedures described by Rocha et al. (2009) and the United States Environmental Protection Agency (USEPA, 1998).

Chemical, physical, and biological parameters of the water and sediments

Water and elutriate samples were analyzed for nine physical and chemical parameters using standardized procedures with a HANNA HI 9829 multiparameter probe. The parameters measured included potential hydrogen (pH), oxidation-reduction potential (ORP), dissolved oxygen (DO) saturation and concentration, electrical conductivity, resistivity, total dissolved solids (TDS), turbidity, temperature, and atmospheric pressure.

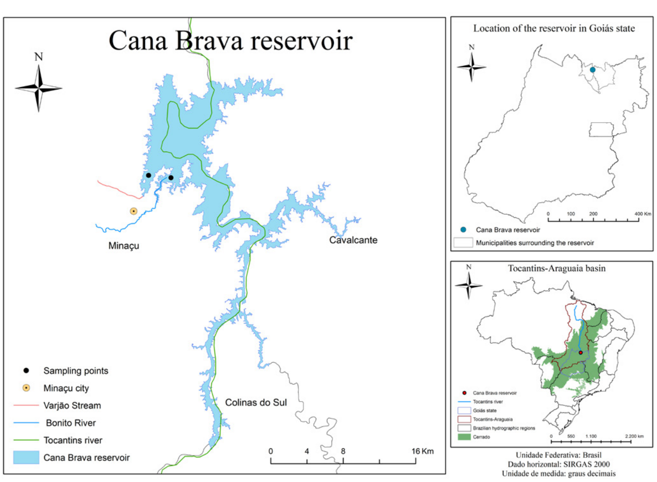

Figure 1 – Location of the Cana Brava reservoir in Northern Goiás, Central Brazil.

Biological analyses involved the collection of periphyton and phytoplankton samples. Phytoplankton samples were obtained using horizontal tows with a 20 µm plankton net, placed in 100 ml dark bottles, preserved in Lugol's solution, and analyzed using the Utermöhl sedimentation chamber method (1958). Periphyton samples were collected *in situ* following Bicudo's (1990) methodology, stored in 200 ml glass bottles, and fixed with 4% formalin. Samples were examined under a Zeiss Axiostar optical microscope at 1000x magnification, and species were identified using standard references (Wetzel and Likens, 2001).

Analysis of contaminants

The atomic absorption method was employed to determine total metal concentrations in water and sediment samples (Perkin-Elmer 2280). Analyses were performed at a certified agricultural laboratory, specialized in water and soil analysis, accredited by the Fertility Laboratory Quality Control Program (PAQLF), and coordinated by the Brazilian Agricultural Research Corporation (Embrapa) Soils, ensuring high-quality standards. The following elements were analyzed: Nickel (Ni), Copper (Cu), Lead (Pb), Cadmium (Cd), Chrome (Cr), Iron (Fe), Zinc (Zn), Manganese (Mn), Sulfur (S), Magnesium (Mg), Calcium (Ca), Potassium (K), Sodium (Na), Aluminum (Al), Phosphorus (P), and Nitrogen (N).

Analysis of toxicity

Water and sediment cytogenotoxicity was assessed using the *Allium cepa* test, according to the methodology described by Fiskesjo (1985). Onion bulbs (*Allium cepa*) of a single variety were acquired from a commercial supplier. Before exposure to the collected water and sediment samples from the Cana Brava reservoir, the viability of the bulbs was verified through a pre-selection process. A total of 16 bulbs were used, separated, and identified as A, B, C, and D, with 4 bulbs per treatment. The bulbs were submerged in distilled water for 24 hours, under a controlled photoperiod of 10 hours of white light (from 8:00 AM to 6:00 PM). After this period, the bulbs showed adequate root growth and were confirmed as viable, and a studied sample of these viable bulbs was selected to compose the negative control group.

The growth of these bulbs—both the roots and the aerial portion of the onion—was monitored for 72 hours, after which they were divided into four quadrants, and after another 48 hours, the growth of the roots was quantified and measured. A root thread from each quadrant was extracted and stored for subsequent analysis. After 72 hours, the remaining roots were measured again, following the established numbering, and a root thread from each quadrant was collected and stored for analysis. After the exposure of the control group, all roots were cut, and the bulbs were reused for subsequent toxicity tests. This experiment involved two distinct treatments (water and sediment). The acute toxicity of the sediment and water was assessed by continuously exposing the onion bulbs to the different experimental substrates, namely water and a 1:4 elutriate of the sediment. For this, sediment samples were dried in an oven at 105°C for 48 hours, sifted to remove leaves and twigs, and then combined with ultrapure water. The water and the elutriate were placed in 1-liter beakers and maintained at a controlled temperature of 25°C for 72 hours (Bertanza et al., 2024).

Of the 16 selected bulbs, four onion bulbs were used per sampling point, with two exposed to water (n=2) and the other two, to the elutriate (n=2). The experiment was conducted in duplicate, totaling eight bulbs per sampling. Eight root threads were collected from each bulb over the 72 hours of experimental exposure, with one thread being collected from each quadrant after 48 hours and another four collected after 72 hours. These roots were fixed in Carnoy's solution (3:1: methanol/acetic acid) for 24 hours and then stored at 4°C before analysis.

The analysis of these fixed roots included a series of steps. They were first immersed in hydrochloric acid (HCl) for 5 minutes to facilitate the hydrolysis of the cell wall and later placed in distilled water for 10 minutes to neutralize the effects of the acid. The roots were then immersed in a 2% acetic orcein solution for 5 minutes to stain the nuclear content and outline the cell walls (Vasquez and Rodríguez, 2023). The slides were prepared with material collected from the cap region, which has constant cell division, avoiding the growth zone, where the cells are more elongated and divide less frequently. A total of 1,000 cells were analyzed per bulb (Bagatini et al., 2007). Since each concentration was duplicated, 2,000 cells were counted for each treatment and 4,000 for each sampling point. The *Allium cepa* cells were analyzed using a Zeiss Axiostar optical microscope at 400x magnification.

We quantified the chromosomal aberrations (CA), nuclear anomalies (NA), and micronuclei (MN) observed in the meristematic cells of *Allium cepa*. The number of cells with chromosomal alterations (CA, NA, and MN) and the mitotic index (MI) were determined following the procedures described by Leme and Marin-Morales (2009). The root growth observed after 48 and 72 hours was analyzed to provide a toxicity index.

Data analysis

Root growth parameters were expressed as mean ± standard error of the mean in millimeters, while cell division data were presented as absolute frequencies along with MI. CA, NA, and MN were also reported as absolute frequencies. The normality of continuous variables was assessed using the Lilliefors test, followed by parametric analysis of variance (ANOVA) or non-parametric Kruskal-Wallis tests as appropriate. Discrete variables were presented as frequencies and analyzed using the chi-square (χ^2) test. Proportional comparisons were performed using the Z-test with Bonferroni adjustment, following MacDonald and Gardner (2000). Additionally, principal component analysis (PCA) was conducted on treatments and water physicochemical parameters, and key alterations were identified in the toxicity test. All statistical analyses maintained a significance level of 5% ($p<0.05$).

Results

Physicochemical parameters of the water

The water quality parameters applied for the study followed the standards established by the Brazilian Council for the Environment in Ordinance (CONAMA, 2005), Resolution 357/2005. According to this resolution, the Cana Brava reservoir is classified as a class 2 water body.

At the site near Bonito River, the water was relatively soft, with a pH of 7.18, while at the site near Varjão Stream, the water exhibited slightly hard characteristics, with a pH of 6.85. These differences can be attributed to the geological composition of the soil surrounding each location, which influences the release of minerals into the water. It is essential to highlight that all physicochemical parameters evaluated at the sampled points (Table 1), besides the concentrations of toxic metals

Table 1 – Physicochemical parameters of surface waters collected in the Cana Brava reservoir near the tributaries of Bonito River and Varjão Stream.

Parameter	Bonito River	Varjão Stream	Reference value (CONAMA 357/2005)	Unit of measurement
pH	7.18	6.85	6–9	pH
ORP	183.4	259	-	mVORP
DO saturation (%)	48.5	50.6	-	% DO
DO concentration	3.93	3.9	>6	ppm
Electrical conductivity	196	84	-	µS/cm
Absolute conductivity	195	88	-	µS/cmᴀ
Resistivity	0.0051	0.0119	-	MΩ.cm
TDS	98	42	<500	ppm TDS
Salinity	0.09	0.04	-	PSU
Turbidity	10.2	3.3	<100	FNU
Temperature	24.66	27.65	-	°C
Atmospheric pressure	14.349	14.411	-	psi
Hardness	85.36	53.31	-	mg/L

pH: potential hydrogen; ORP: oxidation-reduction potential; DO: dissolved oxygen; TDS: total dissolved solids.

(Table 2), were within the limits established by Brazilian legislation, indicating that the quality of the water and sediments does not pose risks to public health or the ecosystem.

Furthermore, the water near Bonito River contained slightly higher levels of S, Ca, Mg, K, and P than the water near Varjão Stream, possibly due to a greater input of nutrients and organic matter resulting from management practices and denser vegetation. In the sediments, the site near Bonito River showed the highest concentrations of S, Na, Zn, Cu, Fe, Mn, and P, while those near Varjão Stream showed higher concentrations of Cr, Ni, B, Ca, Mg, and K. These variations may be influenced by differences in human activities and land use near each water body, which directly affect the dynamics of nutrient cycles and sediment quality (Table 2).

Biological samples: periphyton and phytoplankton

The analysis of biological communities in the study, involving periphyton and phytoplankton, revealed a specific diversity, highlighting Bacillariophyceae, Cyanophyceae, Chlorophyceae, and Zygnematophyceae as the most abundant classes (Figure 2). The area near Varjão Stream showed a richness of 45 algal species, surpassing the 42 species found near Bonito River. However, pollution bioindicator species were identified in Bonito River, such as *Nitzschia sp.*, *Oscillatoria sp.*, and *Euglena sp.*

Additionally, in the area near Varjão Stream, a significant presence of the submerged macrophyte *Chara rusbyana* was observed, possibly representing the first record of this species in the state of Goiás. In contrast, in the area near Bonito River, the floating macrophyte *Salvinia auriculata* predominated. These differences in macrophyte composition and diversity suggest variations in the aquatic environments between these sites, reflecting the influence of specific ecological factors and possible anthropogenic pressures.

Figure 2 – Species richness of the different classes of periphyton and phytoplankton recorded at Bonito River and Varjão Stream, in the Cana Brava reservoir in Northern Goiás, Brazil.

Table 2 – Concentrations of toxic metals and oligoelements found in the samples of surface water and sediments collected in the Cana Brava reservoir near the tributaries of Bonito River and Varjão Stream.

Element	Concentration recorded in the water samples collected from:		Reference value (CONAMA 357/2005)	Unit of measurement	Concentration recorded in the sediment samples collected from:		Reference value (CONAMA 357/2005)	Unit of measurement
	Bonito River	Varjão Stream			Bonito River	Varjão Stream		
Cr	<0.10	<0.10	0.05	mg/L	2.0	2.1	37.3	mg/kg
Ni	<0.100	<0.100	0.025	mg/L	0.7	2.2	18	mg/kg
Cd	<0.100	<0.100	0.001	mg/L	0.1	0.1	0.6	mg/kg
Pb	<0.10	<0.10	0.01	mg/L	0.1	0.1	35	mg/kg
S	0.9	0.6	-	mg/L	2.8	2.0	-	mg/kg
Na	8	8	-	mg/L	3.7	2.6	-	mg/kg
Zn	0.03	0.03	0.18	mg/L	1.5	0.9	-	mg/kg
Cu	0.010	0.010	0.009	mg/L	2.2	2.0	35.7	mg/kg
Fe	0.09	0.10	0.30	mg/L	836	636	-	mg/kg
Mn	0.02	0.02	0.10	mg/L	125	98.6	-	mg/kg
Ca	20	15	-	mg/L	2.1	2.2	-	cmolc/dm³
Mg	8.5	3.8	-	mg/L	0.6	0.8	-	cmolc/dm³
N	0.01	0.01	3.70	mg/L	800	500	-	mg/kg
K	2.3	2.2	-	mg/L	19.4	19.8	-	mg/kg
P	0.20*	0.15*	<0.05	mg/L	10.4	7.7	-	mg/kg
B	-	-	-		0.14	0.23	-	mg/kg
Al	-	-	-		0	0	-	cmolc/dm³
C	-	-	-		5.22	2.90	-	mg/kg

*Values excess of the level recommended by CONAMA Resolution 357/2005.

Analysis of toxicity (Allium cepa test)

No significant variations were observed in the average root length between the treatments and their respective controls after 48 or 72 hours of exposure (p>0.05), as shown in Table 3. The p-values for the average growth among treatments exceeded the 0.05 threshold, indicating that the treatments did not have significant effects on the roots analyzed. These results are consistent with the statistical analysis performed, which used a 95% significance level.

The number of dividing cells varied significantly among treatments, with p<0.0001 in both exposure periods (48 and 72 hours). After 48 hours, all treatments except for Bonito River sediment were significantly different from the control. The behavior of the Bonito River sediment was similar to that of the Varjão Stream sediment (Table 4). After 72 hours, the treatments with sediments maintained higher cell division frequencies than most other treatments. During this period, the water near the Bonito River collection point exhibited behavior similar to that of the sediments, suggesting that, over time, the water may acquire properties comparable to those of the sediments in terms of cellular impact.

A wide range of cytogenetic alterations was detected, indicating the genotoxic and mutagenic effects of the sediments and, to a lesser extent, the water. These alterations included binucleated cells, cells with elongated nuclei, chromosome loss in prophase and metaphase, C-metaphase, anaphase with sticky chromosomes (chromosomal adhesion), chromosomal bridges, and micronuclei (Figure 3). The observed classes of cytogenetic alterations were grouped into a single category—cytogenetic alterations—due to the low frequency of individual stratifications (types of alterations). Consequently, the toxic effect was presented and analyzed using an adjusted data set to enable statistical inference.

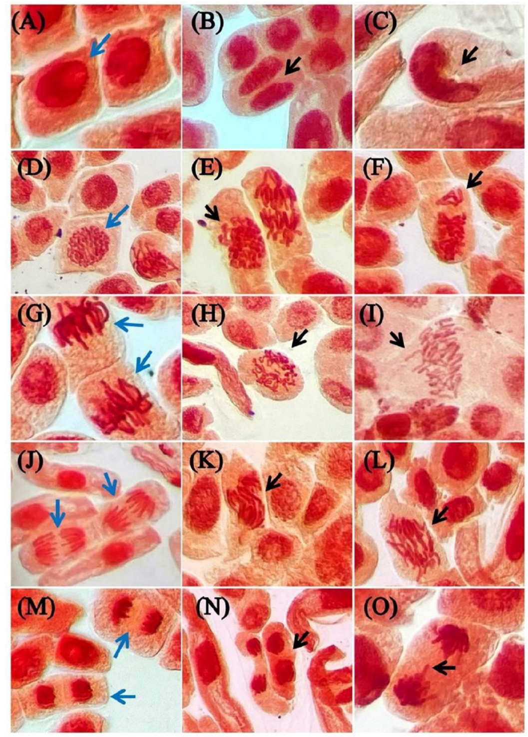

Figure 3 – Meristematic cells of Allium cepa exposed to the water and sediments from the Cana Brava reservoir: (A) Normal interphase; (B) Binucleated interphase; (C) Interphase with elongated nucleus; (D) Normal prophase; (E) Prophase with chromosome gaps; (F) Prophase with loss of chromosomes; (G) Normal metaphase; (H) C-metaphase; (I) Metaphase with loss of chromosomes; (J) Normal anaphase; (K) Anaphase with sticky chromosomes; (L) Anaphase with chromosomal bridges; (M) Normal telophase; (N) Telophase with nuclei of different sizes; and (O) Chromosomal. The first column corresponds to cells in normal phases of cell division (blue arrows), while the next two columns present the alterations observed in the same phases of cell division (black arrows).

Table 3 – Mean±standard deviation of onion roots per treatment, considering all samples analyzed during the two exposure periods.

Exposure time	Mean±standard deviation growth (in mm)					p-value (Kruskal-Wallis test)
	Negative control	Bonito River		Varjão Stream		
		Water	Sediment	Water	Sediment	
48 hours	4.05±2.83	5.99±3.51	2.64±2.59	7.52±6.93	8.59±3.89	0.32
	4.29±3.03	4.44±3.00	8.14±3.93	7.19±3.14	7.23±3.60	
72 hours	3.59±3.51	11.69±8.12	2.59±2.34	7.78±4.44	8.64±3.54	0.30
	3.65±2.19	4.81±4.41	7.44±5.63	9.62±7.57	5.59±5.36	

Table 4 – Number of cells observed in division per treatment and their respective mitotic indices. The values on the same line with different letters in superscript are significantly different (p<0.05) from each other.

Exposure time	Number of cells in division (mitotic index) recorded in the treatment:					p-value
	Negative control	Bonito River		Varjão Stream		
		Water	Sediment	Water	Sediment	
48 hours	275^{a} (13.75)	164^{b} (8.20)	331$^{a, c}$ (16.55)	177^{b} (8.85)	358^{c} (17.90)	<0.0001
72 hours	218^{a} (10.90)	250^{a} (12.50)	343^{b} (17.15)	218^{a} (10.90)	383^{b} (19.15)	<0.0001

The frequency of these alterations was higher in the sediment treatments after 48 hours and remained elevated after 72 hours (Table 5). Although water treatments showed no significant difference from the control after 48 hours, the water near Bonito River showed an increase in cellular alterations after 72 hours, suggesting changes over time.

The *Allium cepa* test revealed different patterns of metabolic activity across treatments. In the water samples, MIs were lower after 48 hours, indicating inhibition of metabolic activity. In contrast, sediments showed increased metabolic activity at both locations (Bonito River and Varjão Stream), which may be associated with a higher frequency of CA, NA, and MN.

The sediment samples presented the highest frequencies of cytotoxic and genotoxic alterations, with a notable increase after 48 hours of exposure. The 72-hour exposure resulted in an even higher frequency of alterations in both sediment and water samples. The sediment elutriates significantly affected cells, especially in the Varjão Stream samples, suggesting the presence of substances with cytotoxic, genotoxic, and mutagenic effects.

The PCA of the treatment parameters, cytogenotoxicity, and elements in the water and sediment samples indicated that the first two PCA axes explained 93.64% of the total variance in the data (Figure 4; Table 6). Negative controls (NC) 1 and 2 were negatively related to the other treatments, while positive associations were found between the cell alterations observed after 48 hours and among elements (Cu, Zn, Fe, Mn) in the sediment at Bonito River, particularly P and K. Cell alterations (after 72 hours) and the mean root length (after both 48 and 72 hours) were associated more positively with the sediment at Varjão Stream.

Discussion

Although the physicochemical parameters in this study are within the legal limits in Brazil, a correlation was identified between electrical conductivity, absolute conductivity, and total dissolved solids in water samples from the Bonito River. Electrical conductivity, reflecting water's ability to conduct electricity through cations and anions produced by the dissociation of substances (Kikuda et al., 2022), indicates pollutants when above 100 µS/cm, as suggested by the Environmental Company of the State of São Paulo (CETESB) (Montovani, 2021; CETESB, 2023). In this study, the recorded values indicate water quality alterations. High concentrations of nitrogen (N) and phosphorus (P), likely from the nearby city of Minaçu, were detected near the Bonito River, which may promote the accumulation of substances that cause cellular stress and genotoxicity in aquatic organisms (Nieder et al., 2018; Ren et al., 2022; Feng et al., 2023).

This nutrient enrichment enables excessive growth of *Salvinia auriculata*, a macrophyte with a high capacity to absorb N and P (Ansari et al., 2010). The proliferation of this plant, which can harm the ecosystem and accumulate N and P, confirms local eutrophication (Medeiros et al., 2016). In contrast, near Varjão Stream, the presence of *Chara rusbyana*, an algae indicator of low-turbidity waters (Palma-Silva et al., 2004), was observed, aligning with data showing lower turbidity (3.3 FNU) and high transparency in this area. *Chara sp.* is considered a bioindicator of water quality, thriving in oligotrophic or mesotrophic conditions (Bueno and Bicudo, 2021).

Microalgal diversity was higher near Varjão Stream, including Zygnematophyceae, an indicator of environmental preservation. In the Bonito River, pollution-indicating diatoms such as *Nitzschia sp.*, Cyanophyceae, and Euglenophyceae were found with some species, such as *Oscillatoria sp.* and *Euglena sp.*, acting as pollution bioindicators (Yusuf, 2020). These bioindicators offer a cost-effective method for water quality assessment. The historical occupation of the Cana Brava reservoir highlights the constant presence of algae and aquatic macrophytes, emphasizing the need for ongoing monitoring to mitigate impacts on water quality and the aquatic ecosystem due to a lack of standardization and continuity in monitoring databases (Pinheiro et al., 2019).

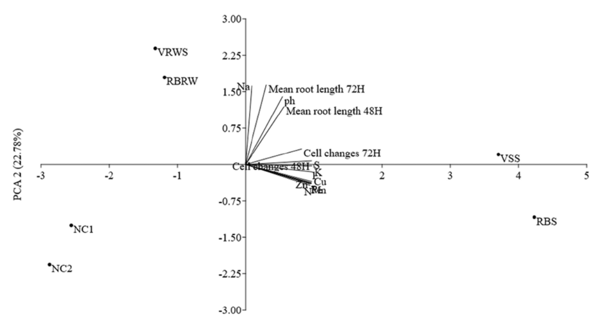

Figure 4 – Plot of the principal components analysis of the relationship between the chemical elements, watercourses, and cytogenotoxicity.
PCA: principal components analysis; NC1: negative control 1; NC2: negative control 2; VSWS: Varjão Stream water and sediment; BRWS: Bonito River water and sediment; VSS: Varjão Stream sediment; 48H: 48 hours of exposure; 72H: 72 hours of exposure.

Table 5 – Frequency of cytogenetic alterations of the onion root meristematic cells exposed to different treatments near Bonito River and near Varjão Stream sampling point in the Cana Brava reservoir, in Northern Goiás, Brazil. The values on the same line with different letters in superscript are significantly different (p<0.05) one from the other.

Exposure time	Total number of cytogenetic alterations recorded in the treatment:					p-value
	Negative control	Bonito River		Varjão Stream		
		Water	Sediment	Water	Sediment	
48 hours	7^{a}	21^{a}	93^{b}	19^{a}	114^{b}	<0.0001
72 hours	9^{a}	80^{b}	85^{b}	18^{a}	161^{c}	<0.0001

Table 6 – Results of the principal components analysis of the treatments, cytogenotoxicity, and element concentrations recorded in the water and sediment samples collected from the Cana Brava reservoir in Northern Goiás, Brazil.

Variable	PC 1	PC 2
Treatment		
NC1	-2.5568	-1.2509
NC2	-2.8724	-2.0580
BRW	-1.1879	1.7943
BRS	4.2334	-1.0831
VSW	-1.3225	2.3905
VSS	3.70630	0.20725
Cytogenotoxic pattern or element		
Cell changes 48H	0.31106	-0.00896
Cell changes 72H	0.26379	0.10362
Mean root length 48H	0.17956	0.38193
Mean root length 72H	0.097043	0.525500
S	0.310220	0.023894
Na	0.029039	0.519560
Zn	0.30233	-0.12842
Cu	0.31066	-0.11145
Fe	0.30850	-0.12583
Mn	0.30917	-0.12347
N	0.30283	-0.13711
K	0.31490	-0.049156
P	0.30891	-0.11922
pH	0.17309	0.44858

NC1: negative control 1; NC2: negative control 2; BRW: Bonito River water; BRS: Bonito River sediment; VSW: Varjão Stream water; VSS: Varjão Stream sediment

Seasonal variations affect the transport of cytotoxic substances, with rainy seasons intensifying runoff and mobilizing pollutants and dry seasons favoring the concentration and prolonged interaction of pollutants in the environment (Ramos et al., 2020). The *Allium cepa* assay is widely recognized as a reliable method for assessing cytotoxic and genotoxic potential (Sacramento et al., 2020). Studies show that samples collected during the dry season exhibit more intense changes, suggesting that seasonal fluctuations and effluent discharges influence the cyto-genotoxic potential of aquatic environments.

The absence of significant variations in mean root length between treatments and controls ($p>0.05$) indicates that exposure to sediments and water from areas near Bonito River and Varjão Stream did not significantly affect root growth in the *Allium cepa* samples. This corroborates findings by Wijeyaratne and Wadasinghe (2019), who also observed no significant variations in root growth with water exposure, although there was progressive inhibition in those exposed to sediment eluate.

On the other hand, the number of dividing cells varied significantly between treatments ($p<0.0001$), highlighting the impact of contaminants, especially in sediments. After 48 hours, all treatments, except the sediment near Bonito River, differed from the control. At 72 hours, the Bonito River water exhibited behavior similar to that of the sediments, suggesting a gradual release of genotoxic substances into the water.

Significant genetic alterations were detected, including binucleated cells, chromosomal adhesion and bridges, as well as micronuclei, indicating exposure to genotoxic and clastogenic agents. Wijeyaratne and Wickramasinghe (2020) highlight that chromosomal bridges may arise from replication errors, leading to chromosomal breaks and aneuploidy (Zachos, 2016). Additionally, chromosome loss and C-metaphase suggest aneugenic effects that may induce abnormal chromosomal segregation (Leme and Marin-Morales, 2008).

The frequency analysis of these alterations showed that treatments with sediments had the highest indices after 48 hours, remaining elevated after 72 hours (Table 4), indicating that sediments act as reservoirs of genotoxic substances. Although water from the Bonito River initially showed no significant impact, an increase in cellular aberrations was observed after 72 hours, suggesting a cumulative effect of soluble pollutants. Jacoboski and Fachinetto (2022) and Kikuda et al. (2022) corroborate these findings, demonstrating cytotoxic and genotoxic potentials in water exposed to domestic and industrial pollutants, even in seemingly clean areas like the Varjão Stream.

Cüce et al. (2022) emphasize that low concentrations of toxic metals in sediments can have combined harmful effects, underscoring the importance of continuous sediment monitoring due to the potential release of toxic substances over time. The efficacy of the *Allium cepa* assay in detecting cytotoxic and genotoxic damage, as confirmed by Kassa (2021), reinforces its relevance in environmental monitoring, endorsed by the International Programme on Chemical Safety (IPCS) and United Nations Environment Programme (UNEP) (Jacoboski and Fachinetto, 2022). Even when physicochemical analyses do not detect specific pollutants, the *Allium cepa* test can diagnose relevant cellular and genetic alterations in sediments, highlighting the need for continuous monitoring, especially seasonal, due to fluctuations in pollutant concentrations and discharges with climate conditions and local water regimes. The water at Bonito River, which showed genotoxic alterations only after 72 hours, exemplifies how prolonged exposure intensifies pollutant effects. Seasonal monitoring is also essential, as pollutant concentrations and their release into the water may vary according to climate conditions and the local hydrological regime.

Conclusions

The study revealed that, despite the physicochemical parameters being within legal limits, electrical conductivity and total dissolved solids in the Bonito River indicated possible pollution, with elevated concentrations of nitrogen and phosphorus likely originating from effluents in Minaçu. These nutrients favored the proliferation of *Salvinia auriculata* near the Bonito River, while *Chara rusbyana*, found near Varjão Stream, indicated good water quality conditions due to low turbidity. The *Allium cepa* test showed a higher frequency of chromosomal abnormalities in sediment samples, especially at Varjão Stream, and a cumulative genotoxic effect in water samples from the Bonito River after 72 hours, suggesting a gradual release of genotoxic substances. The presence of bioindicator species, such as *Nitzschia sp*. and *Oscillatoria sp.*, confirmed signs of pollution, while Varjão Stream exhibited a greater diversity of microalgae, characterizing a relatively preserved environment. These findings underscore the importance of continuous and seasonal monitoring of sediments, which act as reservoirs of pollutants and can release toxic substances over time. The *Allium cepa* test proved effective in detecting cytotoxic and genotoxic damage, complementing physicochemical analyses and reinforcing the need for more comprehensive monitoring.

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Authors'contributions

Benicio, S.H.M.: field sampling, conceptualization, formal analysis, methodology, writing – original draft, writing – review & editing, **Manso**, J.A.X.: conceptualization, formal analysis, methodology, writing – original draft, writing – review & editing. **Souza**, M.B.: conceptualization, formal analysis, methodology, writing – original draft, writing – review & editing. **Silva**, D.M.: funding, methodology, project administration, resources, supervision, validation, writing – review & editing. **Cruz**, A.D.: resources, supervision. **Formiga**, K.T.M.: supervision, writing – review & editing.

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