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Research article

Genotoxic effect of heavy metals on *Astyanax lacustris* in an urban stream

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ABSTRACT

Uncontrolled urbanization growth contributes to the pollution of aquatic environments. Heavy metals released by domestic and industrial effluents can negatively affect aquatic organisms. This study aimed to evaluate the effect of environmental pollutants, such as metals, on fish DNA damage, in stretches of an urban stream. Specimens of the Neotropical fish, *Astyanax lacustris*, were exposed *in situ* for 96 h along the Antas stream, a Brazilian aquatic system deteriorated by anthropogenic factors. Water and sediment samples were collected simultaneously for physicochemical and heavy metal analyses. The comet assay was performed as a biomarker of genotoxicity. Fish located downstream had a higher frequency of DNA damage than in the reference site. We found concentrations of Cr and Ni above acceptable levels in sediment samples. Generally, Ba, Mn, Mg, Zn, Cr, and Ni were the elements most associated with genotoxic damage. Water and sediment of the Antas stream showed genotoxic potential in *A. lacustris* according to the urbanization gradient, demonstrating the importance to prevent the release of environmental pollutants, especially heavy metals in urban areas.

1. Introduction

The management of water resources is a challenge for modern civilizations because of urban growth. The increasing water demand occurs simultaneously with the reduction in this resource qualitatively and quantitatively (Tucci, 2007; Dong et al., 2014). The water quality of a drainage basin reflects the land use and occupation, which is an essential tool to analyze the sources of contaminants in the aquatic system (Sposito et al., 2019). In urban areas, land use includes removal of native vegetation, soil waterproofing, occupation of permanent protected areas, punctual and diffuse discharges in high concentrations, and increased production of solid waste (EPA, 1999; Herngren, 2005). Those factors lead to water quality deterioration and contamination of sediments, which become a source of micro-contaminants boosting the decline of aquatic species.

Studies have shown that urbanization has deteriorated aquatic ecosystems, changed hydro-sedimentological dynamics of rivers, and introduced many contaminants, such as heavy metals, metalloids, and hydrocarbons that cause genotoxic damage to different organisms (Taylor and Owens, 2009; Yi et al., 2015; Šestinová et al., 2017). Among the many chemical elements introduced into drainage basins, metals have an accentuated relevance because of their association with human activities, such as vehicle emissions (Ball et al., 1998; Zhao et al., 2011) and release of domestic and industrial wastewater. For example, elements such as Pb, Cr, Zn, Cu, Cd, and Ni are typical potential contaminants of water and sediments of urban areas (Patel et al., 2017; Kumar and Singh, 2018).

The concern with those elements grows worldwide because they are persistent, bioaccumulative, and toxic (Ali et al., 2019). Besides promoting several morphological, behavioral, reproductive, genotoxic, and mutagenic damages to the aquatic biota (Pulley et al., 2016), they also affect human health. In this sense, biomonitoring is a crucial tool to assess the consequences of aquatic fauna exposure to xenobiotics. In bioassays, many organisms can be used, ranging from bacteria, plants (Kračun-Kolarević et al., 2016), mollusks (Khan et al., 2018), insects (Beghelli et al., 2018), to fish (Souza et al., 2019).

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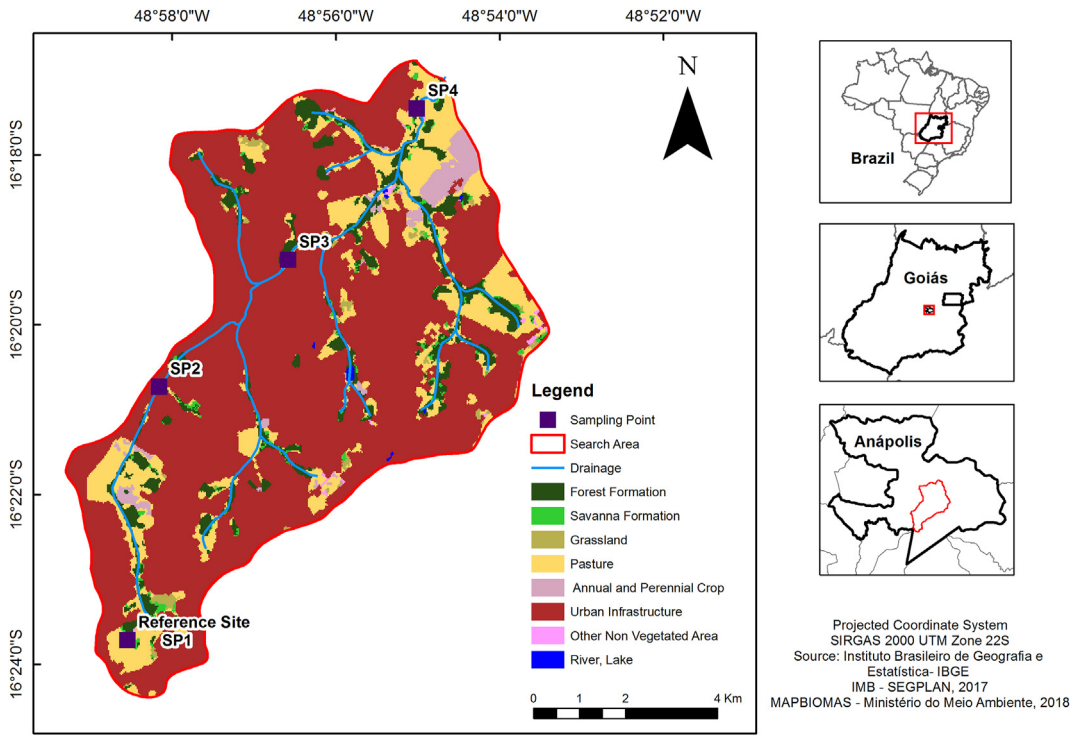

Figure 1. Map of land use and land cover of the Antas basin and location of the sampling points (SP), municipality of Anápolis, State of Goiás, Brazil.

Among those groups, fish is the most used model to estimate the risks to the aquatic environment. They are considered suitable indicators of bioavailability and biomagnification in the environment because they are in the highest trophic level of aquatic ecosystems (Francisco et al., 2019).

In this study, the comet assay was used as a genotoxicity biomarker. It is known as a sensitive and fast method, capable of evaluating repairable and non-repairable DNA lesions in individual cells (Rocha et al., 2009). *Astyanax lacustris* was used as a species model because of its useful characteristics for biomonitoring, such as adequate body size and adaptation in experiments as a test organism (Disner et al., 2017; Stevanato and Ostrensky, 2018; Viana et al., 2018). Furthermore, this species is native to the study basin and widespread in rivers, lakes, and ponds of South America. *A. lacustris* belongs to the family Characidae (Characiformes), which includes small fish called lambaris (Britski et al., 1999). This species has a highly visible humeral point, oval-shaped body, and yellow caudal fin (Botelho et al., 2019). Therefore, this study aimed to evaluate the fish genotoxic response to chemical elements in water and sediment samples in different stretches of an urban stream. Besides, we aim to assess what elements are associated with DNA damage and whether they follow the urban gradient or not.

2. Material and methods

2.1. Study area

The study area was located in the urban portion of the Antas stream basin, in the municipality of Anápolis, State of Goiás, Brazil. This municipality plays an important industrial and logistics role in the Central-West region of the country. As a consequence, the stream is under direct pressure from the urban center in the last decades. The basin's area is 73.3 km^2, and the main stream's length is 15.7 km. The distance between the sampling points SP1 and SP2 is 6.0 km, SP2 and SP3 is 4.2 km, and SP3 and SP4 is 4.8 km. The land use and cover of the drainage basin correspond to 79.9% of the urban area, 11.9% of native vegetation, 7.9% of pasture, and 1.3% of agriculture.

The sampling point 1 (SP1), 16°23′42.3″ S and 48°58′28.2″ W, is located in an area close to the stream source, which is the only point with remnants of native vegetation of medium and large sizes. SP1 is our reference site because it is the area with the least anthropic interference, which provides background conditions for the parameters analyzed subsequently. SP2 (16°20′43.0″ S and 48°58′6.8″ W) is located upstream of a sedimentation basin, and it is subject to erosion, irregular effluent discharge, besides having no riparian forest. SP3 (16°19′12.4″ S and 48°56′33.3″ W) is located after the canalization of the Antas stream, in the most urbanized area of the city, nearby residences and commercial buildings. SP3 has hardly any riparian forest contaminated with plastic materials accumulated in the channel, such as bottles and bags. SP4 (16°17′24.4″ S and 48°55′0.2″ W) is located at the end of the urban area, receiving all upstream garbage. SP4 is close to pasture and agriculture areas, besides having hardly any riparian forest, circulation of animals, and erosion processes on the margins. The monitoring point's location is shown in Figure 1.

2.2. Sampling and physicochemical analysis of water and sediment

Duplicate samples of water and sediment were collected along the Antas stream in June 2019. Collection, preservation, and analysis followed protocols of the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The physicochemical parameters of temperature (T °C), pH, total dissolved solids (TDS), dissolved oxygen (DO), electrical conductivity (EC), turbidity, and oxidation-reduction potential (ORP) were measured in the field using a multiparameter probe (HANNA-HI 9829). In the laboratory, we analyzed the total suspended solids (TSS), ammonia nitrogen (NH_4^+), total phosphorus (TF), and chemical elements (B, Ba, Ca, Cd, Cr, Cu, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Ti, and Zn).

Sediment samples were obtained using a stainless-steel Peterson dredge, stored in plastic packages, and conditioned in a thermal box. In the lab, they were dried in an oven at 50 °C for three days. Then, they were disaggregated and sieved through a 2 mm sieve to remove leaves and rough stones. The sieved material was then subjected to analysis of

Table 1. TSS, NH_4^+, and FT methods of analysis.

Parameter	Sample	Method	Standard
TSS	Water	Gravimetric/filtration in a 0.45μm membrane	APHA (2005) in 2540D
NH_4^+	Water/Sediment	Titrimetric	APHA (2005) in 4500C
TF	Water	Colorimetric/digestion with potassium persulfate/ascorbic acid	APHA (2005) in 4050E
	Sediment	Ignition at 550 °C for 4 h; in 0.5 g, add 25 mL of 1M HCL on a shaking table for 16h, filtration. The extract follows 4050E.	Aspila et al. (1976)
pH	Sediment	Water potentiometric	APHA (2005)

pH, NH_4^+, and FT. The fractions of sediments smaller than 63 μm were used for chemical elements analysis. The sample digestion process followed the protocols 3015A for water and 3051A for sediment (USEPA, 2007a, b), using the combination of HNO_3 and HCL in a microwave digester (ETHOS UP by Milestone). The methods used are summarized in Table 1.

The quantification of chemical elements was performed using an ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy) from Thermo Fisher Scientific (model iCAP 6300 Duo). Sample readings were performed using the axial settings. The parameters used were: pump rotation at 50 RPM, auxiliary (argon) gas flow rate at 0.5 L min-1, argon nebulizer gas pressure at 0.16 Mpa, and power source of 1250 Watts. After optimization, quantification limits were calculated by multiplying by ten the standard deviation of the results of 10 blank analyses divided by the slope of the respective calibration curve. The analysis reading of patterns and samples were performed in triplicate.

2.3. Fish sampling

Forty adult individuals of *A. lacustris* were purchased from a local fish farm and used for the tests *in situ* carried out in June 2019. The tests were performed for 96 h at the sites (SP1, SP2, SP3, and SP4) along the Antas stream. In each location, a fishpond with ten fishes was installed. The physicochemical parameters of water and sediments were measured on the days of installation and removal of the ponds. The experimental ponds of 54 × 27 cm were covered with a 5 mm mesh, which allowed water to circulate while submerged. The fish were not fed during the test. At the end of the exposure, the surviving fish were weighed, measured, and anesthetized within an ice bath to collect biological material (blood). The average ± standard derivation of weight and length of the animals were respectively 10.0 g ± 1.06 and 6.98 cm ± 4.46. In addition, 69.7 % of the individuals were male. The death frequency was one individual in SP2 and SP3, and four individuals in SP4. The project was approved by

Table 2. Comparison of chemical analysis of water from sampling points along the Antas stream, Brazil.

Parameters	Mean ± standard deviation (SD) in sampling points				CONAMA 357/2005 Class 2
	SP1(Ref)	SP2	SP3	SP4	
Temperature (°C)	21.9 ± 0.78	22.1 ± 0.66	23.0 ± 0.63	21.3 ± 3.61	-
pH	5.98 ± 0.42[a]	7.36 ± 0.35	6.88 ± 0.34	7.11 ± 0.24	6–9
Turbidity (UNT)	1.45 ± 0.07	6.70 ± 1.27	18.9 ± 4.03	8.65 ± 0.92	≤100
EC (μS/cm)	7.00 ± 1.41	63.5 ± 0.71	162 ± 0.00	121 ± 1.40	-
TDS (mg/L)	3.50 ± 0.71	31.5 ± 0.71	81.0 ± 0.00	60.5 ± 0.71	<500
TSS (mg/L)	3.85 ± 0.14	8.25 ± 0.35	8.10 ± 0.14	8.20 ± 0.28	-
DO (mg/L)	4.73 ± 0.55[a]	5.43 ± 0.88	3.46 ± 0.14[a]	6.69 ± 0.36	>5
ORP (mV)	113 ± 25.0	45.3 ± 7.40	108 ± 29.4	94.1 ± 0.20	-
TF (mg/L)	0.15 ± 0.50	0.35 ± 0.80[a]	1.23 ± 0.60[a]	0.55 ± 0.40[a]	0,1
NH_4^+ (mg/L)	0.98 ± 0.90	0.30 ± 0.80	1.12 ± 0.50	0.84 ± 0.70	3.7
Metals Concentrations (mg/L)					
B	<LQ[b]	<LQ[b]	<LQ[b]	<LQ[b]	0.5
Ba	0.007 ± 0.001	0.072 ± 0.001	0.059 ± 0.000	0.043 ± 0.000	0.7
Ca	<LQ[b]	5.79 ± 0.18	11.6 ± 0.41	9.82 ± 0.25	-
Cd	<LQ[b]	<LQ[b]	<LQ[b]	<LQ[b]	0.001
Cr	0.004 ± 0.000	0.007 ± 0.000	<LQ[b]	<LQ[b]	0.05
Cu	<LQ[b]	0.004 ± 0.000	<LQ[b]	<LQ[b]	0.009
K	1.60 ± 0.00	0.90 ± 0.06	1.87 ± 0.01	1.16 ± 0.02	-
Li	<LQ[b]	<LQ[b]	<LQ[b]	<LQ[b]	2.5
Mg	0.08 ± 0.00	1.13 ± 0.01	2.33 ± 0.02	2.10 ± 0.01	-
Mn	0.01 ± 0.00	0.16 ± 0.00[a]	0.04 ± 0.00	0.05 ± 0.00	0.1
Mo	<LQ[b]	<LQ[b]	0.004 ± 0.00	<LQ[b]	-
Na	2.02 ± 0.01	4.28 ± 0.02	11.2 ± 0.08	8.47 ± 0.05	-
Ni	0.004 ± 0.000	0.010 ± 0.000	0.008 ± 0.000	0.007 ± 0.001	0.025
Pb	<LQ[b]	<LQ[b]	<LQ[b]	<LQ[b]	0.010
Ti	<LQ[b]	0.008 ± 0.001	0.006 ± 0.000	0.008 ± 0.001	-
Zn	0.015 ± 0.000	0.057 ± 0.000	0.060 ± 0.002	0.058 ± 0.001	0.180

[a] Values above the limit of Brazilian law.
[b] LQ - limits of quantification for chemical elements on ICP-OES; Ref = reference site.

Table 3. Comparison of chemical analysis of sediment from sampling points along the Antas stream, Brazil.

Parameters	Mean ± standard deviation (SD) in sampling points				CONAMA 454/2012 Level 1
	SP1(Ref)	SP2	SP3	SP4	
pH	4.73 ± 0.20	5.53 ± 0.30	6.73 ± 0.10	6.80 ± 0.40	-
TF (mg/Kg)	974 ± 4.50	3716 ± 4.00[a]	4078 ± 13.7[a]	2070 ± 1.30[a]	2000
NH_4^+ (mg/Kg)	140 ± 1.00	84.0 ± 0.70	140 ± 1.20	112 ± 1.50	-
Metals Concentrations (mg/Kg)					
B	50.1 ± 0.05	90.0 ± 0.02	91.0 ± 0.08	67.3 ± 0.02	-
Ba	20.4 ± 0.02	90.9 ± 0.00	113.5 ± 0.11	125.2 ± 0.03	-
Ca	227 ± 0.67	1525 ± 0.51	1772 ± 0.18	2075 ± 1.64	-
Cd	<LQ[b]	<LQ[b]	<LQ[b]	<LQ[b]	0.6
Cr	122 ± 0.12[a]	208 ± 0.03[a]	217 ± 0.20[a]	186 ± 0.22[a]	37.3
Cu	16.2 ± 0.01	28.7 ± 0.03	31.2 ± 0.05	22.6 ± 0.01	35.7
K	127 ± 0.49	423 ± 0.21	660 ± 0.65	1171 ± 0.07	-
Li	1.80 ± 0.00	2.20 ± 0.00	2.50 ± 0.00	3.10 ± 0.00	-
Mg	153 ± 0.30	1064 ± 0.01	1321 ± 1.03	2247 ± 0.63	-
Mn	35.0 ± 0.08	238 ± 0.03	354 ± 0.35	590 ± 0.07	-
Mo	3.20 ± 0.00	2.30 ± 0.00	1.80 ± 0.00	1.50 ± 0.00	-
Na	374 ± 0.81	215 ± 0.03	308 ± 1.34	327 ± 1.61	-
Ni	10.2 ± 0.02	42.0 ± 0.18[a]	39.5 ± 0.04[a]	38.2 ± 0.01[a]	18.0
Pb	5.20 ± 0.01	11.4 ± 0.00	11.1 ± 0.01	9.90 ± 0.14	35.0
Ti	379 ± 0.87	399 ± 0.23	472 ± 0.36	553 ± 0.24	-
Zn	15.9 ± 0.02	66.4 ± 0.00	68.2 ± 0.04	53.4 ± 0.03	123

[a] Values above the limit of Brazilian law.
[b] LQ – limits of quantification for chemical elements on ICP-OES; Ref = reference site.

the Ethics Committee on Animal Use (CEUA) of the State University of Goiás, number 006/2018.

2.4. Comet assay

Blood samples (5 μL) of *A. lacustris* were obtained through a cervical cut. The blood was diluted in a microtube containing 1mL of PBS and one drop of heparin. The material was stored in a thermal box and sent to the laboratory where the protocol proposed by Singh et al. (1988) was followed with some modifications. The samples were then homogenized, where 15 μL of the suspension was pipetted and mixed with 120 μL of low-melting agarose (0.1 g of low-melting agarose and 20 mL of PBS), kept in a thermoblock at 40 °C. The mixture was placed on 1.5% normal melting agarose pre-coated slides (1.5 g of agarose and 100mL of PBS) and covered with coverslips. Then, they were placed in the refrigerator for 5 min to solidify the material. After removing the coverslips, the slides were immersed in a lysis solution (1 mL Triton X-100, 10 mL DMSO, and 89 mL stock lysis solution; pH 10.0) for 24 h in a refrigerator. In the second step, the slides were immersed in electrophoresis buffer (2.5M NaCl, 100mM EDTA, 10mMTris, pH 10; 1mL Triton X-100 and 10mL DMSO) in the refrigerator. The electrophoretic run was performed for 30 min at 1V/cm (vat) and 250 mA. Then, the slides were washed with a neutralization buffer three times at 5-minute intervals, washed twice with distilled water, dried at room temperature, and then fixed with absolute alcohol for ten minutes. For staining, 100 μL of SYBR Green diluted in buffer was used. Then, the slides dried for 30 min at room temperature, followed by immersion in distilled water twice for five minutes, and left for complete drying. All the steps of the test were performed in darkness. The slides were examined using a ZEISS fluorescence microscope at 20x magnification. Fifty nucleoids were analyzed per slide, totaling 100 cells per fish sample. Comet images were measured using the Comet Image 2.2 software, and the parameters Olive tail moment (OTM), percentage of DNA in the tail (% DNA), and tail length (TL) (μm) were used as measures of genotoxic damage. The OTM is the % DNA multiplied by TL.

2.5. Statistical analysis

Genotoxicity data were analyzed using the Shapiro-Wilk and Levene tests to check for normality and homoscedasticity. Since they were not normally distributed, Olive tail moment and tail length data were log-transformed and then tested using a one-way ANOVA, followed by Fisher's LSD post-hoc test using a 5% significance. Data were presented as their mean and standard error values. The chemical elements of water and sediment were analyzed using a Principal Component Analysis (PCA) to verify the correlation of chemical elements and DNA damage among sampling points. All analyses were performed using STATISTICA version 7 and PAST software.

3. Results and discussion

3.1. Chemical analysis of water and sediment

The water physicochemical characterization is summarized in Table 2. The pH ranged from 5.98 to 7.36 and was below the limit permissible by Brazilian legislation in SP1, considered an acidic spring area (CONAMA, 2005). Slightly acidic values in spring areas are associated with the type of soil, the source of groundwater, and decomposition of organic matter from the riparian vegetation. The highest values of turbidity, TDS, and EC were found in SP3. DO was in non-compliance with the legislation in SP1 and SP3, with the lowest concentration (3.46 mg/L) in SP3, the most urbanized area. For phosphorus, only SP1 complied with the legislation, and the highest level was found in SP3. For ammoniacal nitrogen, all locations had levels below the permissible limit, with the highest concentration also found in SP3 (1.2 mg/L).

The general pattern of metals in the water samples were major cations (Ca > Na > Mg > K) and heavy metals (Mn > Ba > Zn > Ni > Ti > Cr ≥ Cu). All chemical elements were within the limit permissible by the legislation. However, even exposure to low concentrations of some environmental pollutants can cause DNA damage and disruption of cellular functions. Some studies show that assessing the individual

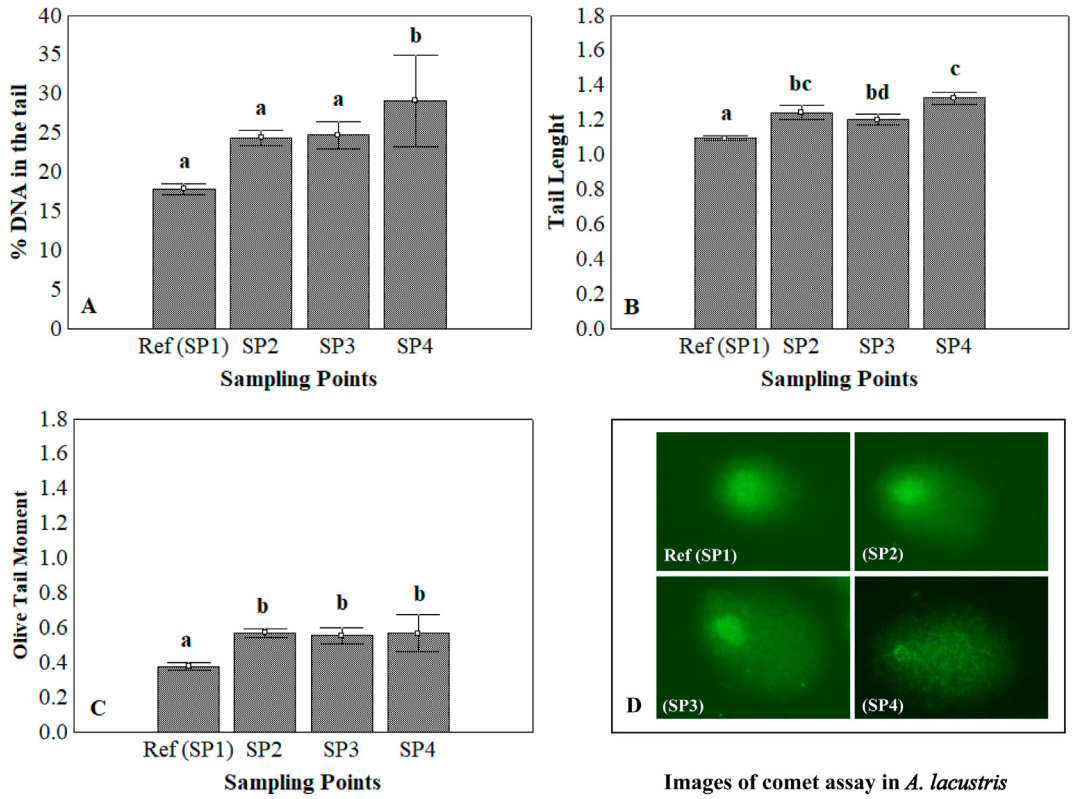

Figure 2. Comet assay parameters of *A. lacustris*, exposed for 96 h in the Antas stream, Brazil. Data are represented as mean ± standard error. Similar letters indicate statistical similarity, while different letters indicate statistical difference (One-way ANOVA Test, LSD). SP = sampling points, Ref = reference site.

concentration of chemical elements, even in low concentrations or under the permissible limits, does not represent the actual ecological risk of these elements in water bodies (Altenburger et al., 2015; Pellegri et al., 2020). That is because the mixture of several elements can produce a combined effect that may enhance the toxicity in the environment, causing damage to the aquatic biota (Enserink et al., 1991).

For Mn > Ba > Ni > Ti > Cr > Cu, the highest concentrations were found in SP2, with Cu found only in this location. We found the lowest redox potential at this site, indicating a reducing environment in comparison to the other sampling points. Lower ORP values facilitate metal solubilization, and this condition is associated with the sediment pH (Table 3). Low pH values cause the release of H^+ ions that compete with metal ions and weaken the metal-sediment association (Ali et al., 2019). For Ca > Na > Mg > K > Zn > Mo, the highest concentrations were found in SP3. High concentrations of Ca and Mg corroborate with EC values in this location, and such ions are associated with water hardness and availability of mineral salts. The water properties influence the mobility of heavy metals in the sediment, and thus, their toxicity in the environment (Kang et al., 2019). Turbidity, TSS, TDS, and EC were associated with the concentration of suspended and dissolved particles. In the water system, the discharge of heavy metals correlates with the transport of suspended particulate material. That release acts as a vehicle of pollutants, which accumulate through decantation in the river bed (Kelderman and Osman, 2007; Rügner et al., 2019). For nutrients, the increase tends to contribute to metals enrichment in sediments. However, this relationship is not direct, and it is associated with other factors, such as the availability of oxygen since aerobic conditions provide for the decomposition of organic compounds and the release of metals.

The sediment parameters analyzed are shown in Table 3. The pH was acidic in SP1 and SP2. TF concentrations were above permissible limits in SP2, SP3, and SP4 (CONAMA, 2012). Cr and Ni were also high in these sites, where, in some cases, the limit exceeded up to 5 times. The concentrations of Mn, Cr, Zn, Ni, Pb, and Ba were at least twice higher in downstream sites than in SP1, reference site. Of these elements, Cr, Zn, Ni, and Pb are among the anthropogenic heavy metals most dangerous to the environment (Ali et al., 2019). In an urban stream in South Korea, Moon et al. (2020) detected Zn, Pb, Cu, and Ni in sediment samples associated with significant DNA damage in the studied fish. Khan et al. (2018), analyzing heavy metals in the River Kabul, found that Zn, Cr, Ni, Mn, Pb, and Cu were the elements with the highest concentrations. Both studies evaluated the metals in urban gradients and reported that downstream regions had higher levels of pollutants than the reference sites. That is attributed to wastewater release, which affects the levels of nutrients, organic matter, TSS, and metals.

Regarding the implications of the metals found at sampling points and the genotoxic effects, elements such as Cu, Pb, and Ni represent moderate to high ecological hazards (Kumar and Singh, 2018). Haile et al. (2016) emphasize that compounds such as Ba and Ti in water and sediment are not regulated and the effect is unknown. However, Carmo et al. (2019) found that fish exposed to Np-TiO_2 had a significant accumulation of Ti in their brains and muscles. Once the element is in the blood, it can cause genotoxic damage to erythrocytes. Delmond et al. (2019) also reported that fish exposed to TiO_2 particles may suffer oxidative stress due to the generation of reactive oxygen species (ROS). For Jadoon and Malik (2017), metals act in the organism producing ROS that causes changes in the DNA repair mechanisms. In this case, the mutation occurs during failures in the repair system, which is an inherited change in the genetic material.

3.2. Genotoxicity

DNA damage in *A. lacustris* was associated with the levels of environmental pollutants in the Antas stream. Fish located in sampling points farther from the reference site showed a higher frequency of DNA

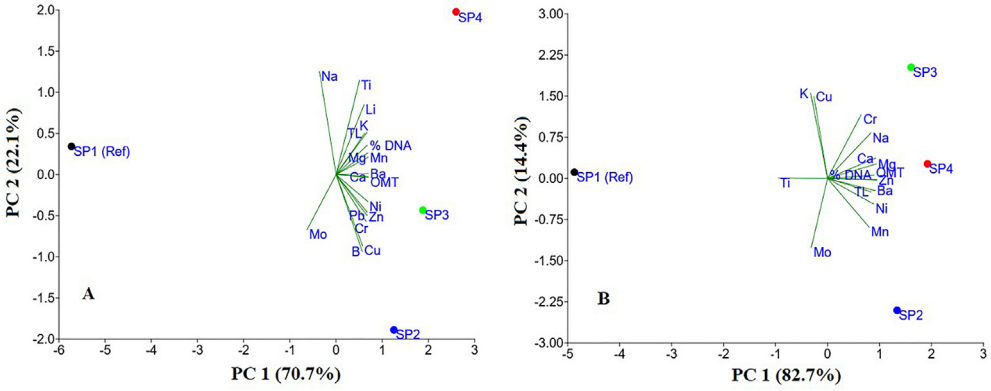

Figure 3. Principal Component Analysis (PCA) of chemical elements in (A) water and (B) sediment samples, in different sampling points (SP), Ref = reference site.

damage for all parameters of the comet assay % DNA in the tail ($F_{(3, 26)} = 2.9250$, $p = 0.05$, Figure 2A), tail length ($F_{(3, 26)} = 9.4256$, $p = 0.0002$, Figure 2B), Olive moment tail ($F_{(3, 26)} = 3.0149$, $p = 0.0480$, Figure 2C). Those results showed that DNA damage on the fish increased according to the urbanization gradient. This fact can be seen in Figure 2D, which shows the comet tails observed in the erythrocyte cells of *A. lacustris* at each sampling point.

Several studies have shown that aquatic organisms exposed to water and sediments contaminated by heavy metals can exhibit genotoxic effects (Turan et al., 2020; Kontaş and Bostancı, 2020). Besides, Viana et al. (2018) reported that A. *lacustris* from impacted sites had higher concentrations of Pb, Cu, Fe, Zn, and Ni in muscle tissue and higher frequency of micronucleus. *Geophagus brasiliensis*, in a Brazilian River, had an increase of DNA damage associated with Fe, Mn, Cd, Cu, and Pb (Gomes et al., 2019). Ghisi et al. (2017) found a higher frequency of DNA damage in *Astyanax* aff. *paranae* located in a site downstream of the urban area, which received a mix of pollutants, including industrial and municipal wastewater. Higher levels of DNA damage in *Astyanax* spp. have also been documented at points disturbed by artificial beaches that received surface runoff, in addition to urban and industrial wastewater (Barros et al., 2017). Thus, it is evident that regions polluted by metals favor genetic instability, leading to DNA damage on the wild fauna, such as on the genus *Astyanax*.

3.3. Multivariate analysis

Chemical elements were analyzed using a Principal Component Analysis (PCA). For water (Figure 3A), components 1 and 2 explained 70.7 % and 22.1% of data variance, respectively. The heavy metals Zn, Ba, Ni, and major cations Ca and Mg showed a strong positive correlation (>0.93) with component 1. Besides, they were the most important elements associated with DNA damage and other parameters of the comet assay. The elements Ti, K, Mo, and Cu showed negative correlations. For sediments (Figure 3B), components 1 and 2 explained 82.7% and 14.4% of data variation, respectively. The negative correlation of Mo with component 1 remained, and a strong positive correlation (>0.95) was observed with Ba, Mn, Ni, Pb, Zn, Ca, and Mg. Component 2 showed a positive correlation with both Na and Ti only. The two analyses showed that SP1, reference site, is quite different from the other sampling points. SP1 was not associated with component 1, which comprises most parameters of water and sediment. In both analyses, all parameters of the comet assay were strongly associated with component 1. That result corroborates the literature regarding polluted locations and genotoxic effects in aquatic organisms.

Regarding the chemical elements associated with genetic damage in *A. lacustris*, Ba can cause multiple harmful effects on the renal and respiratory systems of animals (Lu et al., 2019). That element has been associated with rapid urbanization and, together with Mg, is one of the most common elements in pharmaceutical industrial effluents (Forsido et al., 2020). Ca and Mg cations are essential for fish development. They are associated with water hardness and can reduce the toxicity of other metals, such as Zn and Cu, due to permeability and preferential adsorption. However, Mg can be toxic to some species of Neotropical fish, as addressed by Van Dam et al. (2010). Ni acts as a mutagen on the physiology and behavior of fish (Viana et al., 2018). In *P. Lineatus*, it caused genotoxic effects by accumulating and inducing changes in the tissues that interfered in antioxidant enzymes (Palermo et al., 2015). Pb can interfere with the DNA repair system by inhibiting enzyme activity (Zhang et al., 2019). Despite the individual effects of those elements on the genetic level, we highlight that combined metals, associated with the ambient condition, can increase toxic effects when compared to isolated chemicals (Stankevičiūtė et al., 2017). However, we recognize that this study evaluated acute toxicity responses, requiring additional work to understand the prolonged effects on the water bodies. In this sense, we also agree with Singh et al. (2019) on the importance of an integrative approach, using a battery of tests to determine environmental health risks in ecotoxicological studies.

4. Conclusion

The comet assay in *A. lacustris* showed that water and sediments of the Antas stream exhibited genotoxic effects following the urbanization gradient. The DNA damage correlated with several physicochemical factors that act on the mobility of pollutants in the drainage basin and on the sediment-water interface. A high concentration of Cr and Ni found in sediment samples was above the permissible Brazilian limits. Moreover, we found high values of Mn, Ti, and Mg, which are not regulated, and their genotoxic damage to organisms is yet unknown. Ba, Mn, Zn, Ni, Pb, Ca, and Mg were the elements most associated with genotoxic damage in tested organisms. The difference in DNA damage observed among the sampling points indicates that the urban land use, even at short distances, is detrimental to water and sediment quality and, therefore, may interfere in aquatic species. Furthermore, even in urban areas, some management, such as the maintenance of riparian forest and removal of effluents and garbage, can protect water systems and guarantee the ecology of species.

Declarations

Author contribution statement

Emanoelle Pereira da Silva: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Marcelino Benvindo Souza: Analyzed and interpreted the data; Wrote the paper.

Carlos Filipe Camilo Cotrim: Performed the experiments.

Andreya Gonçalves Costa Motta: Analyzed and interpreted the data.

Matheus Mendonça Luena, Nelson Roberto Antoniosi Filho, Julião Pereira: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Klebber Teodomiro Martins Formiga, Daniela de Melo e Silva: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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