TOXICOGENETIC AND BIOCHEMICAL EFFECTS OF WATER AND SEDIMENT FROM A COASTAL LAGOON (ES/BRAZIL) ASSESSED WITH *ALLIUM CEPA*

EFEITOS TOXICOGENÉTICOS E BIOQUÍMICOS DA ÁGUA E DO SEDIMENTO DE UMA LAGOA COSTEIRA (ES/BRASIL) AVALIADOS COM ALLIUM CEPA

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ABSTRACT

The occurrence of metals in aquatic environments is natural and essential for various metabolic functions. However, when in excess due to contamination, these elements can cause toxic effects. The Mãe-Bá Lagoon, located between the municipalities of Guarapari and Anchieta, in Espírito Santo, has suffered anthropogenic impacts since the 1970s, with the closure of its connection to the sea and the installation of a reservoir for processing iron ore. The aim of this study was to evaluate the toxicogenetic, biochemical, and metal responses of *Allium cepa* exposed to water, elutriate, and solubilized sediment samples collected at three stations in Mãe-Bá Lagoon (ES, Brazil). The results revealed phytotoxicity at the three stations in the first campaign; cytotoxicity in the elutriate from station 1 and in the solubilized water from stations 2 and 3 in the second campaign; genotoxicity in the water from station 1 in the first campaign and in the solubilized water from stations 2 and 3 in the second campaign; as well as mutagenicity in several samples. Alterations were also observed in antioxidant defense enzymes, such as lipid peroxidation and glutathione S-transferase, as well as the accumulation of metals in the roots of the exposed seeds, indicating a potential risk to the local biota.

KEYWORDS: Anthropogenic contamination; genotoxicity; mutagenicity; plant model.

RESUMO

A ocorrência de metais em ambientes aquáticos é natural e essencial para várias funções metabólicas. No entanto, quando em excesso devido à contaminação, esses elementos podem causar efeitos tóxicos. A Lagoa Mãe-Bá, localizada entre os municípios de Guarapari e Anchieta, no Espírito Santo, sofre impactos antropogénicos desde a década de 1970, com o fechamento de sua conexão com o mar e a instalação de um reservatório para processamento de minério de ferro. O

objetivo deste estudo foi avaliar as respostas toxicogenéticas, bioquímicas e de metais de *Allium cepa* exposta a amostras de água, elutriato e sedimento solubilizado coletadas em três estações na Lagoa Mãe-Bá (ES, Brasil). Os resultados revelaram fitotoxicidade nas três estações na primeira campanha; citotoxicidade no elutriado da estação 1 e na água solubilizada das estações 2 e 3 na segunda campanha; genotoxicidade na água da estação 1 na primeira campanha e na água solubilizada das estações 2 e 3 na segunda campanha; bem como mutagenicidade em várias amostras. Também foram observadas alterações nas enzimas de defesa antioxidante, como a peroxidação lipídica e a glutationa S-transferase, bem como o acúmulo de metais nas raízes das sementes expostas, indicando um risco potencial para a biota local.
PALAVRAS-CHAVE: Contaminação antropogênica; genotoxicidade; mutagenicidade; modelo vegetal.

INTRODUCTION

The presence of metals in aquatic ecosystems is mainly associated with geological processes such as weathering and leaching from the soil[1]. Although some metals and micronutrients are essential for the metabolic activities of organisms, high concentrations may compromise ecosystem balance and water quality, in addition to inducing genetic alterations due to their persistence, bioaccumulation, and interaction with DNA[2,3].

Coastal ecosystems are especially vulnerable to contamination by metals and other pollutants, since they are constantly subjected to inputs from anthropogenic activities[2]. These ecosystems have great ecological and socioeconomic importance, contributing to climate regulation and serving as habitats for numerous species, as well as being used by surrounding populations for fisheries, recreation, and domestic purposes[4]. The Mãe-Bá Lagoon, the second largest coastal lagoon in the state of Espírito Santo, Brazil (4.67 km²), is located between the municipalities of Guarapari and Anchieta[5]. Since the 1970s, this lagoon has been exposed to strong anthropogenic pressures, mainly related to industrial activities established in its surroundings[5,6].

Plant bioassays have been widely applied as sensitive tools to monitor environmental contamination and assess the effects of pollutants on living

organisms[10]. Among them, *Allium cepa* L. has been extensively used due to its sensitivity to environmental contaminants and its reliability as a bioindicator of cellular and genetic damage[11,13]. Through this model it is possible to evaluate seed germination, root growth, and mitotic alterations, since the root system is the first structure to be exposed to pollutants in water and soil[14,15]. The relatively large and reduced chromosome number[16] facilitates the observation of the cell cycle[17,18] and the detection of aneugenic and clastogenic alterations[19]. Furthermore, biochemical biomarkers such as antioxidant defense responses are important to detect oxidative stress, an imbalance between the production of reactive oxygen species (ROS) and the antioxidant system[20].

Considering the ecological and social relevance of Mãe-Bá Lagoon and the risks associated with industrial activities in the region, this study aimed to quantify the concentrations of metals in water and sediment samples collected in two campaigns, one shortly after the resumption of steel industry activities and the other one year later and to evaluate their toxicogenetic and biochemical effects using *A. cepa* bioassays.

MATERIAL AND METHODS

The study was conducted in Mãe-Bá Lagoon, located between the municipalities of Guarapari and Anchieta, Espírito Santo, Brazil (-20°45′1.49N -40°34′0.59E). The lagoon is the second largest coastal lagoon in the state, with an area of 4.67 km², and is used for fishing, recreation, landscape harmony, and domestic supply[21]. Water and sediment samples were collected at three stations: EA1 (20°46'07.36"S; 40°34'39.94"W), EA2 (20°45'48.95"S; 40°35'04.13"W), located 0.9 km from EA1, and EA3 (20°45'26.30"S; 40°34'19.63"W), located 1.4 km from EA2. Two sampling campaigns were carried out, in March 2021 and March 2022.

Water and sediment samples from each station were collected in sterile polyethylene containers, stored at 4 °C, and transported to the laboratory for

analysis. Sediment samples were processed to obtain the solubilized fraction and the elutriate. The solubilized fraction followed ABNT NBR 10006, using 12.5 g of dried sediment (42 °C, sieved) mixed with 50 mL of distilled water, stirred for 5 min, and allowed to stand for 7 days before filtering through 0.45 μm membranes. The elutriate followed ABNT NBR 15469, using 12.5 g of sediment mixed with 50 mL of lagoon water, stirred for 30 min, and decanted for 2 h before collection and storage at 5 °C.

The study followed a completely randomized design with five replicates per treatment. Samples of lagoon water, solubilized sediment, and elutriate from each station were tested, with distilled water serving as the negative control[22].

Thirty *Allium cepa* L. (Baia Periforme, Isla Sementes, lot 153901) seeds were placed in Petri dishes lined with filter paper and moistened with each treatment. Seeds were incubated at 24 °C for 96 h in the dark[17]. Root germination and elongation were recorded, and roots were collected for cytogenetic, biochemical, and metal analyses.

Seed germination (%) and root length (digital caliper)[23] were recorded. Roots were fixed in Carnoy's solution (3:1 ethanol:acetic acid) and stored at 5 °C until slide preparation. The Feulgen reaction[24,25] was performed, and slides were prepared by the squashing technique[26]. For each treatment, five slides were analyzed, scoring 1000 cells per slide[17]. Mitotic index (MI), micronuclei frequency, and chromosomal alterations (losses, fragments, bridges, delays, adhesions) were quantified. Aberrations were considered for genotoxicity, and micronuclei frequency for mutagenicity[18].

Water, elutriate, and solubilized samples were acidified with ultrapure HNO_3 (69%), filtered (0.45 μm), and stored at 4 °C. Roots were dried at 40 °C, and 0.1 g of tissue was digested with 5 mL of HNO_3 in a microwave system (Anton Paar Multiwave 3000, Austria) for 40 min. Samples were filtered and analyzed by ICP-MS (Agilent 7500 Series CX) with an autosampler (ASX-100, CETAC). Elements quantified included Al, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ag, Cd, Sn, Ba, La, Ce, W, Hg, Pb, and Bi[19,27,28].

Roots were frozen in liquid nitrogen and stored at 80 °C. Samples were diluted (1:9, w/v) in phosphate buffer (pH 7.8), homogenized for 3 min (IKA T10 Turrax), and centrifuged (10,000 rpm, 10 min, 4 °C). The supernatant was stored at 80 °C. Analyses were conducted in triplicate. Protein concentration was determined by the Bradford method[29]. Antioxidant enzymes were measured: superoxide dismutase (SOD)[30], glutathione S-transferase (GST)[31], and lipid peroxidation (LPO) via the FOX assay[32].

Normality was tested by Shapiro–Wilk. When assumptions were not met, Kruskal–Wallis was applied ($p < 0.05$); otherwise, ANOVA followed by Tukey's test was used ($p < 0.05$). Multivariate analysis was performed by principal component analysis (PCA) using RStudio, considering cytogenetic parameters (MI, chromosomal alterations, micronuclei) and metals bioaccumulated in roots.

RESULTS AND DISCUSSION

The analysis detected 13 metals in water samples, 16 in elutriates, and 15 in solubilized samples. The distribution of metals with the highest concentrations varied between sampling stations (SS) and campaigns. In general, Al, Fe, Mn, Zn and Sr were the most recurrent elements with higher values across the matrices.

The presence of emerging elements such as Ti, Zr, Y, Mo and Ce in aquatic environments can generate harmful effects due to their possible toxicity, persistence in the environment and bioaccumulation, most of which are not essential for plants[38]. These compounds can pose a potential risk to the ecosystem, since they are not covered by routine monitoring programs and lack specific legislation[39]. In fact, some of the concentrations observed in this study, such as Al and Fe, exceeded the limits established by Brazilian environmental legislation (CONAMA Resolution 357/2005) for Class 2 freshwater bodies, reinforcing the potential ecological risks. Comparisons with international guidelines (e.g., WHO, USEPA) also highlight that even concentrations below threshold levels may contribute to sublethal genotoxic and mutagenic effects when organisms are exposed chronically.

Some of the metals quantified, such as Mn, Mo, V, Sr and Zn, are important for carrying out the vital functions of the living organism[10], while other metals, such as Pb, Cd and As, have no physiological function, negatively altering biological processes[40]. In addition to the isolated effects of individual metals, the simultaneous occurrence of several elements in the lagoon suggests possible synergistic or antagonistic interactions. Such interactions, already described in plant and animal models, may explain the variability in toxicogenetic and biochemical responses observed across campaigns. This highlights the complexity of assessing environmental contamination solely based on individual concentrations. In our studies, it was possible to detect these metals in roots exposed to environmental samples, albeit in low quantities, and some metals were between the limit of detection and the limit of quantification. Alberts[41] point out that metals can enter cells by various routes and mechanisms, via membrane proteins, endocytosis or membrane receptors. These transporters act as an initial barrier against changes in the cellular homeostasis of metals, regulating their entry and exit into the cell, but when their concentration is higher in the intracellular environment, the transporters provide the route to expel the excess of their cofactors, preventing toxicity in the cell[42].

Al is not an essential element for living organisms, but its high reactivity enables interfere with physiological and cellular processes. In our study Al showed the highest concentrations mainly at SS2 in both campaigns. This pattern is consistent with reports that Al is mobilized under acidic conditions and accumulates in root tissues and environmental matrices, where it can inhibit root elongation, disturb membrane function and nutrient uptake, and induce oxidative stress and genotoxic effects. Several recent reviews and experimental studies support these mechanisms and the sensitivity of *A. cepa* to Al exposure, including inhibition of mitotic activity, nucleolar and chromosomal alterations and increased reactive oxygen species[72-74,77].

It is important to acknowledge that the spatial scope of the sampling design was somewhat restricted, since all stations were located inside the lagoon and in

proximity to the industrial area. This limitation reduces the extrapolation of the results to the broader lagoon system or to neighboring coastal environments. Future studies should incorporate more distant reference sites, unaffected by direct anthropogenic inputs, to strengthen ecological interpretations.

Overall, the study demonstrates that *A. cepa* bioassays are effective in detecting toxic, genotoxic, and mutagenic effects induced by environmental samples, even when contaminant levels are below some regulatory thresholds. However, the interpretation of the findings should consider the restricted spatial scope of sampling and the influence of climatic variation and industrial activity. By integrating toxicogenetic, biochemical, and chemical analyses, the research provides robust evidence of the ecological vulnerability of Mãe-Bá Lagoon and highlights the need for continuous monitoring using both plant bioassays and complementary models. Future efforts should also include comparisons with legal standards, exploration of synergistic mechanisms among metals, and assessment of risks to other aquatic and terrestrial species associated with the lagoon.

In the roots of A. cepa exposed to water samples, significant differences compared to NC were mainly observed for Al (SS2) and Fe (all stations) in the first campaign, and for Fe (SS1, SS3) and Rb (SS1) in the second campaign. In elutriate samples, Al, Fe, Mn and Rb showed significant increases across stations in the first campaign, while in the second campaign only Al (SS1, SS3) differed from NC. For solubilized samples, Mn, Fe, Ni and Rb were the most affected in the first campaign, whereas Al (SS1) and Fe (all stations) showed significant differences in the second campaign.

There was no change in germination percentage at any sampling station in either campaign when compared to the negative control, indicating that water, elutriate, and solubilized samples had no effect on seed germination. In contrast, during the first campaign, a significant reduction in root growth was observed in SS1, SS2, and SS3 for all sample types. Although some heavy metals act as micronutrients at low concentrations, their beneficial and toxic ranges are very close, meaning that even small increases may trigger toxicity. The *A. cepa* root

growth inhibition test, a simple and efficient method for assessing effluents with metallic compounds, confirmed this sensitivity.

The higher Al values detected in SS1, SS2, and SS3 suggest that root inhibition is linked to aluminium accumulation in the meristematic region, particularly in the symplast, reducing cell proliferation. Aluminium has no role in plant metabolism, which further explains the growth reduction. Similar effects have been reported[46], who showed that high concentrations of heavy metals impair growth and inhibit root development. Additionally, our results point to higher levels of Ti, V, Mn, and Sr in SS1 during the first campaign. Elevated concentrations of these metals, especially in elutriate, reinforce the hypothesis that they contribute to the observed inhibition of root growth.

The results show variations in the levels of metals in the water, elutriate and solubilized samples throughout the different sampling campaigns carried out in Mãe-Bá lagoon. These variations in metal levels were associated with inhibition of root growth at all sampling stations during the first campaign for the water, elutriate and solubilized samples. This effect can be attributed mainly to the accumulation of aluminum in the roots, as described in this study. Although some metals were present in low concentrations, genotoxic and mutagenic effects were observed in the meristematic cells of the roots. These effects may be due to the interaction of metals, even at low concentrations, with cellular DNA. The same applies to antioxidant action, since the presence of metals can act as a stressor for cells, generating reactive oxygen species.

With regard to the stages of cell division and the mitotic index (MI) in the meristematic cells of *A. cepa*, it was found that the MI and the percentage of cells in each stage of the cell cycle showed no significant difference compared to the negative control in the samples with water and elutriate (Table 1). Thus, it can be inferred that there was no manifestation of cytotoxic potential in the exposed A. cepa meristematic cells. However, in the solubilized samples, during the second campaign, in all three sampling stations, there was a significant reduction in MI compared to the negative control and an increase in the number of cells in interphase

with the solubilized sample in SS1, in prophase in SS2 and SS3 and in anaphase in SS1 and SS3 (Table 2).

Table 1. Parameters of mitotic index (MI%) and cell division in meristematic cells of *Allium cepa* exposed for 96 h to water samples collected at the sampling stations of Mãe-Bá Lagoon.

1st Campaign						
	Mitotic Index (%)	Interphase (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
NC	7.57 ± 3.11[a]	90.80 ± 2.84[b]	4.14 ± 3.78[a]	1.94 ± 3.52[a]	1.18 ± 1.56[a]	0.30 ± 1.28[a]
SS 1	14.04 ± 3.11[a]	76.22 ± 2.84[a]	16.04 ± 3.78[a]	3.42 ± 3.52[a]	2.46 ± 1.56[a]	1.64 ± 1.28[a]
SS 2	17.96 ± 3.11[a]	81.62 ± 2.84[ab]	10.86 ± 3.78[a]	3.06 ± 3.52[a]	2.56 ± 1.56[a]	1.46 ± 1.28[a]
SS 3	17.52 ± 3.11[a]	82.18 ± 2.84[a]	11.22 ± 3.78[a]	3.38 ± 3.52[a]	2.14 ± 1.56[a]	0.76 ± 1.28[a]
2st Campaign						
	Mitotic Index (%)	Interphase (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
NC	11.66 ± 1.04[a]	80.24 ± 0.91[a]	3.52 ± 0.91[a]	4.86 ± 0.45[a]	2.48 ± 0.37[a]	0.80 ± 0.15[a]
SS 1	16.38 ± 1.04[a]	88.52 ± 0.91[a]	7.56 ± 0.91[a]	3.42 ± 0.45[a]	2.52 ± 0.37[a]	1.94 ± 0.15[a]
SS 2	17.44 ± 1.04[a]	81.74 ± 0.91[a]	7.84 ± 0.91[a]	4.90 ± 0.45[a]	2.86 ± 0.37[a]	1.84 ± 0.15[a]
SS3	15.26 ± 1.04[a]	84.18 ± 0.91[a]	7.74 ± 0.91[a]	3.88 ± 0.45[a]	2.50 ± 0.37[a]	1.14 ± 0.15[a]

Means ± EP followed by the same letter in the columns do not differ statistically at a 5% significance level using Tukey's test. EP: Standard error. NC: Negative control. SS: Sampling station

Table 2. Parameters of mitotic index (MI%) and cell division in meristematic cells of *Allium cepa* exposed for 96 h to solubilized sediment collected at the sampling stations of Mãe-Bá Lagoon.

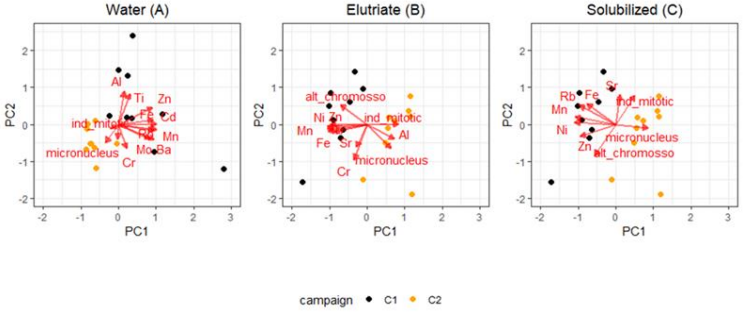
1st Campaign						
	Mitotic Index (%)	Interphase (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
NC	17.58 ± 2.74[a]	82.26 ± 2.61[a]	12.66 ± 5.58[a]	2.40 ± 1.45[a]	1.76 ± 0.81[a]	0.70 ± 1.09[a]
SS 1	15.70 ± 2.74[a]	83.98 ± 2.61[a]	8.92 ± 5.58[a]	3.18 ± 1.45[a]	2.18 ± 0.81[a]	1.42 ± 1.09[b]
SS 2	9.70 ± 2.74[a]	90.04 ± 2.61[b]	3.82 ± 5.58[a]	3.14 ± 1.45[a]	1.92 ± 0.81[a]	0.82 ± 1.09[a]
SS 3	11.90 ± 2.74[a]	87.70 ± 2.61[b]	6.54 ± 5.58[a]	2.78 ± 1.45[a]	1.86 ± 0.81[a]	0.72 ± 1.09[a]
2st Campaign						
	Mitotic Index (%)	Interphase (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)
NC	19.56 ± 0.90[a]	80.30 ± 1.16[a]	13.40 ± 0.77[a]	3.44 ± 0.29[a]	2.12 ± 0.16[b]	0.60 ± 0.15[a]
SS 1	12.04 ± 0.90[b]	87.44 ± 1.16[b]	8.00 ± 0.77[a]	2.50 ± 0.29[a]	1.02 ± 0.16[a]	0.52 ± 0.15[a]
SS 2	15.72 ± 0.90[b]	83.76 ± 1.16[ab]	9.34 ± 0.77[a]	3.70 ± 0.29[a]	1.46 ± 0.16[a]	1.22 ± 0.15[a]
SS3	14.48 ± 0.90[b]	85.02 ± 1.16[ab]	10.50 ± 0.77[a]	2.00 ± 0.29[a]	1.26 ± 0.16[a]	0.72 ± 0.15[a]

Means ± EP followed by the same letter in the columns do not differ statistically at a 5% significance level using Tukey's test. EP: Standard error. NC: Negative control. SS: Sampling station.

The reduction in the mitotic index correlates with an increase in interface cells and a decrease in prophase cells. This may be attributed to the action of the G1/S and G2 selection points of the cell cycle, which initiate DNA synthesis or block cell cycle progression[41]. Regarding the decrease in anaphase cells, 10 we emphasize that this decrease may be associated with the last checkpoint of the cell cycle, known as the metaphase-anaphase checkpoint. Other researchers suggest that the decrease in the mitotic index may be attributed to the effects of environmental chemicals, including heavy metals, on DNA/protein synthesis in the biological system[47].

In the analysis of the water samples (Figure 1 A) in Campaign 1, it can be seen that the mitotic index, chromosomal alterations and the frequency of micronuclei may be associated with the presence of Cr in the water samples. Cr is a metal that causes environmental pollution due to its association with industrial processes and is toxic to microorganisms and plants. It is present in the environment in trivalent [Cr(III)] and hexavalent [Cr(VI)] forms, the latter being considered the most toxic[54-56]. Hexavalent chromium can be genotoxic, causing DNA damage that results in chromosomal alterations and, consequently, the formation of micronuclei[57]. Studies such as those bu autores[58,59] suggest that some heavy metals, such as chromium, cause a decrease in the mitotic index, in addition to causing chromosomal abnormalities.

Figure 1. Principal component analysis (PCA) carried out between chromosomal alterations (alt_chromosome), mitotic index (ind_mitotic) and micronuclei with the metals (Al, Ti, Zn, Fe, Cd, Mn, Mo, Ba, Cr, Rb, Sr, Ni) bioaccumulated in the roots of A. cepa. The black dots refer to campaign 1 and the orange dots refer to campaign 2. Quadrant A refers to the water samples; quadrant B to the Elutriate samples; quadrant C to the Solubilized samples.



For the samples with elutriate (Figure 1 B), during campaign 1, it was possible to observe an association between chromosomal alterations and the presence of Ni, Zn, Mn, Fe and Ti, while in campaign 2 the mitotic index and the presence of cells with micronuclei may be associated with Al. For the solubilized samples (Figure 1 C) during campaign 1, the chromosomal alterations may be associated with the presence of Zn, and the mitotic index, with the presence of Sr. Nickel (Ni), along with other heavy metals, is recognized as an environmental pollutant[60] and may be associated with components of the cell wall and membrane, affecting cell division or elongation[61]. Zn plays a key role in several biological processes, but there is little data available on the effects of zinc in air, water, soil and sediments[62,63]. Some studies on wheat, black cumin, onion and sugarcane species have shown aneugenic and clastogenic effects caused by Zn[64,65]. The results of our work corroborate those described by the aforementioned authors, since inhibition of the mitotic index and chromosomal alterations were identified.

In terms of genotoxic potential, SS1 showed significant chromosomal alterations (CA) during the second campaign for the water samples (Table 4). For the solubilized water, a significant frequency of CA was observed in the first campaign in SS2 and SS3 (Table 4). These results suggest that the metals quantified in the water and solubilized samples had aneugenic and clastogenic action on the genetic material. Silveira et al.[48], points out that the CAs of clastogenic actions include chromosomal breaks and bridges, and the CAs of aneugenic actions include c-metaphase, chromosomal losses, chromosomal delay and multipolarization. The alterations observed in our studies were chromosome delay, categorized as an indicator of aneugenic action due to disturbances in the mitotic spindle, as well as chromosome bridging and chromosome breakage, considered indicators of clastogenesis[49]. It is possible that the metals present in the water and sediment have an affinity with the mitotic spindle, since alterations in chromosome delay were identified. It is worth noting that aluminum (Al) was quantified at all sampling stations during the two campaigns and, according to[50,51]. this metal acts directly on microtubules, leading to chromosomal alterations.

Table 4. Chromosomal alterations (CA) and micronuclei (MN) observed in cytogenetic analyses of *Allium cepa* meristematic cells exposed for 96 hours to elutriate and solubilized water samples collected from Mãe-Bá lagoon.

	1st Campaign					
	Water		Elutriate		Solubilized	
	MN (%)	CA (%)	MN (%)	CA (%)	MN (%)	CA (%)
NC	0.10 ± 0.08[a]	0.00 ± 0.04[a]	0.00 ± 0.06[a]	0.00 ± 0.03[a]	0.00 ± 0.06[a]	0.00 ± 0.08[a]
SS 1	0.16 ± 0.08[a]	0.06 ± 0.04[a]	0.12 ± 0.06[a]	0.10± 0.03[a]	0.16 ± 0.06[a]	0.14 ± 0.08[a]
SS 2	0.26 ± 0.08[a]	0.04 ± 0.04[a]	0.08 ± 0.06[a]	0.06 ± 0.03[a]	0.04 ± 0.06[a]	0.24 ± 0.08[b]
SS 3	0.12 ± 0.08[a]	0.10 ± 0.04[a]	0.06 ± 0.06[a]	0.12 ± 0.03[a]	0.12 ± 0.06[a]	0.28 ± 0.08[b]
	2st Campaign					
	Water		Elutriate		Solubilized	
	MN (%)	CA (%)	MN (%)	CA (%)	MN (%)	CA (%)
NC	0.06 ± 0.42[a]	0.04 ±0.06[a]	0.06 ± 0.38[a]	0.00 ± 0.50[a]	0.02 ± 0.51[a]	0.02 ± 0.32[a]
SS 1	0.92 ±0.42[b]	0.16 ± 0.06[b]	0.46 ± 0.38[a]	0.02 ± 0.50[a]	0.04 ± 0.51[a]	0.06 ± 0.32[a]
SS 2	0.70 ± 0.42[a]	0.10 ± 0.06[a]	0.94 ± 0.38[b]	0.06 ± 0.50[a]	0.46 ± 0.51[b]	0.06 ± 0.32[a]
SS 3	0.46 ± 0.42[a]	0.02 ± 0.06[a]	0.96 ± 0.38[a]	0.02 ± 0.05[a]	0.48 ± 0.51[b]	0.02 ± 0.32[a]

Means ± EP followed by the same letter in the columns do not differ statistically at a significance level of 5% using the Kruskal-Wallis test. EP: Standard error. NC: Negative control. SS: sampling station.

The mutagenic potential of the water, elutriate and solubilized samples was determined by the frequency of cells with micronuclei. Table 4 shows a high frequency of cells with micronuclei in SS1 during the second campaign for the water sample, SS2 during the second campaign for the elutriate sample (Table 4) and in SS2 and SS3 during the second campaign for the solubilized samples (Table 4). According to[52,53] the micronucleus results in DNA fragments resulting from spindle junction failures, originating from various pathways. These pathways include the formation of acentric chromosome fragments due to unrepaired DNA breaks, the simultaneous repair of damaged or incompatible bases incorporated into nearby DNA and interactions in opposing complementary DNA strands; in addition, aneugenic agents can also trigger this condition by causing chromosome

loss, resulting in MN formation. The data from the multivariate analysis indicates similarity between the metals identified in the water samples (Al, Ti, Zn, Fe, Cd, Cr, Mo, Ba, Mn) elutriate (Ni, Zn, Ti, Fe, Mn Sr, Al, Cr) and solubilized (Sr, Fe, Rb, Mn, Ni, Zn) in relation to the changes observed in the cell cycle (Figure 1).

The toxicity of excessive Mn accumulation can reduce the photosynthetic rate, block the biosynthesis of the hormone auxin and chlorophyll pigments and cause oxidative stress and damage to genetic material at the chromosomal or DNA level[66,67,68]. Iron is a necessary micronutrient for almost all organisms due to its fundamental role in biological systems[69,70,71], point out that excess Fe can increase free radical production rates in living organisms, causing DNA damage, as well as cytogenetic alterations in *A. cepa* cells, corroborating the results found in our studies.

For oxidative stress with the roots exposed to the water samples during the second campaign, there was a significant difference for LPO between the three sampling stations, with an increase in activity in SS3. For the roots exposed to elutriate, an increase in GST activity was observed in SS1 during the first campaign and in SS2 during the second campaign. For the roots exposed to the solubilizer during the second campaign, there was an increase in SOD activity in SS1 and in LPO in SS2. Exposure to heavy metals can increase the production of reactive oxygen species (ROS), which are naturally produced in plants but, in excess, can modify the arrangement of membrane lipids, facilitating their peroxidation[46]. The increased level of reactive oxygen species (ROS) is highly reactive and affects a wide variety of cellular, physiological and biochemical functions, such as disruption of the plasma membrane through carbohydrate deoxidation, lipid peroxidation, protein denaturation and destruction of DNA, RNA, enzymes and pigments[78]. Studies[79,80] have demonstrated the importance of the intracellular antioxidant defense mechanism against a variety of stresses, such as metal toxicity, atmospheric pollutants and high doses of pesticides[81].

Enzymes such as catalase, glutathione peroxidase (GPx), glutathione s transferase (GST) and superoxide dismutase (SOD) play an essential role in the

preventive antioxidant system, eliminating reactive oxygen species (H_2 O_2, OH, O_2)[82,83]. Among the main enzymes that function as the first line of antioxidant defense against the stress generated by ROS is superoxide dismutase[84]. SOD catalyzes the dismutation of superoxide into H_2O_2 and molecular oxygen[85], so regulating the activity of this enzyme indicates the ability of plants to eliminate excess ROS in cells[86].

SOD is considered the first enzyme to protect against excess peroxide (H_2O_2)[87]. SOD is initially increased as a result of the formation of ROS, such as the superoxide radical, by exposure to heavy metals[88]. Some authors cite that[89] this increase may be related to heavy metals which, in small quantities, are essential for the most diverse processes of plant metabolism, but in excess negatively affect plant metabolism. Some studies have reported an increase in SOD enzyme activity in green beans under Al stress[90], in *Lactuca sativa*[91], and in *A. cepa* exposed to other metals[92].

GST is a group of enzymes that actively participate in the detoxification of heavy metals[93]. The role of GST is fundamental for the removal of metal-induced ROS, since this enzyme may be involved in the transport of phytochelatin–metal complexes to the vacuole of contaminated plants[94]. Several studies using heavy metals, such as Ni and Pb, have demonstrated the defensive function of this enzyme in the detoxification of H_2O_2[95,97].

Lipid peroxidation (LPO) can be described as the process in which the carbon-carbon double bond in lipids (polyunsaturated fatty acids) is attacked by oxidants, such as free radicals[98], and is used as a biomarker to assess oxidative stress damage in animal and plant tissues and cells[99,100]. Lipids are the main components of the plasma membrane of cells and organelles. Pospisil and Yamamoto[101] point out that, with increased levels of ROS, cellular functions are influenced and oxidative stress is exceeded by the production of lipid-derived radicals, with lipid peroxidation occurring. In this way, external agents can cause ROS, increasing lipid peroxidation.

Although the exclusive focus on *A. cepa* ensured clear and sensitive responses, the toxicogenetic and biochemical alterations reported here may also indicate potential risks to other components of the lagoon ecosystem. Fish, benthic invertebrates, aquatic plants, and even terrestrial organisms interacting with the lagoon could be similarly affected by bioaccumulation and oxidative stress induced by metals. This broadens the ecological significance of our findings, underscoring the vulnerability of Mãe-Bá Lagoon.

The temporal variability observed between the two campaigns can also be linked to environmental drivers. Precipitation was substantially higher in March 2021 compared to March 2022, which may have favored leaching and resuspension of metals from soils and sediments. In contrast, the drier conditions during the second campaign may reflect a higher contribution of concentrated industrial effluents. Thus, climatic patterns, combined with the resumption of industrial activity in 2020, provide important context for the differences observed.

CONCLUSION

The results showed variations in the levels of metals in the water, elutriate and solubilized samples throughout the different sampling campaigns carried out in Mãe-Bá lagoon. These variations in metal levels were associated with inhibition of root growth at all sampling stations during the first campaign for the water, elutriate and solubilized samples. This effect can be attributed mainly to the accumulation of aluminum in the roots, as described in this study. Although some metals were present in low concentrations, genotoxic and mutagenic effects were observed in the meristematic cells of the roots. These effects may be due to the interaction of metals, even at low concentrations, with cellular DNA. The same applies to antioxidant action, since the presence of metals can act as a stressor for cells, generating reactive oxygen species.

Overall, the study demonstrates that *A. cepa* bioassays are effective in detecting toxic, genotoxic, and mutagenic effects induced by environmental

samples, even when contaminant levels are below some regulatory thresholds. However, the interpretation of the findings should consider the restricted spatial scope of sampling and the influence of climatic variation and industrial activity. By integrating toxicogenetic, biochemical, and chemical analyses, the research provides robust evidence of the ecological vulnerability of Mãe-Bá Lagoon and highlights the need for continuous monitoring using both plant bioassays and complementary models. Future efforts should also include comparisons with legal standards, exploration of synergistic mechanisms among metals, and assessment of risks to other aquatic and terrestrial species associated with the lagoon.

CONFLICT(S) OF INTEREST

The authors declare that they have no conflict of interest.

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