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Monitoring Metal Contamination and Genotoxic Effects in Carapebus Lagoon (Brazil) Using bioassays With *Allium cepa*

Monitoramento da Contaminação por Metais e Efeitos Genotóxicos na Lagoa de Carapebus (Brasil) Por Meio de Bioensaios com Allium cepa

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Abstract

Aquatic environments are often contaminated by urban-industrial pollutants, which may induce genotoxic effects in organisms. The Carapebus Lagoon, located in the metropolitan region of Vitória (ES, Brazil), is an urbanized coastal lagoon impacted by sewage discharge. This study evaluated the genotoxic and biochemical effects and the presence of environmental metallic elements in *Allium cepa* roots exposed to samples of water and the elutriate and solubilized fractions of sediment from the lagoon, collected at three stations (SS1, SS2,

and SS3) during two sampling campaigns. Metals were analyzed in the environmental samples and roots, alongside the germination, root growth, cell cycle, antioxidant enzyme activity, and oxidative stress of *A. cepa*. Regarding the first sampling campaign, germination was inhibited by the water samples and the elutriate fraction of the sediment samples from SS1 and SS3 and the sediment samples from SS1, and reduced in all samples from SS2; root growth was reduced by water and the elutriate fraction of the sediment samples from SS2 and the sediment samples from SS2 and SS3; cytotoxicity was induced by the water samples from SS2; genotoxicity was induced by the elutriate fraction of the sediment samples from SS2; and glutathione S-transferase activity changed after exposure to the elutriate fraction of the sediment samples. For the second campaign, the sediment samples from SS2 and SS3 led to cytotoxicity; the sediment samples from all three sites and the elutriate fraction of the sediment samples from SS2 resulted in mutagenicity; and the sediment samples from all three sites altered superoxide dismutase and glutathione S-transferase activity and the level of lipid peroxidation. Metals such as Fe, Ni, Cd, Cr, and Pb possibly contributed to the observed effects. These results indicate the presence of contaminants with toxic potential in the Carapebus Lagoon, reinforcing the importance of environmental monitoring with bioassays.

Keywords: Cell Metabolism. Chromosomal Alterations. Genotoxicity Biomarkers. Sediment Contamination.

Resumo

Os ambientes aquáticos são frequentemente contaminados por poluentes urbano-industriais, que podem provocar efeitos toxicogenéticos nos organismos. A lagoa de Carapebus, situada na região metropolitana de Vitória (ES, Brasil), é uma lagoa costeira urbanizada e impactada pelo lançamento de esgoto. Este estudo avaliou os efeitos toxicogenéticos, bioquímicos e a presença de elementos químicos ambientais em raízes de *Allium cepa* expostas a amostras de água, elutriato e sedimento solubilizado da lagoa, coletadas em diferentes pontos durante duas campanhas. Foram realizadas análises de metais nas amostras ambientais e nas raízes, além de testes de germinação, crescimento radicular, ciclo celular, estresse oxidativo e enzimas antioxidantes. Na primeira campanha, houve inibição da germinação em SS1 e SS3 (água e elutriato), e SS1 (sedimento); redução da germinação em SS2 (todas as amostras); crescimento radicular reduzido em SS2 (água e elutriato) e em SS2 e SS3 (sedimento); citotoxicidade em SS2 (água); genotoxicidade em SS2 (elutriato); e alterações de GST no elutriato. Na segunda campanha, observou-se citotoxicidade em SS2 e SS3 (sedimento), mutagenicidade em SS1, SS2 e SS3 (sedimento) e em SS2 (elutriato), além de alterações de SOD, GST e LPO no sedimento. Os elementos Fe, Ni, Cd, Cr e Pb possivelmente contribuíram para os efeitos observados, incluindo fitotoxicidade, citotoxicidade, genotoxicidade, mutagenicidade e alterações bioquímicas. Esses resultados indicam a presença de contaminantes com potencial tóxico na lagoa, reforçando a importância do monitoramento ambiental com bioensaios.

Palavras-chave: Metabolismo Celular. Alterações Cromossómicas. Biomarcadores de Genotoxicidade. Contaminação por Sedimentos.

1 Introduction

The main sources of contamination of the aquatic environment are untreated urban-industrial and agricultural wastes, which contain non-degradable and potentially harmful toxic substances (Kumar; Bhatti; Nagpal, 2021). These contaminants, including fertilizers and pesticides, have high levels of metals that induce toxic, genotoxic, and mutagenic effects in exposed organisms (Yadav *et al*., 2021). According to Maria, Correia and Santos (2004), most rivers, lakes, lagoons, and estuaries close to cities receive industrial and domestic discharges, leading to contamination of the aquatic environment.

In this context, short-term bioassays can be used as a tool to monitor the environmental risk caused by industrial pollutants (Artico *et al*., 2018). Bioassays based on *Allium cepa* stand out because

this species can be used effectively to monitor environmental samples (Leme; Marin-Morales, 2009) and is highly sensitive to many pollutants such as metals (Fiskesjo, 1988). The use of *A. cepa* is considered suitable to analyze clastogenic and aneugenic damage and chromosomal disorders in the mitotic spindle induced by various classes of contaminants in domestic and industrial sewage as well as water samples from rivers and lakes, which have a complex mixture of substances (Barbério, 2013; Sheikh; Patowary; Laskar, 2020). The *A. cepa* bioassay provides a way to screen pollutants with cytotoxic, genotoxic, and mutagenic potential (Alias *et al*., 2023; Leme; Marin-Morales, 2009; Mazzeo; Marin-Morales, 2015). It employs morphological factors such as germination, root growth, the mitotic index (MI), and biochemical markers as indicators of toxicity, given that the root is the first part of the plant exposed to the environment (Aragão *et al*., 2021; Bernardes *et al*., 2015). The *A. cepa* bioassay should be used in conjunction with regular physicochemical analyses of the water to assess its quality (Düsman *et al*., 2014).

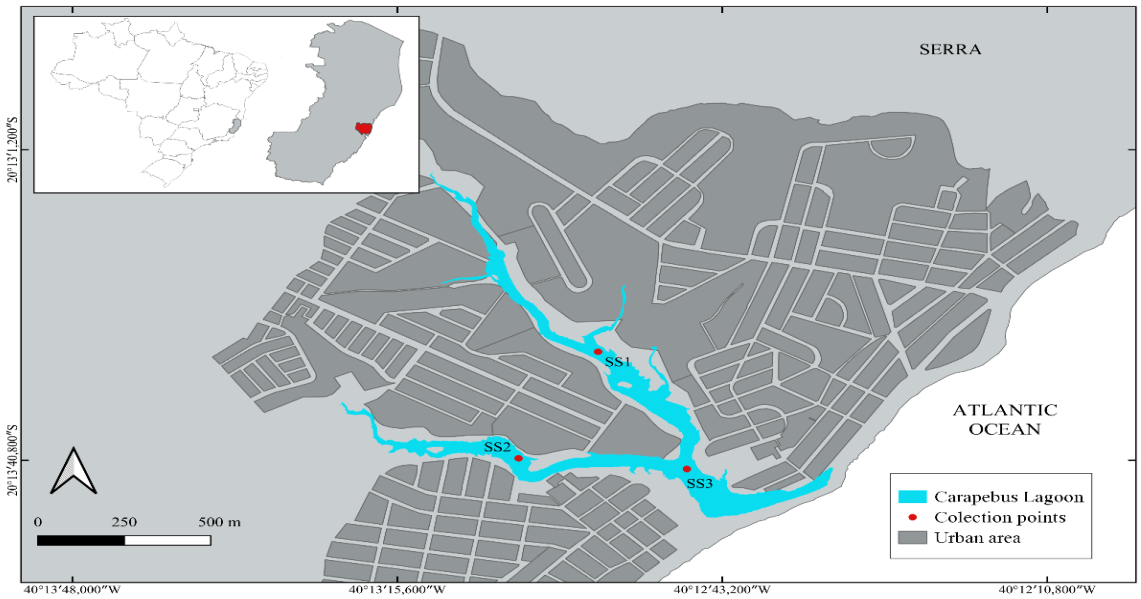
The Carapebus Lagoon is a coastal lagoon located in the metropolitan region of Vitória, ES, Brazil, close to an industrial complex that is a source of atmospheric particulate matter rich in metals and elemental carbon (Santos *et al*., 2017; Soares *et al*., 2019). According to the National Water Agency (ANA), this waterbody has experienced major urbanization, and much of the urban sewage is discharged directly into the lagoon. Consequently, the water of the Carapebus Lagoon is affected by untreated sewage in this area and subjected to the direct influence of atmospheric particulate matter from industry. The aim of this study was to evaluate the genotoxic and biochemical effects of the water and sediment of the Carapebus Lagoon collected during two sampling campaigns. This endeavor used the *A. cepa* bioassay and involved quantification of elements in the water, sediment, and root samples.

2 Material and Methods

2.1 Study area

The study was carried out in the Carapebus Lagoon, located in an environmental protection area in the municipality of Serra, in the Metropolitan Region of Vitória, ES, Brazil. The lagoon has an area of approximately 0.4 km² and is fed by groundwater, rainfall, and small tributary streams (Soares *et al*., 2019). The Carapebus watershed itself extends over 4.8 km². Water and sediment samples were collected at three sampling stations—SS1(20°13'26.96"S, 40°12'55.59"W); SS2 (20°13'40.55"S 40°13'03.5"W), located 0.52 km from SS1; and SS3 20°13'41.91"S 40°12'46.73"W), located 0.49 km^2 from SS2 (Figure 1) during two sampling campaigns at the end of March 2021 and March 2022.

Figure 1 - Collection stations in Carapebus Lagoon. SS: Sampling station. Source: Qgis



Source: research data.

2.2 Preparation of the solubilized and elutriate fractions of the sediment samples

The sediment samples were processed to obtain the elutriate and solubilized fractions, following the ABNT NBR 15469 and ABNT NBR 10006 standards. Briefly, the samples were dried in oven at 42 °C and sieved. For the solubilized fraction, 12.5 g of the dry sample was added to 50 mL of distilled water and incubated at 24 °C for 7 days; then, the supernatant was collected and used for analysis. For the elutriate fraction, 12.5 g of the raw sediment sample was added to 50 mL of water at room temperature, stirred for 30 min, and allowed to settle at 24 °C for 2 h. Then, the supernatant was collected and used for analysis.

2.3 Experimental design

The *A. cepa* bioassays were conducted with a completely randomized design. Cytotoxicity was analyzed with five Petri dishes: the elutriate fraction of the sediment sample, the solubilized fraction of the sediment sample, the water sample, a positive control (0.84 mg/L trifluralin), and a negative control (distilled water)(Lascola *et al*., 2024). The positive control was chosen because its effects and mechanisms have been established based on cell cycle and root growth analyses (Fernandes; Mazzeo; Marin-Morales *et al*., 2007). Thirty seeds of *A. cepa*—the Baia Periforme variety from Isla Sementes (Lot 153901)—were distributed in each plate, followed by the appropriate sample. and as a positive control.

The experiments to analyze the metal contents and oxidative stress analysis were performed in triplicate, with each replicate performed independently, according to Andrade-Vieira, Palmieri and Davide (2017). Thirty seeds were germinated in distilled water and, after the roots reached 1-2 mm

in length, they were transferred to plates with water. Then, they were exposed to the elutriate and solubilized fractions of the sediment samples for 96 h. Composite root sampling was used for the analysis.

2.4 Cytogenotoxicity assessment

Phytotoxicity was assessed by analyzing the percentage of germination and root growth (Aragão *et al*., 2017). The cell cycle was assessed in roots subjected to the Feulgen reaction (Feulgen; Rossenbeck, 1924): They were stained in Schiff reagent for 2 h in the dark (Aragão *et al*., 2024; Grecco *et al*., 2024; Santos *et al*., 2023). A single sample from each replicate was used for cell cycle analysis. The slides were prepared using the gentle crushing technique (Leme; Marim-Morales, 2008). Five slides were prepared per treatment and 1,000 cells per slide were analyzed, for a total of 5,000 cells per treatment (Andrade-Vieira; Palmieri; Davide, 2017). The MI; the number of micronucleated cells; and the percentage of chromosomal aberrations (CA) such as losses, fragments, bridges, delays, chromosomal adhesions, and chromosomal breaks were determined. For genotoxicity, all CA were considered, and for mutagenicity, only cells with micronuclei were considered, according to Santos *et al*. (2022).

2.5 Metal analysis

Chemical analyses were performed according to published protocols (Chappaz *et al*., 2012; Souza *et al*., 2013, 2018) with slight modifications. The digestion process was not performed for the water samples and the elutriate and solubilized fractions of the sediment samples. Composite root samples were completely dried in an oven at 40 °C, and 0.1 g for each sample was added into tubes containing nitric acid. Then, the samples were incubated in a microwave oven for 40 min to complete digestion. The digested material was filtered, weighed, and stored until analysis. The metal analysis was performed using an inductively coupled plasma mass spectrometer (Q-ICPMS, 7500 Series CX, Agilent Technologies, Santa Clara, CA, USA) equipped with an ASX-100 automatic sampler (CETAC Technologies, Omaha, NE, USA) at the Institute of Food Science and Technology of Córdoba (YCITAC, Universidad Nacional de Córdoba; Argentina).

2.6 Oxidative stress analysis

Composite samples of the roots from each triplicate were used to evaluate antioxidant enzyme activity and damage from oxidative stress. The roots were frozen in liquid nitrogen and store at -80 °C until analysis. The total protein concentration was determined with the Bradford method (1976). Superoxide dismutase (SOD) activity was measured according to the protocol described by McCord and Fridovich (1969). Glutathione S-transferase (GST) activity was determined using the method

described by Habig and Jakoby (1981). The lipid peroxidation level was determined using the ferrous oxidation/xylenol orange method, based on the protocol published by Jiang, Woolard and Wolf (1991).

2.7 Statistical analysis

The Infostat program was used for data analysis. The data are expressed as mean and standard error. First, the data were subjected to the Shapiro-Wilk test to determine whether it followed a normal distribution. Normally distributed data were analyzed with an analysis of variance (ANOVA) followed by Tukey's test. Non-normally distributed data were analyzed with the Kruskal-Wallis test followed by Dunn's test. For all analyses, $P < 0.05$ was considered to indicate a significant difference. In the figures and tables, the same letters in the same columns indicate no significant difference.

3 Results and Discussion

The first campaign was characterized by high rainfall (295.2 mm) and the second by low rainfall (38.6 mm), according to data from Cemaden (2023). Electrical conductivity was higher in the first campaign, with values between 55 and 69 µS/cm, while in the second it varied between 3.65 and 4.21 µS/cm (Table 1). The pH was neutral in the first campaign and alkaline in the second, but always within the limits set by CONAMA Resolution 430/2011 (pH between 6 and 9). The water temperature remained constant in both campaigns.

Table 1 - Physical and chemical water parameters measured during the first campaign (March/2021) and the second campaign (March/2022) at the different sampling stations (SS) in Carapebus Lagoon

	1st Campaign			2nd Campaign		
Variáveis limnológicas	**SS1**	**SS2**	**SS3**	**SS1**	**SS2**	**SS3**
Electrical conductivity (µS/cm)	69	57	55	3.65	4.12	4.21
Temperature (°C)	30	28	28.5	28.95	28.70	28.00
Dissolved oxygen (mg/L)	-	-	-	9.73	8.39	8.73
pH	7.18	7.19	7.10	8.36	8.37	8.28

Source: research data.

The electrical conductivity of water reflects the concentration of dissolved ions and is influenced by temperature (Avila-Perez *et al.*, 2023). This parameter indicates possible pollutants and varies according to geological and seasonal factors (Esteves, 2011; Manzano *et al.*, 2015). The higher conductivity for the first sampling campaign, which also had high rainfall, might have been the result of surface runoff and the discharge of domestic effluents, as has been reported for the Carapebus Lagoon (Duarte *et al.*, 2017; Galter *et al.*, 2021; Petrucio; Barbos; Thomaz *et al.*, 2005). The

concentrations of the metals identified in the environmental samples showed seasonal and spatial variations (Figure 2).

Figure 2 - Metal trace element concentrations (µg L^{-1}, log_{10} scale) in water (A), solubilized (B), and elutriate (C) samples collected in different sampling stations in Carapebus Lagoon during the first (1st, March 2021) and second (2nd, March 2022) campaigns. SS: Sampling station

Source: research data.

In the water samples, Al, Ti, V, Cr, Mn, Fe, Rb, and Sr were the main metals solubilized and eluted. The metal concentrations were higher in the samples collected during the first sampling campaign, while during the second sampling campaign there was greater diversity in samples collected at SS1 and SS2. Roots absorbed mainly Al, Cr, Mn, Fe, and Sr (Figure 3).

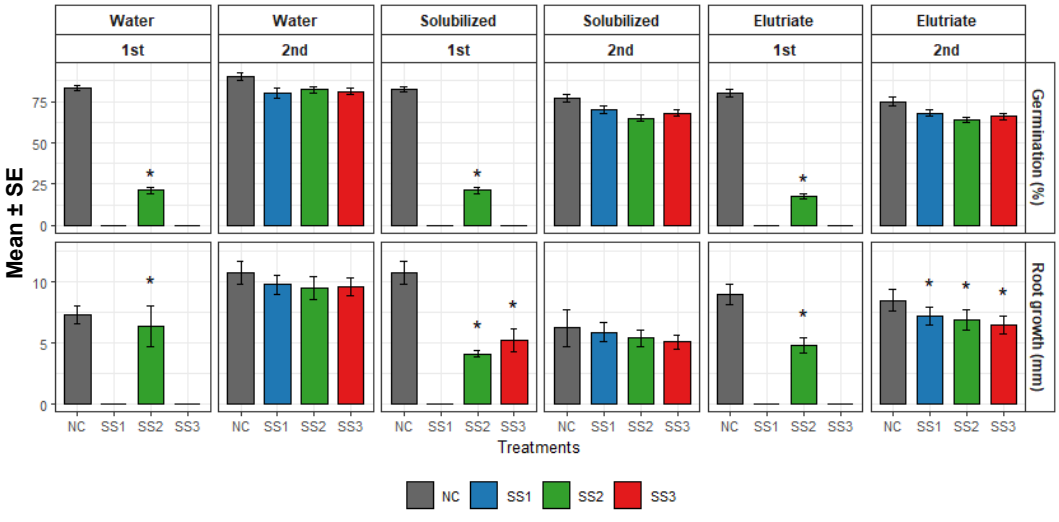
Figure 3 – Metal concentrations (µg g^{-1}, log_{10} scale) in meristematic cells of *A. cepa* roots exposed to the water (A), solubilized (B), and elutriate (C) samples collected in the Carapebus Lagoon sampling stations during the first (1st, March 2021) and second (2nd, March 2022) campaigns. SS: Sampling station

Source: research data.

The metals Mg, Fe, Zn, Mn, Cu, Co, Mo, and Ba are essential to organisms, while As, Hg, Pb, Cd, Ag, Cr, Ni, and Sn can be toxic (Esteves, 2011). As, Cd, Pb, Cr, and Ni are the main pollutants, especially in urban areas (Sabeen *et al*., 2020). There were higher metal concentrations in the solubilized and elutriate fractions of the sediment samples, reflecting the ability of sediment to accumulate metals (Geffard *et al*., 2007; Zhang *et al*., 2016). Fe was detected in samples from all three sampling stations; its presence is linked to natural soil characteristics or industrial activities. The presence of Pb and Cr in the samples suggests anthropogenic influence near the lagoon (Rambo *et al*., 2017).

In the *A. cepa* bioassays (Figure 4), the water and elutriate fraction of the sediment samples collected at SS1 and SS3 during the first sampling campaign and the solubilized fraction of the sediment samples collected at SS1 during the first sampling inhibited germination. Moreover, all samples collected at SS2 reduced germination. Root growth was reduced in the presence of the water samples and the elutriate fraction of the sediment samples collected at SS2 during the first sampling campaign, and in the presence of the solubilized fraction of the sediment samples collected at SS2 and SS3. The results suggest that inhibition of germination and root growth may be linked to non-essential elements (i.e., those with no biological function) (Esteves, 2011), because the root is the first organ exposed to pollutants (Anusha *et al*., 2023). Heavy metals such as Cd, Pb, and Al affect respiration, meristematic division, and cell proliferation, resulting in biochemical changes, genotoxicity, and reduced growth (Liman; Ciğerci; Öztürk, 2015; Silveira *et al*., 2017; Cunha *et al*., 2020).

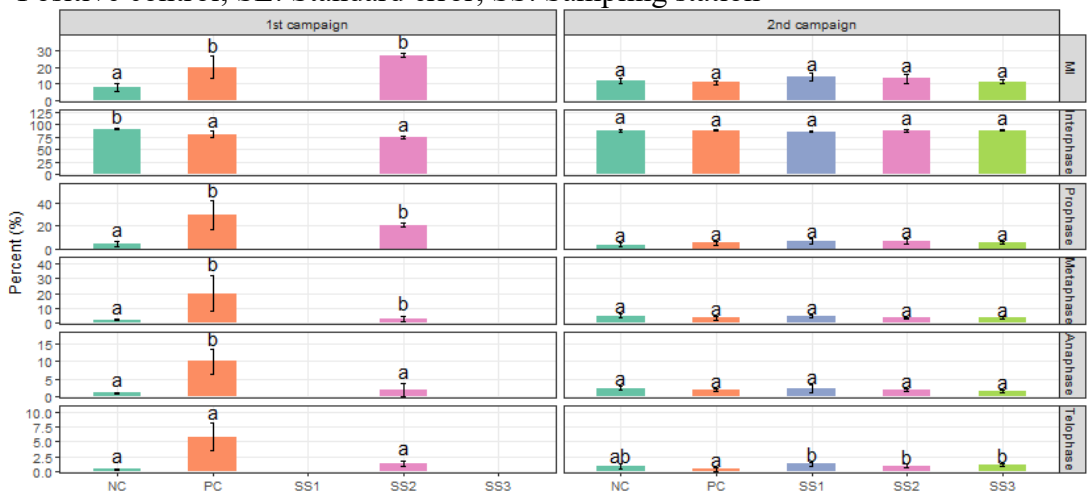
Figure 4 - Germination and root growth percentage of *Allium cepa* exposed to water, solubilized, and elutriated sediment samples collected during the first and second campaigns. Asterisks indicate significant difference from the negative control at the 5% significance level (ANOVA, Tukey's test). Values are the means ± SE. NC: Negative control; SE: Standard error; SS: Sampling station



Source: research data.

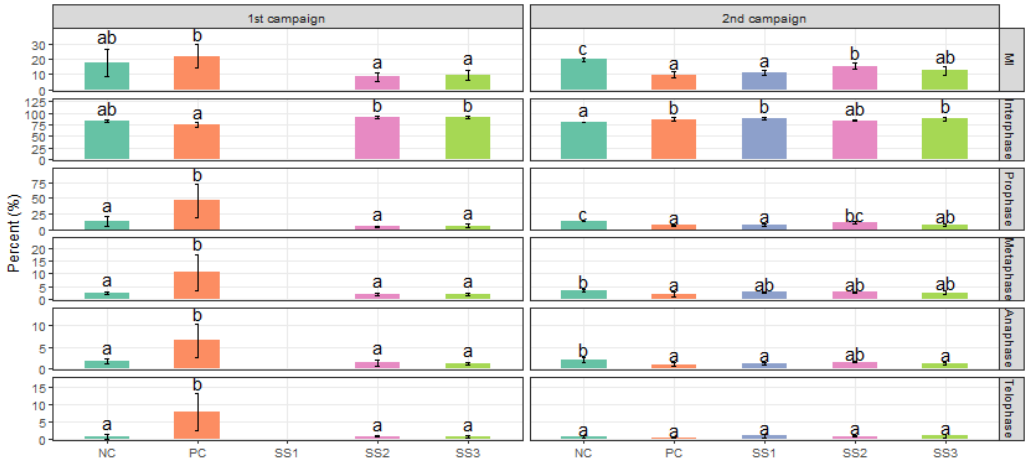
Recent studies have confirmed that metals are phytotoxic to plants (Bona *et al*., 2023; Da Cunha Neto *et al*., 2023), supporting the findings from the present study. There was a significant increase in the MI after exposure to the water samples collected at SS2 during the first sampling campaign (Figure 5) and a significant decrease in the MI after exposure to solubilized fraction of the sediment samples collected at SS2 and SS3 during the second sampling campaign (Figure 6).

Figure 5 - Mitotic index percentage (MI%) and cell cycle phases (prophase, metaphase, anaphase, and telophase) in meristematic cells of *A. cepa* roots exposed for 96 h to water samples collected in Carapebus Lagoon sampling stations during the first (1st, March 2021) and second (2nd, March 2022) campaigns. Different letters indicate significant differences from the negative control at the 5% significance level (ANOVA, Tukey's test). Values are the means ± SE. NC: Negative control; PC: Positive control; SE: Standard error; SS: Sampling station



Source: research data.

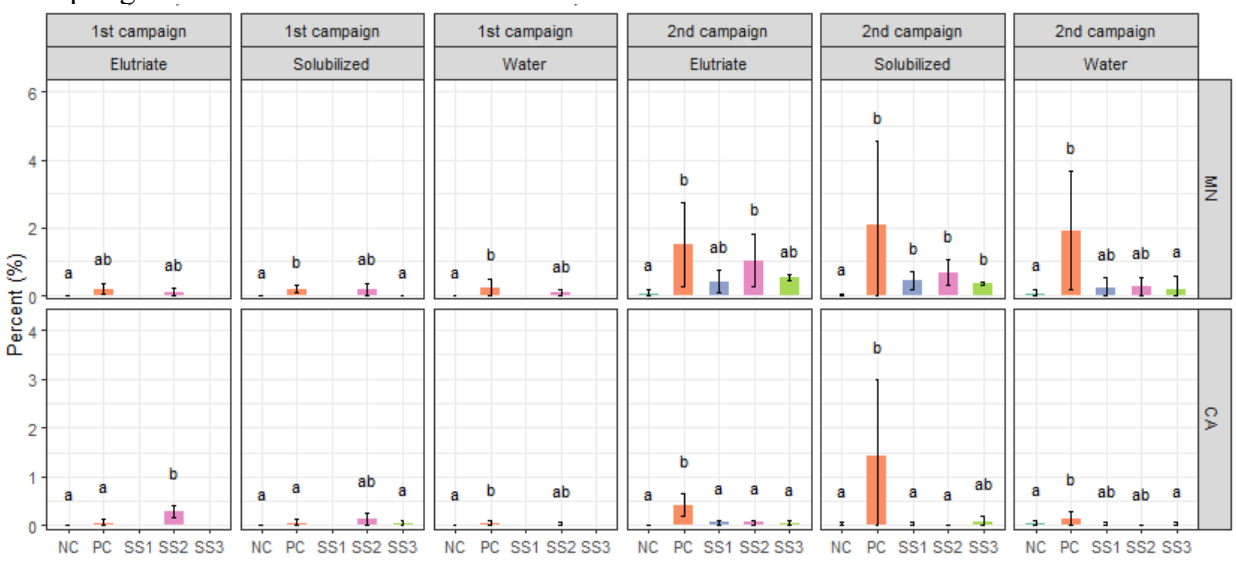
Figure 6 - Mitotic index percentage (MI%) and cell cycle phases (prophase, metaphase, anaphase, and telophase) in meristematic cells of *A. cepa* roots exposed for 96 h to solubilized sediment samples collected in Carapebus Lagoon sampling stations during the first (1st, March 2021) and second (2nd, March 2022) campaigns. Different letters indicate significant difference from the negative control at the 5% significance level (ANOVA, Tukey's test). Values are the means ± SE. NC: Negative control; PC: Positive control; SE: Standard error; SS: Sampling station



Source: research data.

Regarding genotoxicity (Figure 7), the roots exposed to the elutriate fraction of the sediment samples collected at SS2 during the first sampling campaign significantly increased the percentage of cells with CA, indicating possible aneugenic or clastogenic action of elements (Malakahmad *et al*., 2018; Ogunyemi *et al*., 2017). In addition to the genotoxic and mutagenic effects, the environmental samples also induced changes in antioxidant enzyme activity.

Figure 7 - Cytogenetic parameters of chromosomal alterations (CA) and micronuclei (MN) in meristematic cells of *A. cepa* roots exposed for 96h to water, solubilized, and elutriate samples collected at the Carapebus Lagoon sampling stations during the first (1st, March 2021) and second (2nd, March 2022) campaigns. Different letters indicate significant difference from the negative control at the 5% significance level (Kruskal–Wallis; Dunn's test). Values are presented as means ± SE. NC: Negative control; PC: Positive control; SE: Standard error; SS: Sampling station



Source: research data.

Changes such as the reduction in the MI after exposure to the solubilized fraction of the sediment samples collected at SS2 and SS3 may be related to the inhibition of the G1/S and G2 phases of the cell cycle, leading to compromised DNA synthesis and progression (Alberts *et al*., 2017). This reduction has been associated with high concentrations of metals, which disturb the cell cycle (Nefic *et al*., 2013) or lead to dysfunctional chromatin due to metal-DNA interactions (Glińska *et al*., 2007). On the other hand, the increase in the MI after exposure to the water samples collected at SS2 during the first sampling campaign may indicate the presence of a cytotoxic substance that induced abnormal

cell division (Leme; Marin-Morales, 2009). Phytotoxicity may also involve the generation of reactive oxygen species (ROS), which interact with DNA and histones (Yılmaz *et al*., 2023).

Studies with environmental samples show a high frequency of cells with CA and micronuclei (Batista *et al*., 2016; Galter *et al*., 2021). The data from the present study indicate mutagenic potential due to the formation of micronuclei in roots exposed to the solubilized fraction of the sediment samples collected at SS1, SS2, and SS3, and the elutriate fraction of the sediment samples collected at SS2 during the second sampling campaign.

This evidence of chromosomal damage by genotoxic agents (Kisurina-Evgenieva; Sutiagina; Onishchenko, 2016; Lacerda *et al*., 2020) corroborates previously observed clastogenic effects (Castro; Sousa, 2017; Gupta *et al*., 2012; Hemachandra; Pathiratne, 2015). Metals such as Fe, Ni, and Cr can induce chromosomal alterations and inhibit DNA repair enzymes (Majer *et al*., 2002).

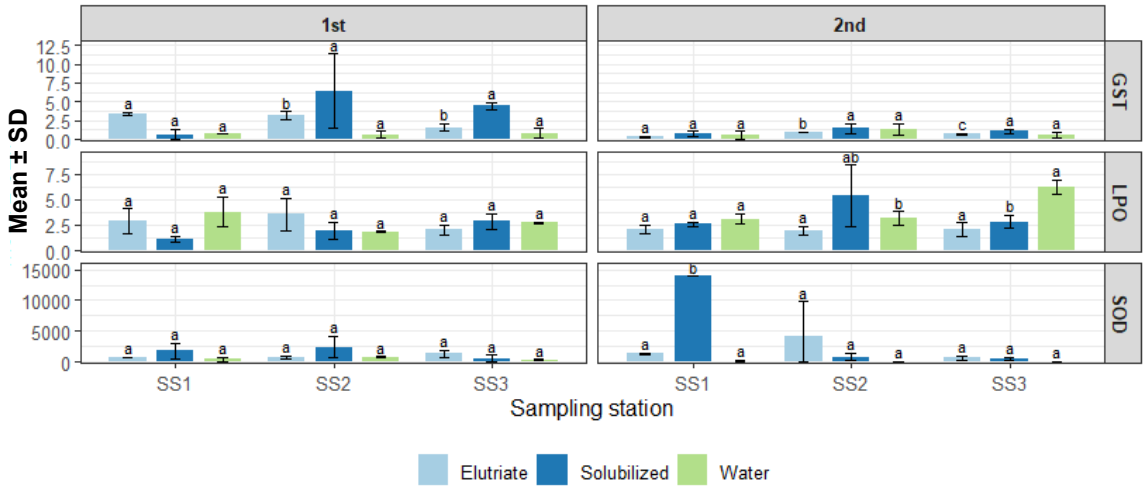
These changes may be related to metals in the roots of *A. cepa* exposed to environmental samples. Metals can affect plant metabolism by altering the cell membrane, reducing photosynthesis, and generating free radicals that damage the cell (Dorlodot; Lutts; Bertin, 2005; LWTAP, 2004; Mahmood *et al*., 2010; Rodrigues *et al*., 2016). The absorption of metals by the cell is influenced by transporter proteins and can be regulated by the inhibition of transport (Hall, 2002).

Some metals quantified in the environmental samples were not detected in *A. cepa* roots. According to Geremias *et al*. (2010) and Geremias *et al.* (2011), this discrepancy may be due to the detoxification system in the cell. The use of plant bioassays to assess the effects of metals in aquatic environments has been well documented in the literature (Duarte *et al.*, 2017; Galter *et al*., 2021; Matos *et al.*, 2017).

Figure 8 shows changes in GST and SOD activity as well as lipid peroxidation. There was a significant difference in GST activity in *A. cepa* roots exposed to the elutriate fraction of the sediment samples collected during the first sampling campaign, and SOD and GST activities and lipid peroxidation in roots exposed to the solubilized fraction of the sediment samples collected during the second campaign. ROS production induced by metals is responsible for fatty acid peroxidation.

Plants have enzyme and non-enzyme antioxidants to neutralize ROS and thus to avoid toxic effects (Aragão *et al*., 2024; Gill; Tuteja, 2010; Khan *et al.*, 2015). An imbalance between ROS production and removal by antioxidants may result in oxidative stress in the cells (Drzymała; Kalka, 2024).

Figure 8 - Enzymatic activities of superoxide (SOD, Unit SOD/mg ptn) and glutathione S-transferase (GST, nM/mg) and level of lipid peroxidation (LPO, nM/mg) in *Allium cepa* roots exposed to water, solubilized, and elutriate samples collected in Carapebus Lagoon sampling stations during the first (1st, March 2021) and second (2nd, March 2022) campaigns. Different letters indicate significant differences from the negative control at the 5% significance level (ANOVA, Tukey's test). Values are the means ± SD. SD: Standard deviation; SS: Sampling station



Source: research data.

The initial enzymes that neutralize ROS are SOD, which converts superoxide (O_2^-) into H_2O_2, and catalase or glutathione peroxidase, which catalyze H_2O_2 into H_2O and O_2 (Batista-Gallep *et al*., 2018; Drzymała; Kalka, 2024). GST, which is a phase II metabolic enzyme, catalyzes the addition of glutathione to metals to produce more water-soluble compounds, preventing reactivity in the cytosol and facilitating excretion (Hasanuzzaman *et al*., 2019; Huber; Almeida; Fátima, 2008). The results from the present study indicate that metals in the samples interfere with plant oxidative metabolism, a finding consistent with previous studies (Chowra *et al*., 2017; Gjata *et al*., 2022; Helaoui *et al*., 2022). The imbalance between ROS and antioxidants leads to lipid peroxidation (Pospisil; Yamamoto, 2017; Vujčić *et al*., 2023). Other pollutants, such as herbicides, fungicides, and pig farming effluents, also negatively affect antioxidant metabolism (Aragão *et al*., 2024; Grecco *et al*., 2024; Santos *et al*., 2023), impairing growth and the response to environment stress.

4 Conclusions

The variations in the metal concentrations between the analyzed environmental samples—with the highest values in the solubilized and elutriate fractions of the sediment samples—are due to the greater capacity of sediment to accumulate metals compared with water. The identified elements, especially non-essential metals, were associated with phytotoxicity, cytotoxicity, genotoxicity,

mutagenicity, and biochemical alterations observed in *A. cepa* roots. High concentrations of Fe, Ni, Cd, Cr, and Pb are related to anthropogenic activities and may also affect cell metabolism.

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