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Investigating the toxicity of tropical reservoir sediments using the Allium test

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Abstract

Water pollution is a global environmental issue, and aquatic sediments are important compartments that might act as sinks or sources of contaminants. Once in the environment, inorganic contaminants such as metals can cause cytogenotoxic effects that damage genetic material and harm the aquatic community. Biological assays such as the Allium test can be used to investigate potential cytogenotoxicity of contaminated sediments based on the alterations of cell cycle indexes and chromosomal aberration frequencies. Therefore, we aimed to assess the toxicity of sediments from four Brazilian reservoirs using the Allium test. Sediments were sampled and elutriates were prepared in a simulating sediment resuspension in the water column. The Allium test was applied to the elutriates, and the metals copper, chromium, cadmium, lead, zinc, and iron were quantified. The elutriates derived from reservoir sediments were able to reduce the mitotic and anaphase index, increase the prophase and metaphase index, and boost chromosomal aberrations compared to the negative control. The cytogenotoxic effects observed may be linked to the presence of copper, zinc, and iron. Therefore, our results showed that the Allium test was a sensitive tool for warning the occurrence of genotoxic contaminants in sediment elutriates from four Brazilian reservoirs.

Keywords: aquatic ecosystems; cytogenotoxicity; chemicals; contaminants; genotoxic; elutriate; metals; water quality.

INTRODUCTION

Anthropogenic activities discharge a pool of contaminants into freshwater ecosystems, and water pollution has become a severe environmental issue worldwide (Schwarzenbach *et al.,* 2010; Quadra *et al.,* 2019a). The mixture of contaminants received by aquatic ecosystems can cause mutagenic effects and harm ecological functions (Lee *et al.,* 2003; Lemos *et al.,* 2009). Adverse effects on human health may also occur due to lifelong exposure to contaminants by drinking water and food consumption (Buschini *et al.,* 2008; Castro-González *et al.,* 2008).

Freshwater reservoirs are created for energy production, irrigation, and water storage (Suen *et al.,* 2006; Tundisi, 2018). Reservoirs can present high sedimentation rates (Mendonça *et al.,* 2017) and become important contaminant sinks (Zoumis *et al.,* 2001; Massoudieh *et al.,* 2010). However, once in the sediment, the contaminants may return to the water column via resuspension processes, causing toxicity to aquatic organisms (Zoumis *et al.,* 2001; Eggleton *et al.,* 2004; Cardoso *et al.,* 2019).

Biological tests can be used to assess the toxicity of individual chemicals or a complex mixture of contaminants present in the environment in an integrated way, such as

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sediments, providing support for safer decisions (Heise *et al.*, 2020). Previous studies have already used the Allium test to assess cytogenotoxicity caused by contaminants in aquatic ecosystems since it is a highly sensitive tool and presents an excellent correlation with other groups of organisms (Rambo *et al.,* 2017; Freitas *et al.,* 2017; Dieterich & Gaete, 2021). For that reason, we aimed to investigate the cytogenotoxicity of sediment elutriates from four freshwater reservoirs in the Brazilian Southeast using the Allium test. Copper (Cu), chromium (Cr), cadmium (Cd), lead (Pb), zinc (Zn), and iron (Fe) were quantified in the sediment elutriates to infer potential causes of detected toxicity.

MATERIAL AND METHODS

Study areas

The sediment samples were collected in four reservoirs located in the Brazilian Southeast: Furnas (FNS, S 21°13'54.84" W 45°57'19.02") and Chapéu D'Uvas (CDU, S 21°35'15.52" W 43°33'10.68") in Minas Gerais state, Santa Fé (SNF, S 22° 3'58.46" W 43° 9'54.40") and Funil (FUN, S 22°31'10.89" W 44°37'30.04") in Rio de Janeiro state (Figure 1). In 1963, FNS was created with a flooded area of 1327 km², and it is mainly used for electricity generation and irrigation (Ometto *et al.,* 2013). In 1995, CDU was created primarily for water supply, with an area of 12 km² (Quadra *et al.,* 2019b). In 2008, SNF was created for electricity generation, with an area of 2.05 km². In 1969, FUN was created with a flooded area of 26 km², and it is mainly used for aquaculture, water, and energy supply (Soares *et al.,* 2008; Ometto *et al.,* 2013).

Sampling and samples treatment

Sediment samples were collected in the dry season near the dam using a gravity corer (Mudroch *et al.,* 1994; Chapman, 1996). After sampling, the treatment was adapted from Dieterich & Gaete (2021), trying to stimulate a lighter resuspension, according to Magdaleno *et al.,* (2008) and Messias (2008). Briefly, after sampling, the sediment samples were dried at 40 °C to remove humidity until constant weight. The surface layer of sediment (20 cm) was then used to prepare sediment elutriates simulating a resuspension to the water column, where distilled water was added to the sediment respecting a proportion of 1:4 (mass: volume, sediment: water) (USEPA, 1998). A total of 100 mL of elutriate was prepared for each sample. Then, the samples were shacked manually for one minute and settled at room temperature (~ 22 °C). Twenty-four hours later, the aqueous supernatant (elutriate) was collected without resuspending the sediment (Magdaleno *et al.,* 2008; Messias, 2008). Distilled water was used as negative control and followed the same steps as elutriate preparation but without sediment. Therefore, five treatments were done and named NC, FNS, CDU, SNF, and FUN (for Negative Control, Furnas, Chapéu D'Uvas, Santa Fé, and Funil, respectively). Sediment elutriates were then used for Allium exposure and metal quantification as specified below.

Metal quantification

Cellulose acetate membrane (Whatman, 0.45µm) was used to filter the sediment elutriates, and Cu, Cr, Cd, Pb, Zn, and Fe were quantified by flame atomic absorption spectrometry

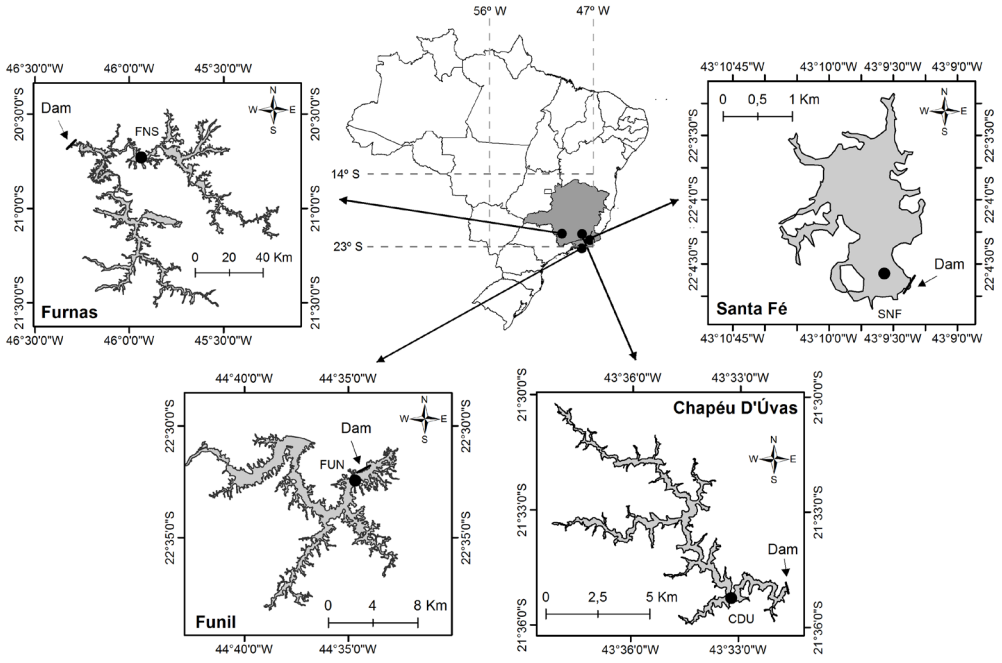

Figure 1. Study area map showing the sampled reservoirs. The gray area indicates the shape of the reservoirs as well as Minas Gerais and Rio de Janeiro states.

(Varian, AAS240FS, Santa Clara, United States). Glassware was pretreated with a neutral detergent (Merck, 5% for 12h) and HNO_3 (Merck, 5% for 12h), and high purity deionized water was employed for analytical purposes (Milli-Q system, 18.2Ωm). Analytical quality control details can be seen in Quadra *et al., (*2019b, 2019c). The limits of detection (LOD) were 0.02 mg L^{-1} (Cu and Cd), 0.2 mg L^{-1} (Cr), 0.7 mg L^{-1} (Pb), 0.05 mg L^{-1} (Zn), and 0.8 mg L^{-1} (Fe).

Allium test

Allium test was developed using *Allium cepa* seeds cv. Baia Periforme (Feltrin), according to Quadra *et al.,* (2019c). The sediment elutriates were directly exposed to the Allium test without filtering. Petri dishes with 3 mL of sediment elutriate solution were prepared, and six pre-germinated Allium seeds were placed in each Petri dish for 24 h, in triplicates. The roots were then fixed in ethanol: acetic acid (3:1) solution for more 24 h. After that, the roots were hydrolyzed for 20 min in 5N HCl, and six meristematic regions per replicate were smashed using the slide and coverslip. The slides were then stained with Giemsa (5% for 2 min), and 2000 cells per slide were counted under an optical microscope (400x magnification). We assessed the mitotic index, phase indexes, and frequency of chromosomal aberrasions taking into account the number of observations divided by the total number of cells counted (Fiskesjo, 1985; Athanásio *et al.,* 2014; Quadra *et al.,* 2019c). The chromosomal aberrations accessed were bridges, chromosomal breaks, c-metaphases, later segregation, chromosomal loss, and micronuclei.

Data treatment

Shapiro-Wilk and Levene tests were performed to confirm normality and variance homogeneity. Then, statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Dunnett's test using $p < 0.05$ as a significance threshold.

RESULTS AND DISCUSSION

The mitotic index was reduced after exposure to all elutriate samples (treatments) compared to NC, except in FNS (ANOVA followed by Dunnett, $p < 0.05$; Table 1). The highest cell inhibition was found in FUN, while in FNS presented the lowest inhibition. Prophase index increased after exposure to all elutriate samples comparing to NC, except in FNS (ANOVA followed by Dunnett, $p < 0.05$). Metaphase index increased significantly in SNF treatment, while anaphase index decreased considerably in SNF and FUN (ANOVA followed by Dunnett, $p < 0.05$; Table 1).

The percentage of chromosomal aberrations significantly increased after exposure to all elutriate samples compared to NC, except in FNS (ANOVA followed by Dunnett, $p < 0.05$, Table 2). The highest increase in the percentage of aberrations was found in SNF and FUN. Fragments were predominantly observed in SNF, while chromosomal bridges in CDU. Aneugenic alterations were mostly found in SNF and FUN. Comparing to NC, only SNF showed a significant result for c-metaphase (ANOVA followed by Dunnett, $p < 0.05$, Table 2). Sticky chromosomes and condensed nuclei were the most frequent changes observed (Table 2). Micronuclei frequency was also higher in all treatments comparing to NC, except in FNS (ANOVA followed by Dunnett, $p < 0.05$, Figure 2). Representative images of the alterations observed during the experiment are shown in Figure 3.

Concentrations of Cr, Cd, and Pb were below their LOD in all sampling sites. The highest Cu concentration was found in FUN, Zn in SNF, and Fe in FNS (Table 3). The presence of metals in the reservoirs is probably associated with anthropogenic activities from the watersheds, resulting in agricultural, urban and industrial effluents discharged in the water resources (Quadra *et al.,* 2019b). FUN is inserted in a profoundly altered watershed that receives an important contribution from domestic and industrial effluents (Neves *et al.,* 2016). SNF reservoir is located downstream of the Juiz de Fora city, and metal contamination can be associated mainly with domestic and industrial effluents from the town (Quadra *et al.,* 2019b). FNS contamination may also be related to industrial and domestic effluents, including the increasing tourism in the region, but agriculture is probably the major source of contaminants to the reservoir (Nogueira *et al.,* 2009; Cavalcanti *et al.,* 2014).

Therefore, the cytogenotoxic effects observed may be related to Fe, Cu, and Zn concentrations found in the reservoirs. The elutriate samples from FUN showed the highest decrease of mitotic and anaphase indexes and the highest increase of micronuclei, fragments, multipolarity, and condensed nuclei. At the same time, FUN showed the highest Cu concentration, which may be related to the cytogenotoxic effects observed. Palacio *et al.,* (2005) showed that Cu around 0.01 mg L^{-1} was able to cause a reduction of 40% of the mean root length of *A. cepa.* The concentrations found in FUN elutriates were higher than the tested by Palacio *et al.,* (2005). Other studies also showed Cu genotoxic effects in plants, although the concentrations tested are higher than those found in the elutriates from the studied reservoirs. For example, Yıldız *et al.,* (2009) found that *A. cepa* exposed to Cu around 0.6 mg L^{-1} showed

Table 1. Mitotic and phases indexes in meristematic cells of *Allium cepa* after exposure to sediment elutriate solutions from the Brazilian reservoirs.

Treatments	M_i (%)	Pro_i (%)	Met_i (%)	Ana_i (%)	Tel_i (%)
NC	7.99	41.37	30.93	16.4	11.3
FNS	7.01	42.78	30.11	15.67	11.44
CDU	5.43*	44.55*	28.9	17.3	9.3
SNF	4.63*	38.39*	37.34*	11.56*	12.71
FUN	3.85*	48.73*	27.45	11.34*	12.48

*: Significantly different from NC (ANOVA followed by Dunnett, $p < 0.05$). M_i (mitotic index); Pro_i (prophase index); Met_i (metaphase index); Ana_i (anaphase index); Tel_i (telophase index); NC (negative control); FNS (Furnas); CDU (Chapéu D' Uvas); SNF (Santa Fé); FUN (Funil).

Table 2. Frequency (%) of chromosomal/cellular aberrations in meristematic cells of *Allium cepa* after exposure to sediment elutriate solutions from the Brazilian reservoirs.

Chromosomal/cellular alterations		**Treatments (negative control and sediments)**				
NC		NC	FNS	CDU	SNF	FUN
Clastogenic effects	Fragments	0.86	0.96	1.85*	1.03	2.57*
	Bridges	1.49	1.6	4.81*	4.70*	3.96*
	C-metaphase	2.34	1.73	2.18	4.54*	2.74
Aneugenic effects	Chromosome loss	1.11	1.38	0.96	0.9	1.35
	Multipolarity	1.03	1.03	1.32	1.51*	1.52*
	Later segregation	1.38	1.38	1.66*	2.02*	1.52*
Toxic effects	Sticky chromosomes	4.98	6.92*	7.52*	12.39*	10.98*
	Condensed nuclei	4.32	5.23*	8.90*	9.39*	10.34*
-	Total percentage of abnormalities	6.55	7.45	10.71*	12.71*	12.59*

*: Significantly different from NC (ANOVA followed by Dunett, $p < 0.05$). NC (negative control); FNS (Furnas); CDU (Chapéu D' Uvas); SNF (Santa Fé); FUN (Funil).

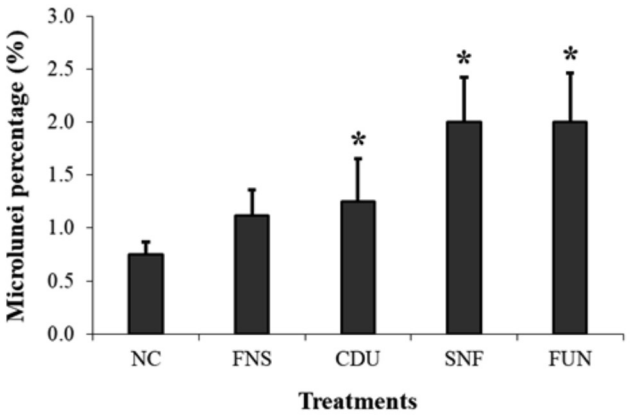

Figure 2. Micronuclei formation in meristematic cells of *Allium cepa* after exposure to sediment elutriate solutions from the Brazilian reservoirs. *: Significantly different from NC (ANOVA followed by Dunnett, $p < 0.05$). NC (negative control); FNS (Furnas); CDU (Chapéu D' Uvas); SNF (Santa Fé); FUN (Funil).

Table 3. Trace metal concentrations of sediment elutriate solutions from the Brazilian reservoirs used in *Allium cepa* exposure experiments.

Treatments	Cu (mg L^{-1})	Zn (mg L^{-1})	Fe (mg L^{-1})
NC	< LOD	0.3	0.4
FNS	< LOD	0.3	75.5
CDU	0.03	0.6	15.1
SNF	< LOD	2.4	51.1
FUN	0.04	0.7	58.7

< LOD (below limit of detection); Cu (copper); Zn (zinc); Fe (iron); NC (negative control); FNS (Furnas); CDU (Chapéu D' Uvas); SNF (Santa Fé); FUN (Funil).

a reduction in mitotic index and increasing the frequency of chromosomal aberrations, inducing DNA damage. Moreover, Muccifora & Bellani *et al.,* (2013) found that *Vicia sativa* seeds exposed to 0.2 mg L^{-1} affected radicle cell division and elongation.

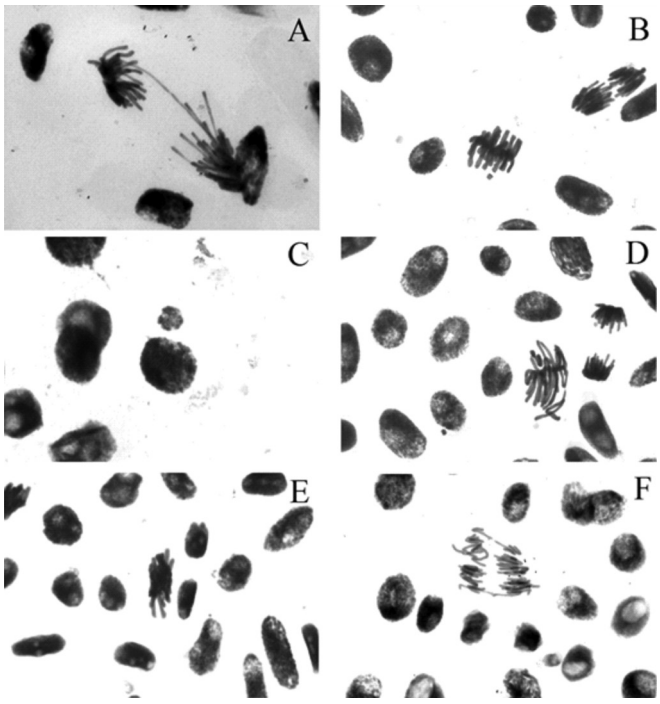

Figure 3. Examples of chromosomal aberrations observed in *Allium cepa* meristematic cells exposed to sediment elutriate solutions from the Brazilian reservoirs. A (bridge); B (fragment); C and D (micronucleus with different size); E (chromosome adherence); F (abnormal segregation).

Similarly, elutriate samples from SNF showed the highest Zn concentration and the highest decrease of prophase index and increase of micronuclei, metaphase index, c-metaphase, later segregation, sticky chromosomes, and the total percentage of chromosomal aberrations. Akbaba (2020) found that Zn concentrations around 20 mg L^{-1} were able to cause genotoxic effects on the *A. cepa*, increasing the frequency of chromosomal aberrations. The tested Zn concentrations were higher than those found in the elutriates from studied reservoirs. However, the high concentration of Fe found in elutriate samples from

FUN and SNF may cause synergetic effects and increase cytogenotoxicity properties. Indeed, previous studies have already documented the synergetic effects of the Zn and Fe mixture (Godet *et al.,* 1996; Olorunfemi *et al.,* 2013). Moreover, Paranaíba *et al.,* (2020) performed an experiment exposing sediments to drought and comparing them to permanently flooded sediments. The authors found a significant mitotic index reduction and aneugenic alteration increase using the Allium test in the sediments exposed to drought, which also showed a higher release of metals (Zn, Fe, Mn, and Cd) to the water column. The maximum concentrations reported in the study were 0.9 mg L^{-1} for Zn and 10.2 for Fe mg L^{-1} (Paranaíba *et al.,* 2020). The Zn concentrations found in SNF elutriates and the Fe concentrations found in all elutriate samples are higher than those reported by the experimental study. Therefore, it is more likely that the elutriate mixture of metals and other contaminants, including those that were not even measured in this study, caused the detected genotoxic effects.

The results from our study show that the input of chemicals may represent a risk to aquatic ecosystems, mainly considering the potential mixture effects, which are, overall, understudied. Biological assays are key tools to understanding the mixture effects in aquatic ecosystems (Magdaleno *et al.,* 2008; Barbério *et al.,* 2009). The Allium test, for example, was already used to evaluate water and sediment quality in different countries (Geras'kin *et al.,* 2011; Matsumoto *et al.,* 2004; Barbério *et al.,* 2009; Athanásio *et al.,* 2014). Therefore, our findings reinforce the applicability of the Allium test to warn about genotoxic chemicals present in freshwater ecosystems.

CONCLUSIONS

Cytogenotoxicity effects were observed using the Allium test after exposure to sediment elutriate solutions from Brazilian reservoirs. The results are probably related to the Cu, Zn, and Fe concentrations found in the sediment elutriates, but more likely that the mixture of metals and other contaminants, including chemicals not measured in the present study, are causing the detected genotoxic effects. Therefore, the Allium test was a sensitive tool for alerting the occurrence of genotoxic contaminants in sediment elutriates simulating resuspension to the water column in aquatic ecosystems.

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