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Assessment of the genotoxicity of sediment elutriates from an aquatic ecosystem on *Allium cepa*: Limache stream in central Chile

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Abstract The aim of this study was to assess the genotoxic effects of sediment elutriates of an aquatic ecosystem. Sediment samples were taken from Limache stream, located in central Chile. The tests were carried out on sediment elutriates. Genotoxicity was determined by bioassay with *Allium cepa*. The percentage of germination, root growth, mitotic index, and frequency of chromosome aberrations were determined. The results show a significant increase in chromosome aberrations and decrease of the mitotic index in *Allium cepa* in all the sediment elutriates compared to the control. No significant differences were observed in the percentages of germination or root growth among the sediment elutriates. A negative correlation was found between the mitotic index and chromosomal aberrations. In conclusion, genotoxic variables are more sensitive than growth variables. The sediments contain chemical agents in bioavailable concentrations that produce genotoxic effects. *Allium cepa* test proved to be a sensitive indicator of genotoxic contaminants in sediment elutriates of the Limache stream in central Chile.

Keywords Toxicity · Bioassays · Risk · Micronuclei

Introduction

Rivers and streams present growing contamination problems produced by anthropic activities (mining and agriculture) (Batista et al., 2016; Ibeh & Umeham, 2018; Silveira et al., 2018). The sediments of these aquatic ecosystems have environmental relevance due to their capacity to accumulate chemical agents such as metals and pesticides in concentrations greater than those found in the water (García-Alonso et al., 2015; Geffard et al., 2007; Ortíz-Ordoñez et al., 2016; Zhang et al., 2016). Chemical agents can be released to surface water from the sediments through biological and chemical processes and provoke toxic effects in aquatic organisms (Xiao et al., 2019; Mondal et al., 2017). A relation has been found between metal concentrations in sediment and the aquatic fauna as a result of bioaccumulation and biomagnification. Due to this, there is growing interest in evaluating the toxicity of sediments of aquatic ecosystems (Biruk et al., 2017; García-Alonso et al., 2015; Guimarães et al., 2019; Zhang et al., 2016). There has also been an increase in toxicity studies of elutriates, which are the aqueous fraction of the sediments (Garmendia et al., 2009; Haring et al., 2010; Jardim et al., 2008; Ortíz-Ordoñez et al., 2016;

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Ramírez & Mendoza, 2008). Jardim et al. (2008) found bioavailable metals (Cu, Zn, Cr, Cd, Pb) in sediments of the Río Corumbataí in Brazil in concentrations that caused acute and chronic toxicity in *Daphnia magna* and *D. similis*. Míguez et al. (2010) reported toxicity associated with metals and organic compounds with elutriates of sediments of the Río Uruguay in larvae of *Pimephales promelas*, neonates of *Ceriodaphnia dubia* and in *Phosphobacterium leioghnati*. Morais et al. (2013) found chronic toxicity in sediments of the Río Ribeira de Iguape in Brazil—where there was mining activity in the area from 1918 to 1995—to *Daphnia similis*; however, they did not find toxicity of individuals exposed to superficial water of the same river. This study suggests that, in spite of being in a restoration process, the recovery of sediment quality is not as great as that of the water, due to the accumulation capacity of the chemical agents present in the sediments.

Determining the exposition to the individual concentrations of chemical agents is not enough by itself to predict their toxic effects, since there are environmental factors (i.e., pH, organic material, redox, etc.) that affect their bioavailability. Chemical agents in complex mixtures such as surface water, sediments, and elutriates may also have additive, antagonistic, or synergic interactions that affect their individual toxicity. For this reason, bioassays are used to evaluate the toxicity of these complex mixtures, since the response of the organisms measures the overall effect of the chemical agents present in the mixture (Barbosa et al., 2010; Castillo, 2004; Hassan et al., 2016). *Allium cepa* has been used to evaluate effects of chemical agents, because it is considered as an efficient bioindicator of genotoxicity (Wijeyaratne & Wadasinghe, 2019). The bioassay with *Allium cepa* is an internationally validated method to evaluate the genotoxic potential of chemical agents in the environment (Batista et al., 2016; Silveira et al., 2018; Tedesco et al., 2017). It is a rapid and low-cost method that has demonstrated high sensitivity to chemical agents and good correlation with other bioassays. *A. cepa* also has good karyotype characteristics, since it has a low number of chromosomes ($2n = 16$) that are easily visualized under a microscope, which allows uncomplicated identification of chromosome alterations (Rainho et al., 2010; Bianchi et al., 2011; Biruk et al., 2017; Silveira et al., 2017).

Limache stream, located in the Región de Valparaíso in central Chile, is considered a category I priority site in the Regional Strategy for Biodiversity Conservation (CONAMA & PNUD, 2005); its water is stored in the Los Aromos reservoir, which is one of the main sources of water for human consumption. It is influenced by different anthropogenic activities, especially agricultural activity and the effluent discharge from a treatment plant of residual urban water (Córdova et al., 2009; Fuente et al., 2014). Agricultural activities may generate diffuse contamination due to the use of pesticides. These may reach water bodies due to runoff, infiltration, and erosion of soils where they were applied and by atmospheric transport, contaminating water and sediments (Hernándes-Antonio & Hansen, 2011). Although there have been studies of environmental quality of the water in the Limache stream, the bioavailability of potentially genotoxic agents in sediments is unknown. This study postulates that elutriates of the sediments in the Limache stream contain a complex mixture of chemical agents in bioavailable concentrations that provoke genotoxicity in *A. cepa*. The aim of this study was thus to evaluate the genotoxic effects of sediment elutriates of the Limache stream on *Allium cepa*.

Materials and methods

Sampling

The sampling was carried out in autumn, 2018. Five sampling sites were selected in the Limache stream (Fig. 1). The site selection criterion was the level of anthropization of the sector (agricultural, urban, and industrial). The sediments were extracted superficially, three replications per station and were transported in plastic containers and stored at −4 °C.

Sample preparation

Sediment elutriates were obtained according to Ramírez and Mendoza (2008). Sediment samples were dried at 60 °C to remove all humidity; then, the dry material was placed in 14-mL centrifuge tubes with distilled water in a 1:3 v/v relation. The tubes were agitated in a stirring rack for 1 h to generate a homogeneous mixture, then allowed to settle in a

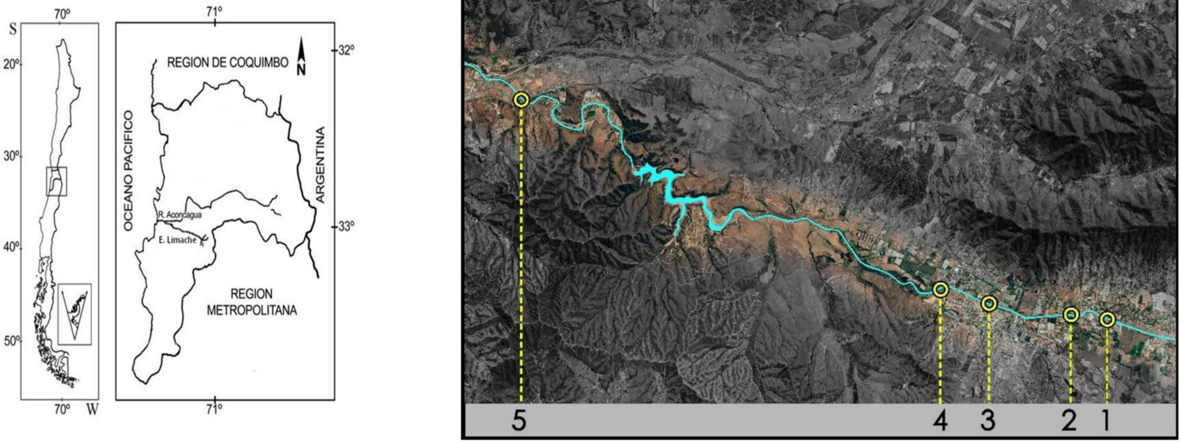

Fig. 1 Location of sampling sites in the Limache stream. The sites had the following anthropic activities: (1) agricultural, (2) urban and agricultural, (3) road and urban, (4) agricultural, and (5) tourism

refrigerator. Before performing toxicity tests, tubes were centrifuged for 15 min at 3000 rpm, and the aqueous supernatant (elutriate) extracted without re-suspending the sediment.

Characterization of sediment and elutriates

The organic matter in the sediments was determined by weight loss after heating at 500 °C for 4 h. pH and redox potential were measured in situ. The granulometry of the sediments was determined using six-sieve magnetic stirrer with the following aperture diameters: 4750 µm (No. 4), 2000 µm (No. 10) for medium-coarse sand, 425 µm (No. 40) for medium sand, 250 µm (No. 60) for fine sand, 106 µm (No. 140) for medium-fine sand, and 75 µm (No. 200) for silt, and analyzed with the GRADISTAT software. Phosphate and nitrate in the elutriates were determined by colorimetry with a Hanna spectrophotometer model HI83399-02.

Bioassay with *Allium cepa*

Commercial seeds of *Allium cepa* were used from the same variety and lot. Seeds were scarified with 5% sodium hypochlorite for 15 min and washed with distilled water for 10 min. We prepared Petri plates with filter paper disks of the same diameter as the plates. Three milliliter distilled water was added, distributing it homogeneously and taking care that there were no air bubbles below the filter paper. Twenty seeds per plate were used for the analysis of germination and growth and 5 per plate for the genotoxic analysis, separated to insure adequate growth. Seeds were incubated in an environment chamber at room temperature in total darkness until the roots reached a length of 2 mm. As_2O_3 at a concentration of 10 mg/L was used as positive control (PC) and distilled water as negative control (NC) (Ramírez & Mendoza, 2008; Silveira et al., 2018).

Determination of germination percentage and relative growth index

Germination percentage was estimated according to Njoku et al. (2008), germination index (%G) and relative growth index (RGI) according to Biruk et al. (2017). When radicle length reached 2 mm, seedlings were treated with 3 mL of test solution (elutriates). Root length was measured every 24 h for five days. RGI and $\%G$ were calculated using the formulas:

$$\mathrm{RGI} = \frac{\mathrm{RLI}}{\mathrm{RLC}}$$

$$\%G = \frac{RLI \times GSI \times 100}{RLC \times GSC}$$

RLI is the radicle length of seeds treated with elutriates, RLC is the radicle length of seeds of the negative control, GSI is the number of germinated seeds after treatment with elutriates, and GSC is the number of germinated seeds in the negative control. RGI values were placed in three categories according to the toxic effects observed:

- Inhibition of radicle elongation (I): $0 < \mathrm{RGI} < 0.8$
- No significant effects (NSE): $0.8 \leq \mathrm{RGI} \leq 1.2$
- Stimulation of radicle elongation (S): $\mathrm{RGI} > 1.2$

Determination of genotoxicity

To determine genotoxicity, seedlings germinated to 2-mm radicle length were treated with 3 mL of the test solution (elutriates) for 48 h. Then, roots were harvested and fixed in Carnoy's solution, and stored at 4 °C for 24 h. Roots were hydrolyzed in HCl for 9–10 min at 60 °C to soften the tissues. Roots were stained with 2 drops of lacto-propionic orcein. The meristematic region, the final 2 mm of the root was cut, placed on a slide and carefully squashed with the cover slip with a drop of 2% acetocarmine. Finally, the cover slip was sealed.

To estimate the percentage of mitotic phases and the mitotic index, we observed meristematic cells. Six slides were prepared for each trial per elutriate, and 1000 meristematic cells were observed under a microscope per slide. The different stages of mitotic division (prophase, metaphase, anaphase, and telophase) were recorded. The mitotic index was calculated for each as

$$\mathrm{MI} = \frac{\text{Total cells in mitosis}}{\text{Total cells counted}} \times 100$$

We also quantified the following chromosome aberrations (CA): micronuclei (MN), nuclear buds, anaphase bridges, chromosome fragmentation, and C-mitosis in 1000 cells. To estimate genotoxicity, we obtained the frequency of chromosome aberrations and micronuclei, using the following formula:

$$\text{Frequency MN or CA} = \left(\frac{A}{B}\right) \times 100$$

where A is the number of cells with the parameter analyzed (MN or CA) and B is the total mitotic cells analyzed (Bianchi et al., 2011; Biruk et al., 2017; Silveira et al., 2018).

Statistical analysis

ANOVA analysis followed by Tukey tests was performed to compare variables between sediment elutriates, with a significance level of $p < 0.05$. Pearson correlation was used to relate variables. The statistical procedures and tests were performed in the software Minitab 17.

Results and discussion

Table 1 shows that the pH was alkaline; similar values were reported by García-Alonso et al. (2015) in the Segura River in Spain. This alkalinity could be

Table 1 Physical and chemical parameters and granulometry of the sediments and elutriates

Parameter	Unit	Sampling sites				
		1	2	3	4	5
pH		8.9	8.8	8.8	9.0	8.8
Redox	mV	234	229	195	145	114
Organic matter	%	9.7	14.4	31.7	1.8	9.8
$\text{Phosphates}_{\text{elutriates}}$	mg/L	4.7	1.3	10.5	< LD	< LD
$\text{Nitrates}_{\text{elutriates}}$	mg/L	4.1	2.7	< LD	< LD	0.9
Gravel	%	5.5	2.2	6.1	1.4	9.7
Sand	%	94.5	97.8	93.9	98.6	90.3
Fine gravel	%	0.7	0.5	1	0.1	4.4
Very fine gravel	%	4.8	1.7	5.2	1.2	5.3
Medium sand	%	71	57.6	52.4	84	56.5
Fine sand	%	4.4	0.4	1.8	3.6	5.3
Very fine sand	%	19.1	39.7	39.7	11.1	28.4

< *LD* below detection limit

to the presence of bicarbonates and the geological characteristics of the study area. The redox potential suggests a low tendency to formation of metal oxides, favoring the solubility of metal salts in the interstitial water of the sediments; similar values were reported by Gaete et al. (2017). The greater amount of organic matter in the elutriate sediments of some sampling sites is due to the sedimentation of vegetable matter (Cogua et al., 2012), which may be favored by a decrease in flow, which in the study area varied from 0.01 to 0.76 m^3/s (Carreño et al., 2018; Córdova et al., 2009). This parameter is important since the amount of organic matter in the sediment influences the adsorption of metals in the aquatic ecosystem (Guimarães et al., 2019).

The concentrations of phosphates and nitrates, 10.5 mg/L and 4.1 mg/L, respectively, suggest that they are an important source of the internal load of nutrients into the water body, which can accelerate the eutrophication process (Villalobos et al., 2003). The variation in nutrient concentrations among sampling sites may be associated with changes in soil use (Fuente et al., 2014; Córdova et al., 2009. The sampling sites with greatest anthropic activity were sites 1, 2, and 3, which showed the highest values for all parameters. The lower concentrations of nutrients in the other sites below the detection limit could be due to the dilution effect of the Lliu Lliu tributary stream.

Germination percentage was greater than 85% for all sediment elutriates. No significant differences were found in germination percentage, RGI, or %G between the sediment elutriates and the negative control (Table 2). This may be due to the nutrients favoring development of the seedlings and plant growth, inhibiting the effects of chemical agents. It may also be due to the low sensitivity of *Allium cepa* to some chemical elements; Iannacone and Alvariño (2005) reported that vascular plants, especially *Allium cepa* and *Raphanus sativus*, have low sensitivity to heavy metals. Our results are similar to those of Biruk et al. (2017), who used seeds of *Lactuca sativa* exposed to different extracts of sediments of the Río Matanza—Riachuelo, Argentina.

There was no significant difference in radicle length between the negative control and seedlings exposed to sediment elutriates (Table 2). This could be due to an antagonistic interaction between chemical agents, which inhibits the individual toxicity of the chemical agents in these complex mixtures. Iannacone and Salazar (2007) found that the binary mixtures Cd/Hg and Hg/Pb had antagonistic interactions on *Chironomus calligraphus*. Gaete and Chávez (2008) assessed the toxic effect of binary mixtures of copper, zinc, and arsenic on *Daphnia obtusa*, finding that certain combinations of the binary mixtures had antagonistic interactions, such as the Zn/Cu mixture.

Sediment elutriates did not affect radicle length (Table 2). This suggests that this response variable is not sensitive to the chemical agents present in the mixtures. This agrees with the report of Silveira et al. (2017), who exposed seeds of *Allium cepa* and *Lactuca sativa* to different chemical agents (SPL, cadmium, atrazine, and metal methanosulfonate). They did not find significant effects on radicle length in *A. cepa*, but did find differences for *L. sativa*; they considered *A. cepa* to be an insensitive model for phytotoxicity tests.

A decrease in the mitotic index was found for all sediment elutriates compared to the control (Table 3). There was a significant increase in chromosome aberrations, dominated by micronuclei (MN) and C-mitoses (Table 3, Fig. 2). The sediment elutriates

Table 2 Mean germination percentage (±) standard deviation, inhibition percentage, germination index (%*G*), relative growth index (RGI), and radicle length in seeds of *Allium cepa* exposed to sediment elutriates

Sampling sites	% Germination	% Inhibition	Germination index (%G)	Relative growth index (RGI)	Radicle length 120 h
NC	96.7 ± 2.9	3.3 ± 2.9	100	1	3.2 ± 0.3
1	96.7 ± 5.8	3.3 ± 5.8	87.3 ± 12.4	0.9 ± 0.1	2.8 ± 0.1
2	88.3 ± 2.9	11.7 ± 2.9	93.7 ± 19.8	1.0 ± 0.2	3.2 ± 0.4
3	86.7 ± 2.9	13.3 ± 2.9	95.7 ± 11.3	1.1 ± 0.1	3.4 ± 0.4
4	90 ± 0	10 ± 0	98.6 ± 18,4	1.1 ± 0,2	3.4 ± 0.4
5	85 ± 13.2	15 ± 13.2	90.1 ± 55,6	1.0 ± 0.4	3.0 ± 1.0

NC negative control

Table 3 Mean ± standard deviation of the frequency of chromosome aberrations and mitotic index in radicles of *Allium cepa* exposed to sediment elutriates. Different letters indicate significant differences ($p < 0.05$) between the sampling sites and negative (NC) and positive (PC) controls

Sampling sites	Micronuclei	Nuclear buds	Anaphase bridges	Chromosome fragmentation	C-mitosis	Total aberrations	Mitotic index
PC	1.3 ± 0.2^{BC}	0.2 ± 0.0	0.3 ± 0.2	0.5 ± 0.1	1.9 ± 0.8	4.1 ± 0.8^{B}	5.4 ± 0.2^{B}
NC	0.4 ± 0.0^{c}	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.9 ± 0.1^{A}	11.6 ± 0.8^{A}
1	3.5 ± 0.7^{AB}	0.8 ± 0.1	0.4 ± 0.2	0.8 ± 0.1	1.0 ± 0.1	6.5 ± 0.2^{B}	7.0 ± 1.4^{B}
2	2.0 ± 1.3^{AB}	0.6 ± 0.4	0.3 ± 0.3	0.4 ± 0.3	0.4 ± 0.2	4.5 ± 1.0^{B}	5.7 ± 1.7^{B}
3	1.6 ± 0.6^{ABC}	0.4 ± 0.1	0.5 ± 0.3	0.4 ± 0.1	1.2 ± 0.4	4.1 ± 0.5^{B}	7.4 ± 1.6^{B}
4	2.4 ± 0.7^{ABC}	0.7 ± 0.8	0.4 ± 0.2	0.2 ± 0.1	0.7 ± 0.3	4.3 ± 1.6^{B}	4.4 ± 0.8^{B}
5	3.6 ± 1.3^{A}	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.2	0.6 ± 0.1	5.1 ± 1.6^{B}	5.3 ± 0.9^{B}

from sampling sites with more anthropic intervention had higher frequencies of MN. This may be related to the pesticides and metals from agricultural activities in the Limache stream watershed. Similar result was reported by García-Alonso et al. (2015), who found that the sediments of lower sectors of the Segura River basin caused genotoxicity in *Saccharomyces cerevisiae*. This suggests that micronucleus frequency allows detecting changes in environmental conditions and that there are chemical agents in sediment elutriates in bioavailable concentrations that produce genotoxic effects. This is similar to the report of Bianchi et al. (2011), who exposed seedlings of *A. cepa* to samples of the water of the Río Monjolinho in Brazil, which has high levels of heavy metals; they found a significant presence of chromosome aberrations. The frequency of MN may be increased by the expulsion of extra genetic material in C-mitosis cells. It may also be the result of structural changes between sister chromatids or different chromosomes due to breaks and terminal deletions, or due to the adherence of ribosomes or nucleoli. At the end of cell division, chromosome bridges may break and produce fragments that are converted to MN in the daughter cells (Bianchi et al., 2016, 2011). The mitotic index and chromosome aberrations were negatively correlated ($r = -0.67$; $p < 0.05$), but there was not a significant correlation between radicle growth (cm) and the expected mitotic index. Similar results were reported by Silveira et al. (2017). In conclusion, the sediments contain chemical agents in bioavailable concentrations that provoke genotoxic effects in *A. cepa*. Genotoxic

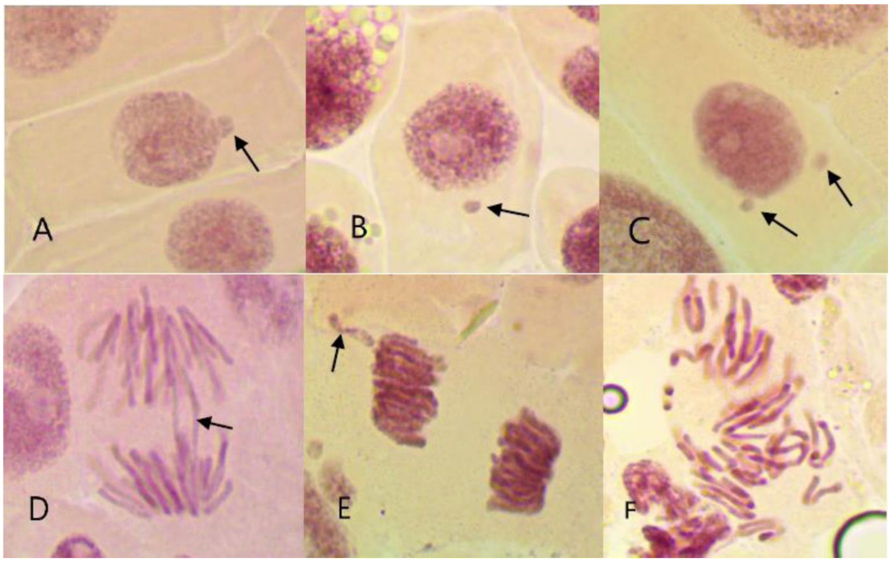

Fig. 2 Chromosome aberrations found. **A**: Nuclear bud; **B** and **C**: Micronuclei; **D**: Anaphase bridge; **E**: Chromosome fragmentation and **F**: C – Mitosis

variables are more sensitive than growth variables. *Allium cepa* test proved to be a sensitive indicator of genotoxic contaminants in sediment elutriates of the Limache stream in central Chile.

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interest The authors declare no conflict of interest.

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