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Full Paper

Cytotoxicity, Genotoxicity and Mutagenicity of Aluminum, Manganese and Lead in Meristematic Cells of Root *Allium cepa*

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Abstract: Metals are chemical contaminants that are not normally biodegradable and accumulate in living organisms. In addition, they may present toxic effects to plants and affect human health causing mutations and DNA damage of exposed organisms. The objective of this study was to evaluate the cytotoxicity, genotoxicity and mutagenicity of the aluminum, manganese and lead using *A.cepa* test. Solutions in three concentrations with the metals and controls were prepared for the genetic analyzes. The seeds of *A. cepa* were exposed to germination, and evaluation of cytotoxicity, genotoxicity and mutagenicity. The results showed that the concentrations of the metals did not interfere in the growth of the radicle, but the increase in the concentration of the metals was directly proportional to the increase of the number of seeds germinated. For the mitotic index, it was observed that the increase in aluminum concentration provided the increase of cells in cell division, but for manganese and lead there was reduced. The metals at the concentrations evaluated did not present genotoxic and mutagenic potential for meristematic cells of *A. cepa*. The *A. cepa* test it showed to be effective for performed analyzes, however, further studies are needed using other biological assays.

Keywords: average root growth; ecotoxicology; germination; metals

1. INTRODUCTION

Anthropic actions related to the dischard of industrial, agricultural and domestic sewage effluents into streams and rivers are promoting the contamination of these by metals [1]. The existence of metals in aquatic ecosystems arouses interest in the scientific community, especially in relation to the effects that can cause human health. Some metals at low concentrations are considered essential elements because they participate in several physiological processes [2, 3], however, in high concentrations they present risks for both ecosystems and human health [4].

Metals are non-biodegradable chemical contaminants that accumulate in living organisms [5, 6]. They present toxic effects in plants, microorganisms, and may affect human health causing the appearance of mutations and DNA damage of the exposed organisms [7, 8]. It's assumed that the metals found in soil and in aquatic environments can be derived from agricultural activities, anthropic or by the geological characteristics of the soil itself.

Among the metals, we can highlight the aluminum, manganese and lead that can come from effluents of steel and automobile industries and processes of fertilization of the soil. Besides that they can be found in utensils, such as tools, stacks and batteries, which in most cases do not present appropriate discard, contaminating soil, air and water. Due to the toxic potential of these metals it is relevant to evaluate their cytotoxicity, genotoxicity and mutagenicity by bioassays, which are important tools for the evaluation of the responses of organisms when exposed to environmental contaminants [9].

The test of *Allium cepa* is a bioassay used for environmental assessments, because this specie presents large chromosomes in a small number, which facilitates its analysis, besides the ease of cultivation at any time of the year [10]. In addition, the *A. cepa* has sensitivity for identification of cytotoxicity, genotoxicity and mutagenicity in meristematic cells

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originating from the root tissue [11, 12].

In this context, the objective of this study was to evaluate the cytotoxic, genotoxic and mutagenic effects of aluminum, manganese and lead using the test *A. cepa*.

2. MATERIAL AND METHODS

Solutions with the metals aluminum, manganese and lead were prepared using the monoelementary stock standards of 1000 mg L^{-1} (SpecSolo, SEM-682, USA) of each metal. The Ordinance nº 2914/2011 [13] of the Ministry of Health recommends that for the metals aluminum (Al), manganese (Mn) and lead (Pb), the maximum allowed values are 0.2; 0.1 and 0.01 mg L^{-1}, respectively. Our experiment was conducted with the values 0.1, 0.2 and 0.3 mg L^{-1} for Al, 0.05, 0.1 and 0.2 mg L^{-1} for Mn, and 0.005, 0.01 and 0.05 mg L^{-1} for Pb. As a negative control was used distilled water and as a positive control was used the Trifuralin® (0.84 ppm). The solutions were prepared at the time of use.

Allium cepa Test

Sixty seeds of *A. cepa* (Periform Bay variation) were placed in Petri plates and 3 mL of each solution to be tested (aluminum, manganese and lead) were added for germination (Fig. 1). The seeds were exposed to the treatments under controlled conditions, with temperatures of 23±3 ºC per 96 hours using a germination chamber (Quimis).

The germinated seeds were counted manually and the roots sizes were measured with digital caliper (Digmess). Then the roots were collected and fixed in Carnoy 3:1 (ethanol:acetic acid). The microscope slides were prepared as described in [14], stained with the Schiff reagent based on Feulgen method for 1h30min. For each treatment, 10 slides were prepared with *A.cepa* root meristem, 500 cells were counted from each slide, totaling 5000 cells per treatment. The slides were analyzed in optical microscope with magnification of 400x.

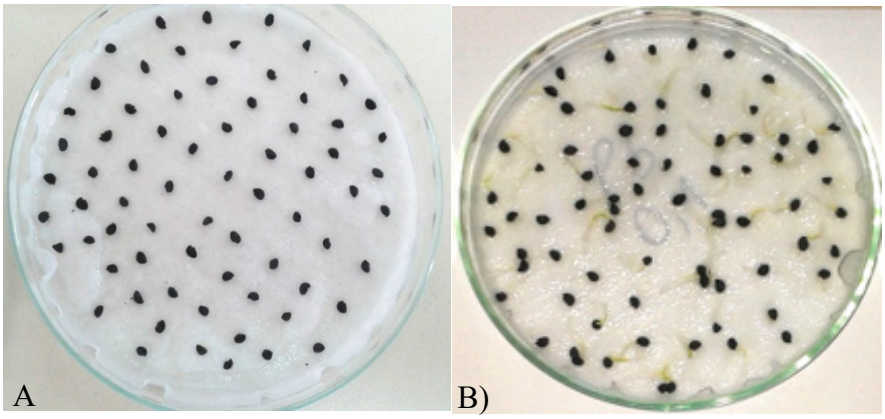

Figure 1. Demonstration of plaques with seeds of *Allium cepa* arranged in sheet of filter paper. (A) Moistened seeds with the solution for germination (B) Germinated seeds after 96 h.

Cytotoxicity analysis

The percentage of germination (%GR) and mean root growth (MRG) were used to determine the cytotoxic potential of solutions of metals Al, Mn and Pb at different concentrations. Mitotic index (MI) and cell death index (CDI) were also evaluated. The indices were calculated using the formulas described below:

$$\%GR = \frac{\text{number of seeds germinated in the test solution}}{\text{number of seeds germinated in control}} \text{ X } 100$$

$$MI = \frac{\text{number of cells in cell division}}{\text{number of total counted cells}} \text{ X } 100$$

$$CDI = \frac{\text{nmber of cells in cell death)}}{\text{number of total counted cells}} \text{ X } 100$$

The mean root growth was determined by the average size of all germinated roots for each solution.

Analysis of mutagenicity and genotoxicity

Chromosomal Alterations Index (CAI) was evaluated by counting chromosomal alterations (multipolar anaphases, chromosomal breaks, C-metaphase, adhesions, losses and chromosomal bridges) in the meristematic cells of the root. Micronuclei were counted to evaluate the Mutagenicity Index (MTI). The images of normal cells and with alterations of roots of *A. cepa* were presented in Figures 2 and 3.

The indices were calculated according to the formulas described below:

$$CAI = \frac{\text{number of cells with chromosomal alteration}}{\text{number of total counted cells}} \text{ X } 100$$

$$MTI = \frac{\text{number of cells with micronucleus}}{\text{number of total counted cells}} \text{ X } 100$$

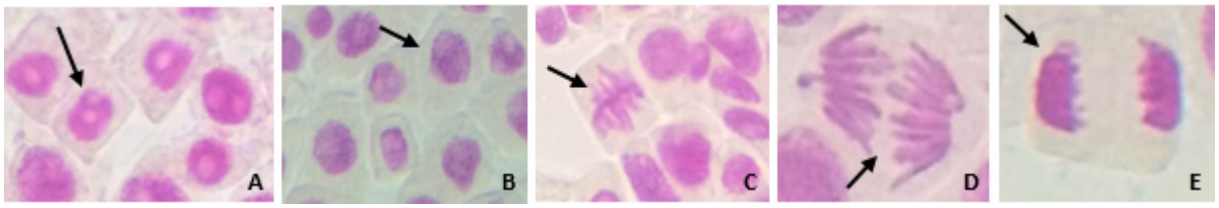

Figure 2. Examples of *Allium cepa* roots cells in normal cell division: (A) interfase; (B) prophase; (C) metaphase; (D) anaphase and (E) telophase at 400x magnification.

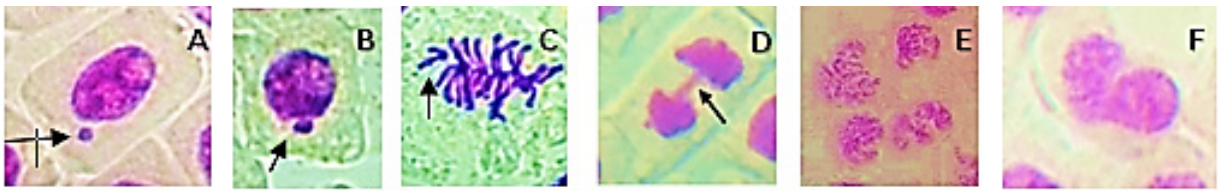

Figure 3. Examples of chromosomal alterations found in *Allium cepa* root cells treated with solutions: (A) micronucleus; (B) budding; (C) chromosomal loss; (D) chromosome bridge; (E) cell death and (F) lobed nuclei in 400x magnification.

Statistical analysis

For the statistical analysis of *A. cepa*, was used the non-parametric Kruskal-Wallis test, with Dunn's posteriori. All tests were performed using the software R [15] platform, with a significance level of 0.05.

3. RESULTS AND DISCUSSION

The Table 1 shows that in none of the concentrations evaluated had statistical difference for RGR (Relative Growth Rate). Therefore, it is possible to infer that at these concentrations, the metals did not inhibit root growth. For the %GR (germination) parameter, the Mn metal showed a difference between CN (negative control) with $Mn_{0.05}$ and $Mn_{0.5}$ and between $Mn_{0.1}$ and $Mn_{0.5}$. For Al, there was a significant difference between $Al_{0.1}$ with the other solutions and between CN and all metal concentrations. The Pb metal showed a significant difference between the CN and all the metal concentrations, between $Pb_{0.005}$ and all other solutions, and between $Pb_{0.1}$ and $Pb_{0.5}$. The results indicated that the increase of the concentration of the metals caused the increase of germination, although it has not caused toxic effect with the capacity to alter the metabolism of the seedling. According to [16], the first effect caused by Al in plants is the reduction of root growth, causing inhibition of stretching, cell expansion and mitotic index.

Mitotic index acts as a tool to analyze the cytotoxic potential of a determined substance [17, 18]. For this index, there was a significant difference between $Al_{0.3}$ and $Al_{0.1}$, $Al_{0.2}$ and positive control, and between $Al_{0.1}$ and negative control (Figure 4a). For manganese, the MI showed a statistical difference between the positive control and $Mn_{0.05}$ and $Mn_{0.1}$, and

between $Mn_{0.5}$ and $Mn_{0.1}$ and the negative control (Figure 4b). The metal lead did not show a significant difference for this index (Figure 4c).The increase in Al concentration was directly proportional with increase in MI. In a study performed with *A. cepa*, the extract of Al in the concentration of 0.0046 g/L presented reduction of the mitotic index in 28% when compared to the control [19]. For the cell death index (CDI), no significant difference was observed between the solutions of the metals, demonstrating that the treatments did not alter the longevity of the analyzed cells (Figure 5 (a), (b) and (c)).

Table 1. RGR (Relative Growth Rate), Germination (%GR), of *Allium cepa* exposed to the concentrations of the metals.

Concentration	RGR	%GR
Mn 0.005	6.04±2.86a	54.28±6.38bc
Mn 0.1	7.75±2.99a	77.14±3.19ab
Mn 0.5	6.59±4.00a	84.28±3.19c
CN	8.32±1.21a	100±0a
Al 0.1	9.87±2.76a	37.14±3.19c
Al 0.2	8.01±3.13a	82.85±6.38b
Al 0.3	8.55±1.84a	84.28±3.19b
CN	8.32±1.21a	100±0a
Pb 0.005	9.88±2.51a	77.14±3.19c
Pb 0.1	8.10±2.52a	84.28±3.19b
Pb 0.5	9.22±2.52a	84.28±3.19b
CN	8.32±1.21a	100±0a

Note: Same letters are not significantly different among the treatments for each metal.

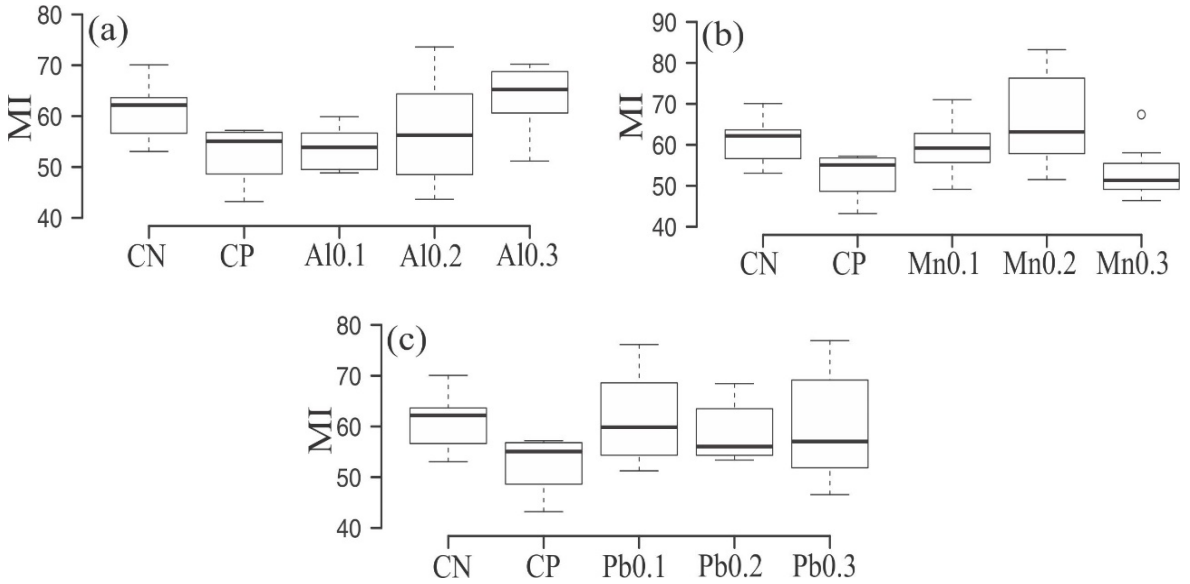

Figure 4. Mitotic index (MI) in *Allium cepa* cells exposed to different concentrations of: (a) Aluminum, (b) Manganese and (c) Lead.

Based on the analysis performed for the index of chromosomal alterations, it is possible to observe a significant difference for Al, Mn and Pb, only between the positive control and the other solutions (Figure 6 (a), (b) and (c)). The chromosomal alterations index analyzes the presence of chromosomal alterations present in the observed cells, they can be observed at all stages of the cell cycle and are described as alterations in the normal structure of a chromosome or in its total number [20].

According to [21], toxicity may not necessarily be associated with a genotoxic effect, because alterations related with the mitotic index, cell death,

germination and root growth indicate cytotoxicity, while genotoxicity is indicated by chromosomal alterations. The results of this study indicated absence of genotoxicity, because no significant difference was detected between the treatments when compared to the negative control. This fact corroborates with the previously mentioned research, although differences have occurred for the mitotic index.

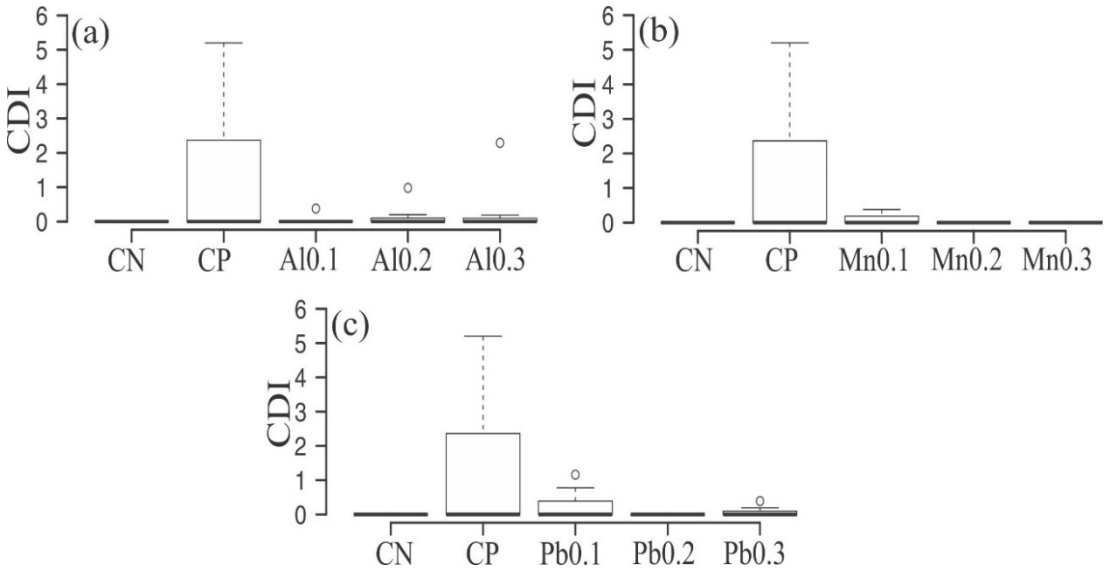

Figure 5. Cell death index (CDI) in *Allium cepa* cells exposed to different concentrations of (a) Aluminum, (b) Manganese and (c) Lead.

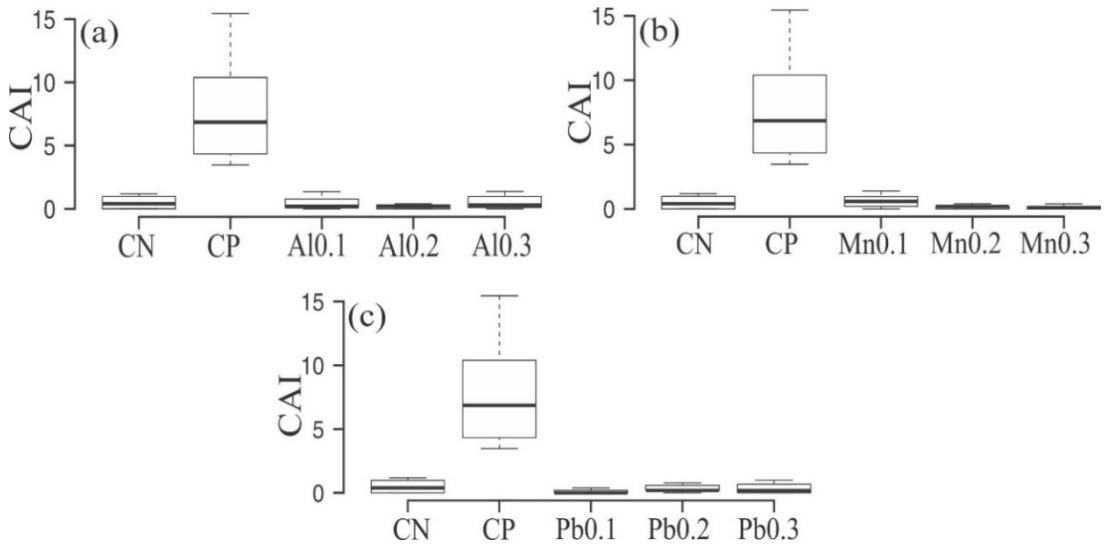

Figure 6. Chromosomal alterations index (CAI) in *Allium cepa* cells exposed to different concentrations of (a) Aluminum, (b) Manganese and (c) Lead.

The micronucleus is a parameter used to evaluate the mutagenic potential of substances, because is the result of the absence or incorrect repair of alterations in the parental cells, being considered an effective and simple test for mutagenicity analysis [22].

According to [23] micronuclei can arise spontaneously, but most of the time its appearance is due to the action of mutagens, characterizing the genetic alterations. In our study, for micronuclei only a significant difference was obtained between the positive control and the other solutions, which revealed a smaller amount of micronuclei (Figure 7 (a), (b) and (c)).

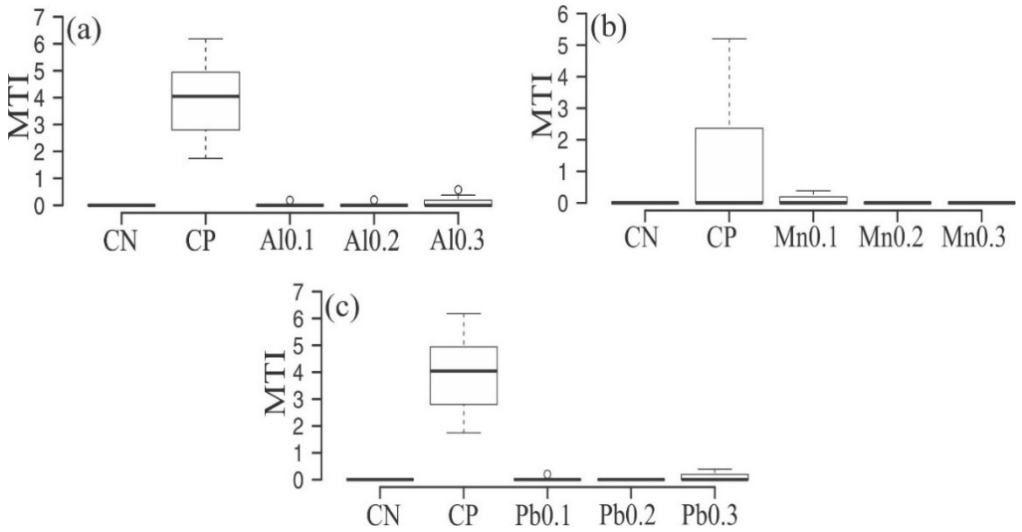

Figure 7. Mutagenicity index (MTI) in *Allium cepa* cells exposed to different concentrations of (a) Aluminum, (b) Manganese and (c) Lead.

4. CONCLUSION

The metals at the concentrations evaluated did not present genotoxic and mutagenic potential for meristematic cells. The *A. cepa* test proved to be effective for the analysis performed, serving as a basis for further research to evaluate these metals, the results found serve as an initial alert for the possible effects that these metals may represent on living organisms and on the ecosystem, since altered the number of cells in cell division according to the increase of the concentrations of the metals analyzed. However, further studies are needed using other biological assays, so that the results referring to effects of these metals be better characterized.

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