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Cadmium toxicity on seed germination and initial growth of chia

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ABSTRACT. In recent years, there has been a growing concern related to soil and water contamination due to the constant dispersal of toxic metals. In addition to their ecotoxicological potential, these elements exhibit a cumulative character that favors their permanence in soil and passage to living organisms, which can lead to an ecological imbalance. Among toxic metals, cadmium (Cd) is an obstacle to agriculture because it can adversely affect food quality and human health, as well as diminish plant growth and productivity. Thus, the objective of this work was to evaluate the toxicity of cadmium on seed germination and initial growth of chia. The ecotoxicological effects of four Cd concentrations (15; 30; 45; and 60 mg L^{-1}) were evaluated. The response variables were germination percentage, first count, germination speed index, total length, shoot length, root length, seedling dry mass, and tolerance index. It is concluded that the presence and accumulation of Cd in the culture substrate played an inhibitory role in seed germination and initial seedling growth of chia starting at 15 mg L^{-1}. On the other hand, no significant effect was observed for the treatments in relation to dry mass of the chia seedlings.

Keywords: cadmium chloride; *Salvia hispanica*; toxic metal.

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Introduction

The contamination of water and soils with toxic metals is a major environmental problem that can have negative consequences on the health of humans and other animals. The increasing use of technological products and their inadequate disposal, as well as environmental disasters and the excessive use of fertilizers, have increased the levels of these elements in the environment. Among metals, cadmium (Cd) is a highly toxic element that is released into the environment through the indiscriminate use of agricultural fertilizers, phosphate fertilizers, fungicides, pesticides containing metals, inadequate incineration of garbage with nickel-cadmium rechargeable batteries (e.g., in calculators and cell phones), and many industrial processes (Amirjani, 2012; Alloway, 2013).

Agricultural soils are continuously polluted with harmful toxic metals that contaminate water and impede the absorption of nutrients by plants, causing morphological, biochemical, physiological and structural changes (Augusto, Bertoli, Cannata, Carvalho, & Bastos, 2014). Consequently, this hampers growth, causing a significant reduction in yield and loss in productivity (Yadav, 2010). In plants, the concentration of Cd can vary from 0.03 to 0.1 mg kg^{-1} and it is estimated that phytotoxic concentrations vary between 5 to 10 mg kg^{-1} in sensitive species, which accumulate mainly in the roots (Kabata-Pendias, 2010).

Toxic elements, such as Cd, are agents of stress alter the functions of various organisms and can significantly damage plants. The main symptoms of toxicity are the following: inhibition of seed germination; disturbance of plant metabolic activities; reduction in the root system and stem; changes in the structure of chloroplasts; inhibition of photosynthesis; reduction of plant biomass; lipid peroxidation; decrease in respiration rate and growth; inhibition of the action of enzymes; reduction in cell division; and interference in cellular membrane permeability, which reduces the ability to absorb and transport water and essential elements (e.g., Ca, Mg and K) inside the plant, reducing tolerance to stress (Kabata-Pendias, 2010; Rahoui, Chaoui, & El Ferjani, 2010; Gill, Hasanuzzaman, Nahar, Macovei, & Tuteja, 2013; Chen et al., 2014).

Studies of plant development under different Cd contamination levels have been conducted with the common bean (Santos, Fagan, Teixeira, Soares, Reis, & Corrêia, 2013), *Jatropha curcas* (Chaves & Souza, 2014), wheat (Ahmad, Akhtar, Zahir, & Jamil, 2012; Guilherme, Oliveira, & Silva, 2015), leaf mustard - *Brassica juncea* (Augusto et al., 2014; Alfiya & Dheera, 2015), crambe (Hu et al., 2015), *Brachiaria brizantha* and *B. decumbens* (Borges, D'avila, Campos, Coelho, Miquelluti, & Galvan, 2016), wheat and beans (El Rasafi, Nouri, Bouda, & Haddioui, 2016), chickpeas (Ahmad, Abdel-Latef, Abd-Allah, Hashem, Sarwat, Anjum, & Gucel, 2016), and basil - *Ocimum basilicum* - (Gharebaghi, Haghighi, & Arouiee, 2017), among others. However, no studies were found that evaluated the toxic effects of Cd on the seed germination of chia.

Chia (*Salvia hispanica* L.) is an herbaceous plant propagated from seed and belongs to the family Lamiaceae. Its native distribution is from southern Mexico to northern Guatemala and it has been shown to have beneficial effects on human health due to high levels of proteins, omega 3 and omega 6, soluble fibers, vitamin B, calcium, zinc, phosphorous, potassium, copper and natural antioxidants (Ayerza & Coates, 2011). In Brazil, the regions of northeastern Rio Grande do Sul, western Paraná, and some areas of Minas Gerais and Mato Grosso have started to invest in cultivating chia over the last few years (Migliavacca, Silva, Vasconcelos, Mourão Filho, & Baptistella, 2014). The results have been promising; however, there is no information on the nutritional requirements and cultivation characteristics of this plant, since most studies have concentrated on the benefits to human health and nutritional composition of the seeds (Migliavacca et al., 2014).

Considering that high concentrations of Cd negatively affect seed germination, and believing that it is beneficial to know the effects of this toxic metal on germination, growth and development of chia plants, the goal of this study was to evaluate the toxicity of cadmium on seed germination and initial growth of chia with the objective of providing information that can be used when cultivating this species under this condition.

Material and methods

To evaluate the toxic effect of Cd on the seed germination process of chia seeds, acquired from a company that produces and sells seeds in Burzaco, Argentina, were sown on a paper substrate moistened with aqueous solutions of cadmium chloride, at concentrations of zero (control), 15, 30, 45, and 60 mg L^{-1} (adapted from Ahmad et al., 2012). For the control (level zero), only distilled water was used. The experimental design was completely randomized, where treatments consisted of different concentrations of the solutions. The toxic effect of Cd was evaluated using the tests listed below:

Germination: conducted based on four repetitions of 100 seeds distributed in plastic boxes (gerbox), on germitest paper moistened with distilled water or cadmium chloride solution (2.5 times the weight of the paper). After sowing the seeds, the plastic boxes were maintained in BOD chambers at a constant temperature of 20°C and 8 hours of light and 16 hours of dark. Counts were made on days seven and 14 (adapted from Stefanello, Neves, Abbad, & Viana, 2015) and the results were expressed as percentages (Brasil, 2009).

First count: conducted together with the germination test, where the percentage of normal seedlings was determined on day seven of the test.

Germination speed index (GSI): germinated seeds were counted daily at the same time. The criterion for germination was the protrusion of the radicle and the germination speed index was calculated based on the formula in Maguire (1962).

Seedling length: normal seedlings were obtained by sowing four repetitions of 20 seeds. Rolls of paper containing the seeds were kept in a germination chamber for seven days, at a temperature of 20°C. Total length, shoot length and root length of 10 seedlings were randomly evaluated for each repetition using a millimeter ruler. The average length of the seedlings was obtained by adding the number of measurements of each repetition and dividing this by the number of normal seedlings measured, with the results expressed in centimeters (Nakagawa, 1999).

Dry mass of seedling: first, the fresh weight was obtained from 10 previously measured seedlings, for four replicates, which were then maintained in papers bags in a dryer at a temperature of 60°C until reaching a constant weight (48 hours). Subsequently, the seedlings were weighed on a precision balance (to 0.001 g) and the results were expressed in milligrams (Nakagawa, 1999).

Tolerance index (TI): was determined with the formula given by Iqbal and Rahmati (1992) (Ahmad et al., 2012) (Equation 1).

$$\text{Tolerance index (T.I.)} = \frac{\text{Mean root length in metal solution}}{\text{Mean root length in control}} \text{ X } 100 \qquad (1)$$

Data analysis: the variables, expressed as percentages, were converted to arc sine $\sqrt{x/100}$. The data were submitted to an analysis of variance using the F test and, when significant, a regression analysis was performed using the program Sisvar (Ferreira, 2011).

Results and discussion

The analysis of variance indicated significant differences ($p < 0.05$) as a function of the treatments for all the variables, except seedling dry mass (Table 1).

Table 1. Summary of the analysis of variance for the variables germination (G), first count (FC), germination speed index (GSI), total length (TL), shoot length (SL), root length (RL), tolerance indices (TI), and dry mass (DM) of chia seedlings exposed to different concentrations of cadmium.

Source of variation	Degrees of freedom	Mean square							
		G	FC	GSI	TL	SL	RL	TI	DM
Treatment	4	3347.2*	3764.1*	1117.4*	11.81*	0.84*	6.534*	2684.9*	0.0057
Residue	15	10.05	8.016	4.40	0.092	0.046	0.061	24.11	0.009
CV (%)		4.93	4.88	2.68	6.80	12.57	8.97	8.78	18.21

* Significant at 5% probability by the F test. CV = Coefficient of variation

In the absence of Cd, the seeds had an average of 95 and 92% normal seedlings for the germination test and first count, respectively (Figure 1), and there was a slight reduction for the 15 mg L^{-1} and values below 50% (42%) starting at 45 mg L^{-1}. In the presence of Cd the seedlings showed typical symptoms of toxicity. Among the seedling abnormalities observed for the highest concentrations used (60 mg L^{-1}), atrophy or the absence of a primary root were notable, as well as roots darkened at the apices and a thin, weak, and contorted primary root (Figure 2).

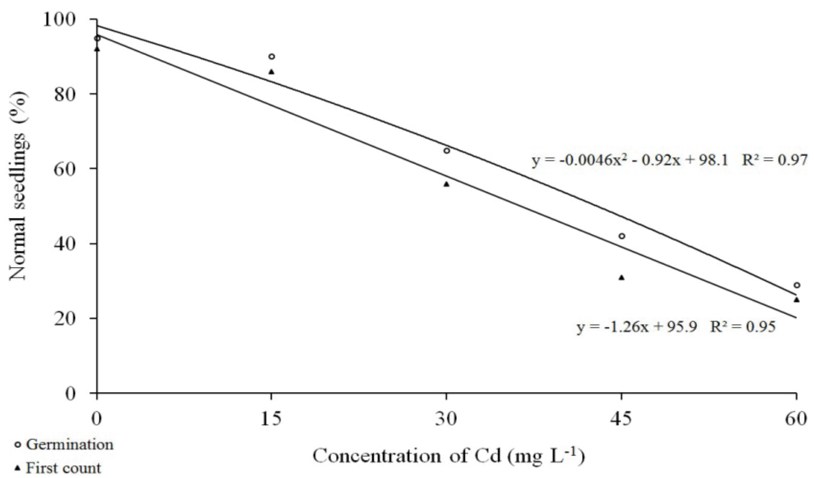

Figure 1. Germination and first germination count of chia seeds exposed to different concentrations of cadmium.

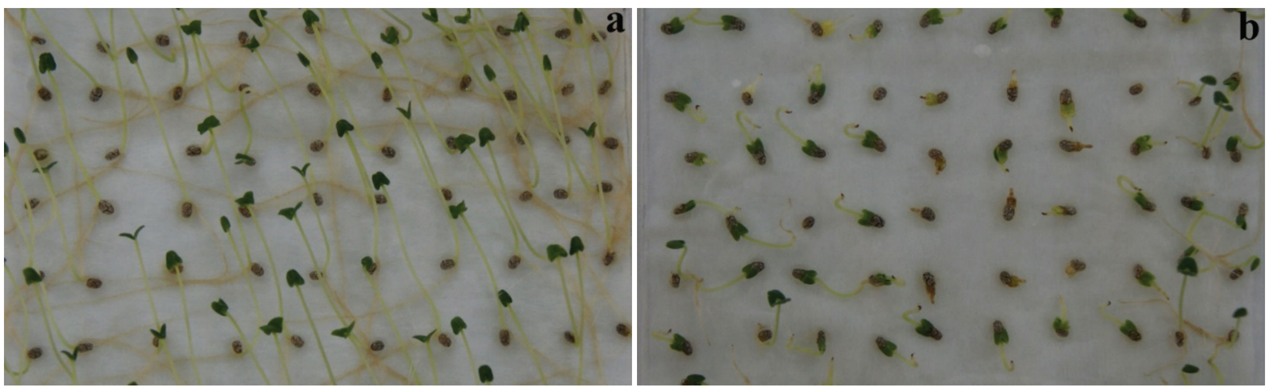

Figure 2. Effect of different concentration of Cd on the germination of chia seeds for 7 days: (a) control; (b) 60 mg L^{-1} of Cd.

Similar results were obtained by El Rasafi et al. (2016) for bean seeds exposed to 10 to 1000 mg L^{-1} of Cd. The authors verified that this metal significantly affected seed germination starting at 50 mg L^{-1}. In the same way, Ahmad et al. (2012) studied the tolerance of wheat seeds to Cd (0 to 80 mg L^{-1}) and observed a significant reduction in the percentage of normal seedlings starting at 20 mg L^{-1}. In addition, Gharebaghi et al. (2017) evaluated the response of basil (*Ocimum basilicum*) to Cd treatments (0 to 20 mg L^{-1}) and verified that germination was inhibited compared to the control. According to El Rasafi et al. (2016), inhibition by toxic metals depends on the concentration used, the metal itself, and the plant species. The effect of toxic metals can also be influenced by the ability of a metal to interact with the tegument and embryo tissues of the seeds of different species and depends the physical-chemical properties of these elements. Different plant species have varying types of seed teguments, structure and anatomy; therefore, among species, the same concentration of an element can have different toxic effects (Ko, Lee, & Kong, 2012).

Generally, toxic elements negatively affect germination and compromise plant development, mainly due to the lack of defense mechanisms at this stage (Yang et al., 2010). After the solution enters the tegument, the seed counts on nutrient reserves to supply metabolites for germination. One of the primary effects of Cd toxicity can be observed during this process, where activity of β amylases is significantly reduced, harming respiration, which inhibits growth of the embryonic axis and radicle (He, Ren, Zhu, & Jiang, 2008). Therefore, the inhibition of these enzymes might be indicative of the toxicity mechanisms of toxic elements. Plants sensitive to exposure to these metals can be used as indicators in contaminated environments because they respond rapidly to the deleterious effects of pollutants (Kong, 2013).

Starting at 30 mg L^{-1}, germination speed was reduced as the Cd concentration in the substrate increased (Figure 3). The reduced speed is probably related to a decrease in enzyme activity linked to embryo growth and the protrusion of the radicle, because Cd disrupts development, cellular differentiation and growth through changes in the activity of the enzyme peroxidase (Santos et al., 2013). A delay in germination can occur due to the protective role of the integument, which can block and retain toxic metals on its surface (Sun & Luo, 2014).

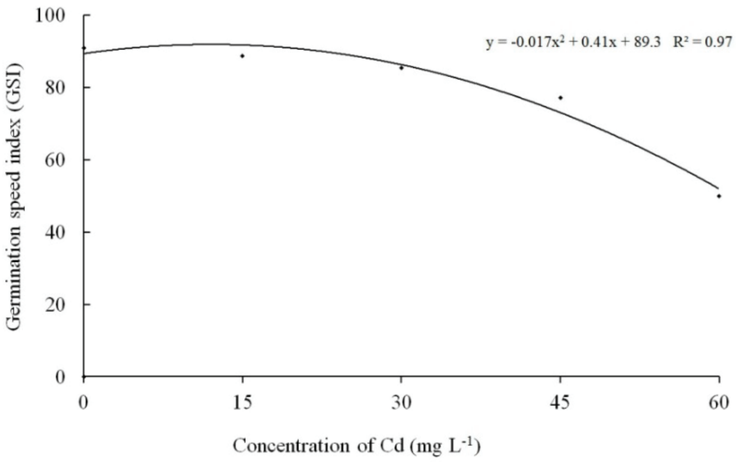

Figure 3. Germination speed index of chia seeds exposed to different concentrations of cadmium.

For total length of the aerial part and roots of chia, starting at 15 mg L^{-1} there was a reduction in size with an increase in the metal concentration (Figure 4a). There was a reduction in total length of the seedlings from 7.32 cm (control) to 3.1 cm (60 mg L^{-1}). Similarly, stem length decreased from 2.38 cm (control) to 1.31 cm and root length decreased from 4.93 cm (control) to 1.79 cm for the highest Cd concentration used (60 mg L^{-1}). In addition, there was no difference in the dry mass of the seedlings (Figure 4b).

The results of this study corroborate Al-Qurainy (2010), who verified that in Eruca sativa root length decreased 28% and stem length decreased 44% for 50 mg L-1 of Cd. Also, Borges et al. (2016) observed a reduction in root length of 2.5 and 1.0 cm in Brachiaria brizantha and B. decumbens when exposed to 180 mg L-1 of this metal. Similarly, Grigoraş and Stratu (2015) concluded that the Cd concentrations used (1.10; 1.6 and 2 mg L-1) did not affect seed germination, but negatively influenced seedling growth (especially the lengthening of the root) of Dianthus barbatus L. and D. chinensis L. According to these authors, the effect of the seedling growth delay can be attributed to metabolic and physiological disturbances caused by the Cd concentrations used in the treatments.

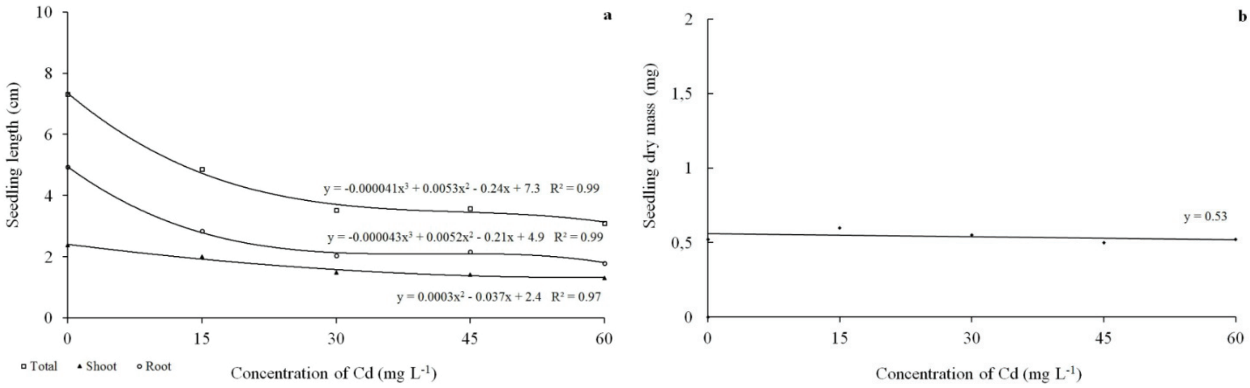

Figure 4. Length (a) and dry mass (b) of chia seedlings exposed to different concentrations of cadmium.

On the other hand, Guilherme et al. (2015) observed that wheat seeds exposed to Cd exhibited a decrease in percentage of normal plants starting at a concentration of 0.03 mM compared to the experimental control. During initial seedling growth, concentrations of 0.06 mM and 0.12 mM inhibited root and stem growth, respectively, which reduced the dry mass of the seedlings. Similarly, Alfiya and Dheera (2015) evaluated the effects of various Cd concentrations (0.5, 1, 1.5 and 2 mM) on *Brassica juncea* and found that as the metal concentration increased all of the parameters (root and stem length, seedling size, fresh and dry mass, germination percentage and phytotoxicity) decreased. In addition, Ahmad et al. (2016) used 200 uM of Cd and found that the stress from the metal significantly decreased the length of the stem and root of chickpeas, and Hu et al. (2015) observed a 48% reduction in root length, 34% reduction in stem length and 13% reduction in fresh mass of crambe seedlings exposed to 0.1 mM of Cd (11.2 mg L^{-1}).

In addition to the reduced root length, an increase in epicotyl thickness was observed in response to the substrate contaminated with Cd. According to Rodrigues, Santos, Santos, Pereira, and Sobrinho (2016), the plant increases its filtering capacity by increasing its negative charges to protect internal tissues from the damage caused by toxic metals. Various studies point to the endoderm and exoderm as the main barriers and retention sites of toxic metals in plant roots, which minimize the translocation of these ions and favor tolerance to this stress (Gomes, Marques, Nogueira, Silva, Castro, & Soares, 2011). Our results are in agreement with Mondal, Das, Roy, Datta, and Banerjee (2013), who reported that a reduction in root growth could be due to the direct interference of Cd with some hydrolytic enzymes, which play a fundamental role in the transport of nutrients to the primary root and stem. The authors also noted that a decrease in stem length could be due to the direct inhibition of cellular elongation or division, retarded root growth, and reduced transport of nutrients and water.

The tolerance index for 15, 30, 45 and 60 mg L^{-1} was 58, 41, 44 and 36% (Table 2), indicating that the Cd concentration in the substrate caused a decrease in root length. This index reflects the ability of seedlings to grow in environments with high concentrations of a metal and, consequently, indicates that the chia plants should only be recommended for cultivation in areas with low Cd levels.

Table 2. Tolerance index of roots of chia seedlings exposed to different concentrations of cadmium.

Concentration of Cd (mg L^{-1})	Tolerance index (%)
0	100 a*
15	58 b
30	41 c
45	44 c
60	36 c

* Significant at 5% probability by the F test.

Many studies have reported that even low concentrations of Cd are toxic to most plants. The roots are the primary parts of the plant that are in contact with the contamination and are more sensitive to the toxicity of metals compared to the stem (Yang, Qi, Jiang, & Chen, 2013). Cadmium is absorbed by the roots, causing phytotoxicity, primarily by influencing cellular growth in the elongation zone of the root, which impedes the mechanisms of absorption and conduction of water and nutrients (Bian, Zhou, Sun, & Li, 2013). According to Heidari and Sarani (2011), a decrease in root length can be caused by the inhibition of mitosis and reduction in the synthesis of components of the cell wall and metabolism of polysaccharides.

Finally, toxic metals adversely interfere with the growth, distribution and biological cycle of plant species, which makes it necessary to search for plants that have tolerance mechanisms to use in contaminated areas as an alternative for environmental remediation. The same plant may exhibit different behaviors in a contaminated soil, and the response to contamination can vary based on the characteristics of the species, the stress, and the conditions of the specific area (Souza, Silva, & Ferreira, 2013). Thus, more detailed studies are needed to establish the maximum amount of Cd that chia seeds can tolerate for germination and growth in environments contaminated with this toxic metal.

Conclusion

The presence and accumulation of Cd in the substrate played an inhibitory role in seed germination and initial seedling growth of chia starting at 15 mg L^{-1}. On the other hand, no significant effect was observed for the treatments in relation to dry mass of the chia seedlings.

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