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Effects of cadmium, copper, and zinc on germination and post-germination growth of chia seeds

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ABSTRACT

In recent years, concerns regarding environmental pollution and its impact on agricultural production have intensified due to the ongoing dispersal of heavy metals. This study investigated the ecotoxicological effects of three heavy metals (cadmium (Cd), copper (Cu), and zinc (Zn)) on the germination and post-germination growth of chia (*Salvia hispanica*). Except for the notable 18.2% decrease in germination rate with Zn treatment on the first day, the germination rates of metallic-stressed chia seeds did not significantly differ from the control for the other metals. However, the parameters related to post-germination growth were negatively impacted in varied ways by the metal treatments, causing a significant hindrance in radicle elongation (ranging from 56.3% to 81.6%) and hypocotyl elongation (from 27.1% to 76.5%), as well as a notable decrease in both fresh and dry biomasses. The most significant reduction in radicle and hypocotyl elongation occurred with Zn and Cd treatment. The largest decrease in fresh and dry biomass was seen in the radicle and hypocotyl under Zn exposure. The tolerance index demonstrated that chia showed the greatest ecotoxicological tolerance to Cu, followed by Cd and Zn. This suggests that chia has potential for cultivation in polluted areas, particularly those contaminated with Cu.

Keywords: heavy metal stress, biomass, chia, tolerance, radicle, hypocotyl

INTRODUCTION

In recent years, industrialization and urbanization have contributed immensely to environmental degradation by introducing pollutants like heavy metals into natural resources such as water, soil, and air. Heavy metals are well-known environmental pollutants that have the potential to induce harmful effects in different plants (Farouk and Al-Amri, 2019a, 2019b; Xing et al., 2020; Chen et al., 2021). While low concentrations of certain heavy metals like copper (Cu) and zinc (Zn) are essential for plant growth, they can become toxic at high concentrations. In contrast, unlike copper, metals like cadmium (Cd) and lead (Pb) are toxic even in small concentrations (Kiran and Sharma, 2022). Plants under heavy metal stress show a noticeable sign of toxicity owing to their strong connection with metabolic and ultrastructural modifications (Farouk and Al-Amri, 2019a, 2019b; Noor et al., 2022). These symptoms can appear at various stages of the plant's life span, including seed germination (Muszyńska and Labudda, 2019). Germination and post-germination growth assays are critical as they dictate crop establishment and productivity (Kiran and Sharma, 2022).

Seed germination is a sensitive physiological process in plants and is highly susceptible to heavy metal contamination, particularly when these metals reach the embryo, which lacks enough defense mechanisms (Sethy and Ghosh, 2013). Germination is the start of a plant's life, and in stressful environments, this process can be severely impeded (Moreira et al., 2020). Indeed, inhibiting germination is the seeds' primary defense mechanism to withstand adverse environmental conditions. The impact of heavy metals on seed imbibition and radicle elongation is influenced by their ability to penetrate the seed coat and alter the physiological processes involved in germination (Seneviratne et al., 2019). Excessive amounts of heavy metals have been shown to inhibit germination, damage membranes, and disrupt the mobilization of food reserves. This disturbance is characterized by higher proportions of cotyledon/embryo in overall soluble sugars, glucose, fructose, and amino acids. Additionally, the presence of heavy metals induces ion imbalance and evokes oxidative stress by hyperaccumulation of reactive oxygen species (Sethy and Ghosh, 2013; Farouk and Al-Amri, 2019a, 2019b). Soil contamination with heavy metals disrupts plant metabolism, associated with qualitative and quantitative declines in agricultural yields, triggering major worries regarding the safety of food. They are a global environmental issue that can enter the food chain through cereals, fruits, and vegetables, posing a threat to human health (Alengebawy et al., 2021). Climate change is expected to further intensify heavy metal contamination by affecting factors related to their bioavailability, fate, and toxicity, including their eco-physiological properties (Alengebawy et al., 2021; Rharbi et al., 2023).

Chia (*Salvia hispanica* L.) is a pseudocereal known for its highly valued seeds, which offer significant nutritional and medicinal benefits. Chia is now familiar as a promising new crop because of its high oil content and impressive levels of Omega-3 fatty acids (Kulczynski et al., 2019). Furthermore, chia seeds are rich in proteins, dietary fiber, vitamins, carbohydrates, antioxidants, and nutraceuticals (Grancieri et al., 2019). This unique chemical profile and health-promoting potential have led to greater interest in incorporating chia seeds into the food industry and enhancing nutrition security (Knez Hrnčič et al., 2020). Although chia seeds have become increasingly popular as dietary supplements, there is little research on their responses to heavy metal contamination during germination.

The purpose of this research was to evaluate the effects of three metal treatments (Zn, Cu, and Cd) at different concentrations on the germination and seedling growth of chia, a species whose seeds are widely consumed in the human diet, in order to assess potential risks to food safety and plant development associated with heavy metal exposure.

MATERIAL AND METHODS

Plant material and germination conditions

Seeds of chia were disinfected with 5% sodium hypochlorite for 10 min, then washed repeatedly with distilled water before being germinated in glass Petri dishes (12 cm in diameter) lined with double filter paper (Whatman International Ltd.) moistened with 20 mL of metallic solution. Germination kinetics and post-germination growth were followed for 7 days at 27 °C. Petri dishes were incubated in the dark for the first four days before being moved to ambient light conditions. We opted for 20 seeds per Petri dish and 10 Petri dishes for each treatment. The experiment was conducted at the Faculty of Science in Rabat (-6.83823880°/N, 34°00796730°/W, and 43.80 m altitude).

Metal treatments and experimental design

The impact of different concentrations of Zn, Cu, and Cd on chia seed germination was tested. Preliminary tests were conducted on five different concentrations of Zn (5, 7, 9, 11, and 13 mM) and Cu/Cd (0.5, 0.7, 0.9, 1.1, and 1.3 mM) to determine threshold concentrations based on relative water content (%) as a limiting factor (Tab. 1). Zinc sulfate ($ZnSO_4$), copper sulfate ($CuSO_4$), and cadmium sulfate ($CdSO_4$) are used as a source of metallic Zn, Cu, and Cd.

Germination and post-germination traits

To investigate chia seeds' germination and post-germination characteristics under selected concentrations of heavy metals as indicated from preliminary tests. Four sets of Petri dishes lined with double filter paper (Whatman International Ltd.) moistened with 20 mL of either distilled water, 9 mM Zn solution, 0.9 mM Cu solution, or 1.2 mM Cd solution. The parameters studied were measured every 24 hours over 7 days.

Counts of germination were checked daily to determine germination percentage (G) using the formula:

germination rate (%) = (number of germinated seeds / total number of seeds) × 100.

The calculation of germination energy (GE) involved dividing one-fourth of the maximum number of seeds that germinated in a day by the total number of seeds and multiplying by 100. The germination period (GP) corresponds to days from seeding to when the maximum number of seeds germinated (Kumar et al., 2011).

The length of the hypocotyl and radicle was measured in germinated seeds (under control and metal stress conditions), every 24 hours per treatment, with a graduated ruler and their fresh and dry biomass with a precision balance scale. All parameters were measured with ten replicates.

The tolerance index (TI) was determined using the formula provided by Stefanello et al. (2019) as follows:

$$TI = \frac{\text{Mean radicle length under metal stress}}{\text{Mean radicle length in control}} \times 100$$

Statistical analysis

All data were subjected to analysis of variance (ANOVA) to assess the effects of metallic treatments on the growth traits studied. Means were compared at the 5% significance level using Tukey's post hoc test. Statistical analyses were conducted using SPSS, version 25 for Microsoft Windows.

RESULTS

Based on statistical analysis (ANOVA) of the preliminary test results (Table 1), we recorded a significant decrease ($P \leq 0.05$) in relative water content (%) of chia seeds from 9 mM concentration for Zn, 0.9 mM for Cu, and 1.2 mM for Cd, compared to the control. These three concentrations were used to prepare the metal solutions.

The germination percentage was similar between the control and metal stress treatments, except for Zn on the first day of germination, where there was a significant difference (Table 2). Compared to the control, Zn caused a significant reduction in germination percentage by approximately 18.2%. Regarding the timing, the germination percentage significantly increased from the initial to the subsequent day only in chia seeds treated with Zn and Cu treatments. However, there was no significant discrepancy observed after 48h ($P>0.05$). The maximum germination percentage was 86.7%, 83.3%, 80.0%, and 77.5% for the control, Cu, Zn, and Cd, respectively. There was relatively consistent germination energy across all treatments, including the control group.

The mean germination percentage across metallic treatments ranged from 73.6 to 79.2% (Table 2). Among metallic treatments, Cd exhibited the lowest mean germination percentage (73.6%) and was significantly different from the control. Independent of metallic treatment, it has also increased considerably over time from 66.9 to 81.9%. A similar trend was observed for germination energy percent, too (Table 2).

By graphical extrapolation (data not shown), the time taken to reach 50% of germinated seeds was approximately 16 h for control seeds, 18 h for Cu and Cd-treated seeds, and 20 h for Zn-treated seeds.

The three heavy metal elements studied significantly inhibited the elongation of the radicle (from the first day) and hypocotyl (from the 2nd day) of chia seeds, compared to the control ($P \leq 0.05$) (Table 3). The most significant inhibition was detected when plants were exposed to Zn and Cd, dropping root growth by 81.6% and 72.9% on day 7 of germination.

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Table 1. Results of the analysis of variance of different concentrations of Zn, Cu, and Cd on the relative moisture content (%) of chia seeds after 4 days of germination

Zn	Control	5 mM	7 mM	9 mM	11 mM	13 mM
	94.91 ± 1.80^{a}	92.90 ± 0.94^{a}	92.79 ± 1.03^{a}	88.88 ± 0.71^{b}	87.42 ± 0.56^{b}	87.80 ± 0.54^{b}
Cu	Control	0.5 mM	0.7 mM	0.9 mM	1.1 mM	1.3 mM
	94.91 ± 1.80^{a}	93.66 ± 0.83^{ab}	93.51 ± 1.74^{ab}	88.17 ± 1.51^{bc}	87.67 ± 0.74^{bc}	86.72 ± 6.45^{c}
Cd	Control	0.5 mM	0.7 mM	0.9 mM	1.1 mM	1.3 mM
	94.91 ± 1.80^{a}	92.91 ± 0.60^{ab}	92.69 ± 1.42^{ab}	92.20 ± 0.73^{ab}	85.02 ± 4.01^{b}	84.34 ± 8.49^{b}

Means followed by different lowercase letters in each row are significantly different at the 5% significance level (Tukey's test)

Table 2. Germination percentage (G%) and germination energy (GE) of chia seeds under three heavy metal treatments (Zn, Cu, and Cd) over 7 days (d)

		1d	2d	3d	4d	5d	6d	7d	Mean
G%	C	73.33 ± 2.89^{Aa}	80.00 ± 10.00^{Aa}	83.33 ± 10.41^{Aa}	83.75 ± 7.50^{Aa}	85.00 ± 7.07^{Aa}	86.67 ± 7.64^{Aa}	86.67 ± 7.64^{Aa}	**82,68 ± 4,71^{A}**
	Zn	60.00 ± 5.00^{Bb}	76.67 ± 2.89^{Aa}	77.50 ± 2.89^{Aa}	78.75 ± 2.50^{Aa}	79.17 ± 2.04^{Aa}	79.17 ± 2.04^{Aa}	80.00 ± 2.89^{Aa}	**75,89 ± 7,10^{AB}**
	Cu	67.50 ± 5.00^{ABb}	78.33 ± 2.89^{Aa}	78.75 ± 2.50^{Aa}	81.67 ± 2.89^{Aa}	81.67 ± 2.89^{Aa}	83.33 ± 2.89^{Aa}	83.33 ± 2.89^{Aa}	**79,23 ± 5,54^{AB}**
	Cd	66.67 ± 5.77^{ABa}	70.00 ± 8.66^{Aa}	72.50 ± 8.66^{Aa}	75.00 ± 7.07^{Aa}	76.25 ± 7.50^{Aa}	77.00 ± 6.71^{Aa}	77.50 ± 6.12^{Aa}	**73,56 ± 4,04^{B}**
Mean G		**66,88 ± 5,46^{b}**	**76.25 ± 4.38^{ab}**	**78.02 ± 4.45^{a}**	**79.79 ± 3.80^{a}**	**80.52 ± 3.72^{a}**	**81.54 ± 4.31^{a}**	**81.88 ± 3.99^{a}**	
GE	C	18.33 ± 0.72^{Aa}	20.00 ± 2.50^{Aa}	20.83 ± 2.60^{Aa}	20.94 ± 1.88^{Aa}	21.25 ± 1.77^{Aa}	21.67 ± 1.91^{Aa}	21.67 ± 1.91^{Aa}	**20,67 ± 1,18^{A}**
	Zn	15.00 ± 1.25^{Aa}	19.17 ± 0.72^{Aa}	19.38 ± 0.72^{Aa}	19.69 ± 0.63^{Aa}	19.79 ± 0.51^{Aa}	19.79 ± 0.51^{Aa}	20.00 ± 0.72^{Aa}	**18,97 ± 1,77^{AB}**
	Cu	16.88 ± 1.25^{Aa}	19.58 ± 0.72^{Aab}	19.69 ± 0.63^{Aab}	20.42 ± 0.72^{Aa}	20.42 ± 0.72^{Aa}	20.83 ± 0.72^{Aa}	20.83 ± 0.72^{Aa}	**19,81 ± 1,39^{AB}**
	Cd	16.67 ± 1.44^{Aa}	17.50 ± 2.17^{Aa}	18.13 ± 2.17^{Aa}	18.75 ± 1.77^{Aa}	19.06 ± 1.88^{Aa}	19.25 ± 1.68^{Aa}	19.38 ± 1.53^{Aa}	**18,39 ± 1,01^{B}**
Mean GE		**16,72 ± 1,36^{b}**	**19.06 ± 1.10^{ab}**	**19.51 ± 1.11^{a}**	**19.95 ± 0.95^{a}**	**20.13 ± 0.93^{a}**	**20.39 ± 1.08^{a}**	**20.47 ± 1.00^{a}**	

Means followed by different lowercase letters in each row and different uppercase letters in each column are significantly different at the 5% significance level (Tukey's test)

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Table 3. Change in radicle and hypocotyl elongation (mm) in germinated chia seeds under three heavy metal treatments (Zn, Cu, and Cd) over 7 days (d)

		1d	2d	3d	4d	5d	6d	7d
Radicle	C	4.33 ± 0.39^{Ad}	17.30 ± 1.57^{Ac}	26.40 ± 4.38^{Ab}	28.70 ± 6.78^{Ab}	32.67 ± 4.85^{Ab}	39.22 ± 6.12^{Aa}	43.43 ± 3.91^{Aa}
	Zn	1.40 ± 0.52^{Cc}	1.50 ± 0.53^{Cc}	2.20 ± 0.79^{Cc}	2.28 ± 0.91^{Cc}	2.66 ± 0.54^{Cc}	5.20 ± 1.14^{Cb}	8.00 ± 1.63^{Ca}
	Cu	2.83 ± 0.56^{Bc}	7.00 ± 2.00^{Bbc}	11.33 ± 2.25^{Bb}	12.00 ± 2.83^{Bb}	12.38 ± 2.83^{Bb}	12.60 ± 3.13^{Bb}	19.00 ± 7.21^{Ba}
	Cd	2.00 ± 0.67^{Cd}	2.35 ± 1.29^{Ccd}	5.80 ± 2.39^{bCcd}	6.17 ± 2.14B^{Cbc}	8.57 ± 4.65^{BBab}	9.50 ± 3.66^{BCab}	11.75 ± 1.67^{Ca}
Hypocotyl	C	0.00 ± 0.00^{Af}	5.80 ± 1.30^{Ae}	11.80 ± 2.90^{Ad}	12.63 ± 2.62^{Ad}	27.63 ± 5.10^{Ac}	36.30 ± 2.91^{Ab}	43.88 ± 2.59^{Aa}
	Zn	0.00 ± 0.00^{Ad}	0.00 ± 0.00^{Bd}	4.80 ± 1.10^{Cc}	7.50 ± 3.78^{Bbc}	8.29 ± 0.95^{Cb}	8.80 ± 2.28^{Cb}	20.00 ± 1.00^{Ca}
	Cu	0.00 ± 0.00^{Ad}	5.20 ± 1.48^{Acd}	8.70 ± 2.00^{Bc}	15.90 ± 3.75^{Ab}	21.20 ± 4.39^{Bb}	30.80 ± 5.81^{Ba}	32.00 ± 4.18^{Ba}
	Cd	0.00 ± 0.00^{Ac}	4.00 ± 1.73^{Ab}	4.90 ± 0.99^{Cb}	5.80 ± 1.69^{Bb}	6.30 ± 3.50^{Cb}	6.70 ± 1.77^{Cb}	10.33 ± 4.55^{Da}

Means followed by different lowercase letters in each row and different uppercase letters in each column are significantly different at the 5% significance level (Tukey's test)

In comparison, Cu treatment resulted in a 56.3% reduction in radicle elongation. Regarding the length of hypocotyls, Cd had the greatest inhibitory effect compared to the control (76.5% on the 7th day). Nonetheless, the length of the hypocotyl was reduced by about 27.1% under Cu and 54.4% under Zn compared to the control (Table 3).

The fresh and dry biomass of radicle and hypocotyl (Tables 4 and 5) was significantly reduced in the germinated chia seeds subjected to heavy metal stress compared to control conditions ($P \leq 0.05$). The difference among treatments regarding the fresh biomass of radicle and hypocotyl became significant on the 3rd day of germination. Among the three metal treatments, Zn exhibited the most pronounced inhibitory effect, with an inhibition percentage of approximately 95.5% for the radicle and 85.9% for the hypocotyl compared to the control on the 7th day ($P \leq 0.05$). Under Cd and Cu treatments on the 7th day, the fresh biomass of radicle was significantly reduced by about 90.9 and 85.3%, respectively, while the fresh biomass of hypocotyl decreased by approximately 69.7 and 22.7%, compared to the control (Tab. 4). Significant variations in dry biomass were noted among treatments, including the control group, after 24 hours for the radicle and 48 hours for the hypocotyl during germination. By the seventh day of germination, the three metal treatments significantly decreased the dry biomass of the radicle and hypocotyl compared to the control. Zinc exhibited the most inhibitory effects, leading to decreases of around 47.1% for the radicle and 65.1% for the hypocotyl. In addition, a decrease in dry biomass of approximately 42.9% and 62.5% was recorded under Cd, while about 42.9% and 28.4% under Cu for the radicle and hypocotyl, respectively, compared to the control (Table 5).

The tolerance indices for Zn, Cu, and Cd were 32.3, 65.4, and 46.2% on the first day, and 18.4, 43.8, and 27.1% on the 7th day, respectively (Table 6). Chia radicle exhibited the highest tolerance index to Cu compared to the other metallic elements.

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Table 4. Change in radicle and hypocotyl length (cm) of radicle and hypocotyl in germinated chia seeds under three heavy metal treatments (Zn, Cu, and Cd) over 7 days (d)

		1d	2d	3d	4d	5d	6d	7d
Radicle	C	0.74 ± 0.48^{Ae}	2.97 ± 0.53^{Ad}	5.85 ± 0.95^{Ac}	8.80 ± 0.80^{Ab}	10.30 ± 1.56^{Aab}	10.65 ± 0.73^{Aa}	11.13 ± 0.72^{Aa}
	Zn	0.72 ± 0.20^{Ab}	2.00 ± 0.62^{Aa}	1.70 ± 0.46^{Ba}	0.90 ± 0.34^{Cb}	0.81 ± 0.41^{Cb}	0.74 ± 0.43^{Cb}	0.50 ± 0.17^{Cb}
	Cu	0.88 ± 0.15^{Ab}	2.17 ± 0.90^{Aa}	2.30 ± 0.76^{Ba}	2.49 ± 0.49^{Ba}	2.76 ± 0.68^{Ba}	2.34 ± 0.79^{Ba}	1.64 ± 0.62^{Bb}
	Cd	0.73 ± 0.17^{Ac}	1.98 ± 0.62^{Aab}	2.08 ± 0.17^{Bab}	2.41 ± 1.07^{Ba}	2.08 ± 0.55B^{Cab}	1.43 ± 0.49^{Cabc}	1.01 ± 0.39^{BCbc}
Hypocotyl	C	0.00 ± 0.00^{Ae}	3.30 ± 0.62^{Ad}	7.10 ± 1.00^{Ac}	11.57 ± 2.21^{Ab}	12.35 ± 0.53^{Ab}	15.11 ± 1.53^{Aa}	17.09 ± 2.09^{Aa}
	Zn	0.00 ± 0.00^{Ab}	0.00 ± 0.00^{Bb}	3.08 ± 0.85^{Ca}	3.17 ± 0.93^{Ba}	3.24 ± 0.63^{Da}	2.58 ± 1.24^{Ca}	2.41 ± 0.96^{Da}
	Cu	0.00 ± 0.00^{Af}	3.13 ± 0.29^{Ae}	5.53 ± 1.20^{Bd}	9.61 ± 1.39^{Ac}	10.72 ± 1.58^{Bbc}	12.29 ± 1.66^{Bab}	13.21 ± 1.60^{Ba}
	Cd	0.00 ± 0.00^{Ad}	2.88 ± 0.58^{Ac}	3.79 ± 0.69^{Cbc}	5.26 ± 1.43^{Ba}	4.88 ± 1.04^{Cab}	4.56 ± 1.16^{Cab}	5.17 ± 1.00^{Cab}

Means followed by different lowercase letters in each row and different uppercase letters in each column are significantly different at the 5% significance level (Tukey's test)

Table 5. Change in dry biomass (mg) of radicle and hypocotyl in germinated chia seeds under three heavy metal treatments (Zn, Cu, and Cd) over 7 days (d)

		1d	2d	3d	4d	5d	6d	7d
Radicle	C	0.17 ± 0.03^{Ab}	0.43 ± 0.10^{Aab}	0.58 ± 0.08^{Aa}	0.60 ± 0.20^{Aa}	0.53 ± 0.15^{Aa}	0.61 ± 0.12^{Aa}	0.70 ± 0.17^{Aa}
	Zn	0.10 ± 0.03^{Bb}	0.27 ± 0.11^{Ab}	0.47 ± 0.10^{Aa}	0.48 ± 0.05^{Aa}	0.50 ± 0.26^{Aa}	0.37 ± 0.06^{Ba}	0.37 ± 0.06^{Ba}
	Cu	0.14 ± 0.02^{ABb}	0.36 ± 0.11^{Aab}	0.44 ± 0.16^{Aa}	0.50 ± 0.17^{Aa}	0.49 ± 0.09^{Aa}	0.47 ± 0.10A^{Ba}	0.40 ± 0.10^{Ba}
	Cd	0.11 ± 0.03^{Bb}	0.32 ± 0.08^{Aab}	0.43 ± 0.06^{Aa}	0.50 ± 0.00^{Aa}	0.47 ± 0.06^{Aa}	0.48 ± 0.15A^{Ba}	0.40 ± 0.17^{Ba}
Hypocotyl	C	0.00 ± 0.00^{Ad}	0.40 ± 0.10^{Ac}	0.58 ± 0.15^{Abc}	0.60 ± 0.12^{Abc}	0.73 ± 0.06^{Aab}	0.87 ± 0.15^{Aa}	0.88 ± 0.08^{Aa}
	Zn	0.00 ± 0.00^{Ac}	0.00 ± 0.00^{Cc}	0.43 ± 0.06^{Aab}	0.53 ± 0.23^{Aa}	0.35 ± 0.13^{Cb}	0.36 ± 0.11^{Cab}	0.30 ± 0.08^{Cb}
	Cu	0.00 ± 0.00^{Ae}	0.23 ± 0.05^{Bd}	0.46 ± 0.05^{Ac}	0.48 ± 0.15^{Abc}	0.53 ± 0.12^{ABabc}	0.66 ± 0.09^{Ba}	0.63 ± 0.10^{Bab}
	Cd	0.00 ± 0.00^{Ab}	0.27 ± 0.12^{Ba}	0.36 ± 0.13^{Aa}	0.43 ± 0.09^{Aa}	0.37 ± 0.12^{Ca}	0.35 ± 0.11^{Ca}	0.33 ± 0.15^{Ca}

Means followed by different lowercase letters in each row and different uppercase letters in each column are significantly different at the 5% significance level (Tukey's test)

Table 6. Tolerance index of germinated chia seeds based on radicle growth under three heavy metal treatments (Zn, Cu, and Cd) on days 1 and 7

Treatment	Tolerance index (%)	
	1d	7d
C	100.00	100.00
Zn	32.33	18.42
Cu	65.36	43.75
Cd	46.19	27.06

A higher tolerance index indicates better tolerance to heavy metals, while a lower one indicates a reduction in tolerance and, therefore, a more negative impact of heavy metals on radicle growth

DISCUSSION

Inhibition of germination is one of the most well-known and common adverse effects of toxic mineral elements (Kiran and Sharma, 2022). Many plants are sensitive to toxicity during seed germination and seedling development. Consequently, changes in plant growth during these stages under heavy metal stress are often regarded as a significant indicator for assessing plant tolerance to heavy metals (Sethy and Ghosh, 2013). Except for Zn, the other metallic elements (Cu and Cd) did not show any inhibitory effect on chia seed germination. Zn significantly reduced the germination rate within the first 24 hours. Many researchers have previously demonstrated the inhibition of seed germination under metallic elements stress across various species, including turnip under Cd, Cr, and Pb (Siddiqui et al., 2014), wheat exposed to Pb and Cu (Jańczak-Pieniążek et al., 2023), alfalfa subjected to Cd, Cr, Cu, and Ni (Aydinalp and Marinova, 2009), as well as barley, rice, and wheat exposed to Cu (Mahmood et al., 2007). However, in contrast to our findings regarding Zn, some studies, such as those on barley, rice, and wheat (Mahmood et al., 2007) and alfalfa (Aydinalp and Marinova, 2009), have not reported a negative effect of Zn on seed germination. The differences observed in germination inhibition rates between our findings and those of other studies for the same metallic element suggest that seeds from different plant species exhibit varying germination capabilities under metal stress (Abbas et al., 2017). Generally, seed germination inhibition depends on the metallic element, its concentration, the duration of seed exposure, as well as the plant species (whether sensitive or tolerant), and even the variety and nature of the seed coat (Munzuroglu and Geckil, 2002). Heavy metals inhibit seed germination primarily by disrupting key physiological processes. They damage cell membranes, reduce enzymatic activity involved in digestion and mobilization of food reserves such as proteins and carbohydrates, and disturb ionic balance, affecting germination (Sethy and Ghosh, 2013; Kiran and Sharma, 2022). Additionally, heavy metals induce oxidative stress by generating free radicals that damage cellular structures and impair seedling development (Seneviratne et al., 2019).

During germination, the applied concentrations of the three heavy metals inhibited radicle and hypocotyl growth differentially, resulting in reduced length and decreased fresh and dry biomass. The growth of the hypocotyl and radicle is susceptible to the toxic effects of metals, often being completely inhibited at low concentrations that have little effect on germination (Kranner and Colville, 2011). Sanjosé et al. (2021) reported that at 4000 µM of Cu, the length of the hypocotyls in *Salsola vermiculata* was significantly reduced to 46% of the control, while the radicle length was significantly affected from 250 µM, decreasing by 71% at that concentration and 28% at 4000 µM. In contrast, Zn did not affect hypocotyl length at any concentration; however, it caused a significant reduction to 49% of the control at 4000 µM. In another study on chia, stem and root lengths decreased by 44.9% and 63.7%, respectively, at the highest cadmium concentration tested (60 mg/L) (Stefanello et al., 2019). This delay in seedling growth observed in several studies is linked to the metabolic and physiological disturbances caused by heavy metal concentrations in several plant species, including *Dorycnium pentaphyllum* (Lefèvre et al., 2009), *Solanum nigrum* (Al Khateeb and Al-Qwasemeh, 2014), *Triticum aestivum* (Guilherme et al., 2015), *Crambe abyssinica* (Hu et al., 2015), *Eruca sativa* (Zhi et al., 2015), *Cicer arietinum* (Ahmad et al., 2016), and *Lactuca sativa* (Moreira et al., 2020). In most cases, heavy metals' toxic

effects on plant growth depend on both time and dose. Our results on post-germination parameters in chia suggest that the reduction in radicle growth may be attributed to the direct interference of metals with hydrolytic enzymes, necessary for nutrient transport to the primary root and stem (Sethy and Ghosh, 2013; Muszyńska and Labudda, 2019). Meanwhile, the decrease in hypocotyl length could result from either the direct inhibition of cellular elongation or division, combined with slower root growth and diminished transport of nutrients and water, which ultimately impacts biomass (Muszyńska and Labudda, 2019). In general, the inhibition of seed germination and the dynamics of growth are influenced by several factors, including the specific type of metal, its concentration, the length of time the seeds are exposed, the species of plant (whether they are sensitive or tolerant), as well as the variety and properties of the seeds, particularly the composition of their seed coats (Munzuroglu and Geckil, 2002).

The germination rates and the tolerance index calculated for the different metal treatments reveal that chia seedlings are capable of thriving in environments containing elevated levels of Cd, Cu, and Zn. Consequently, this suggests that chia could be a suitable choice for cultivation in polluted regions, particularly those with elevated levels of copper.

CONCLUSION

The present study evaluated the effects of three metal treatments (Zn, Cu, and Cd) on the germination and post-germination growth of chia seeds. The final germination rate of chia showed no significant change under any of the three metal treatments. However, the studied parameters related to post-germination growth were negatively and differentially affected by the metal treatments, leading to a notable inhibition of radicle and hypocotyl elongation, as well as a significant decrease in both fresh and dry biomasses. Comparatively, chia demonstrated a high tolerance index to Cu, followed by Cd, and then Zn. Additional physiological and biochemical tests are required to improve our comprehension of how metal stress affects the germination of chia seeds.

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