



Effect of calcium and organic matter as an alternative for reducing cadmium toxicity in maize

Efecto del calcio y la materia orgánica como alternativa de reducción en la toxicidad de cadmio en maíz

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Abstract

Cadmium (Cd) contamination in maize is crucial for ensuring food security, as this heavy metal can accumulate in the grains and pose a significant risk to human health. The objective of this research was to evaluate calcium (Ca) sources and organic matter (OM) as alternatives to reduce cadmium uptake in maize. A completely randomized design with a 2 x 4 factorial arrangement was used, where different treatments combining OM application and Ca sources were evaluated. Soil preparation included the addition of compost in the OM treatments. Cd contamination was induced by adding cadmium chloride (CdCl_2), followed by the application of Ca sources through irrigation. Maize seeds were planted in pots with consistent irrigation to maintain adequate moisture. Over a period of 60 days, various variables were monitored and recorded, including dry weight and Cd concentration in roots and stems. The results showed highly significant differences in the dry biomass of maize leaves and roots exposed to cadmium contamination, both in simple effects and in the interaction between OM and Ca sources. It was observed that the incorporation of 2% OM and calcium nitrate $\text{Ca}(\text{NO}_3)_2$ had a significant impact on biomass production and Cd concentration in maize plants exposed to the contaminant, both independently and in combination.

Keywords: heavy metal; cadmium uptake; dry biomass.

Resumen

La contaminación por cadmio (Cd) en maíz es crucial para garantizar la seguridad alimentaria, dado que este metal pesado puede acumularse en los granos y representar un riesgo significativo para la salud humana. El objetivo fue evaluar fuentes de calcio (Ca) y materia orgánica (MO) como alternativa de reducción en la absorción de cadmio en maíz. Se empleó un diseño completo al azar con un arreglo factorial de 2 x 4, donde se evaluaron diferentes tratamientos que combinaban la aplicación de MO y fuentes de Ca. La preparación del suelo incluyó la adición de compost en los tratamientos con MO. La contaminación con Cd se realizó adicionando cloruro de cadmio (CdCl_2), posteriormente, se aplicaron las fuentes de Ca mediante riego. Las semillas de maíz se sembraron en macetas con un riego constante para mantener la humedad a capacidad de campo. Durante un periodo de 60 días, se monitorearon y registraron diversas variables, incluyendo la biomasa seca y concentración de Cd en raíces y tallos. Los resultados mostraron diferencias altamente significativas en la biomasa seca foliar y de la raíz de las plantas de maíz expuestas a la contaminación por cadmio, tanto en los efectos simples como en la interacción entre la MO y la fuente de Ca. Se observó que la incorporación de un 2% de MO y nitrato de calcio $\text{Ca}(\text{NO}_3)_2$ tuvo un impacto significativo en la producción de biomasa y en la concentración de Cd en las plantas de maíz expuestas al contaminante, tanto de manera independiente como en conjunto.

Palabras clave: metal pesado; absorción de cadmio; biomasa seca.



Introduction

Zea mays L., better known as corn, is one of the most widely recognized crops globally, both for its production and its crucial role in food security, as well as for human and animal consumption. Its versatility and widespread use make it an essential dietary component in many parts of the world (Rouf et al., 2016). However, the presence of heavy metals, particularly cadmium (Cd), in the soil poses a threat to the safety and quality of crops, human health, and environmental balance (Rashid et al., 2023).

Cadmium, known for its high toxicity, is a significant concern for both human health and ecosystems. Unlike other elements, Cd does not play any essential biological role in the environment, and its presence is merely a byproduct of human activities such as mining, industry, and intensive agriculture (Hayat et al., 2019). It can accumulate in soils and be absorbed by plants, ultimately leading to contamination of the food chain and human exposure through the consumption of contaminated food (Angon et al., 2024). This contamination raises various environmental and health issues.

In the international context, as awareness of the risks associated with Cd contamination has increased, several countries have implemented stricter environmental regulations to reduce its effects. These regulations aim to reduce exposure to heavy metals in food and promote the development of more sustainable agricultural practices to protect both the environment and human health (Hembrom et al., 2020). Regulations that set limits on allowable levels of Cd in food products have been established, leading to more attention being paid to agricultural practices that may influence the accumulation of this metal in crops.

Nationally, contamination by heavy metals is becoming an increasingly serious problem in agricultural ecosystems. Metals such as Cd accumulate in the food chain and can have detrimental effects on the physiological functions of living organisms (Luo et al., 2019). Anthropogenic activities can raise Cd concentrations in soils and groundwater, which are essential for maintaining a healthy supply of food and drinking water (Daripa et al., 2023).

Given these challenges, it is necessary to explore viable options to reduce the risks associated with Cd absorption in agricultural crops. Among the potential approaches, the use of soil amendments such as calcium (Ca) and organic matter (OM) has become a promising strategy. Each nutrient is essential for plant growth; Cd not only competes with Ca for root transport membranes due to their similar physicochemical properties but can also form insoluble complexes in the soil, reducing its availability to plants (Hayat et al., 2019). On the other hand, organic material derived from plant and animal residues can improve soil

structure and nutrient retention capacity, reducing the amount of cadmium absorbed by plants and, in some cases, facilitating its movement toward less accessible forms (Genchi et al., 2020).

In light of these considerations, the study aimed to evaluate the potential of calcium and organic matter as alternatives to reduce cadmium toxicity in corn, a widely consumed crop globally.

Materials and methods

The study employed a mixed research methodology, integrating a statistical and mathematical approach. A completely randomized design (CRD) with a 2 x 4 factorial arrangement was used, where factor A was the application of organic matter (0% and 2%), and factor B consisted of calcium sources (no calcium, calcium nitrate, calcium sulfate, and 50% calcium nitrate + 50% calcium sulfate), resulting in a total of eight treatments. Each treatment included a population of 144 plants distributed in four replications. Each replication contained a group of 36 plants, from which 10 were selected to form the sample, according to the guidelines of Arispe et al. (2019). The remaining 26 plants were maintained as part of the border effect, as recommended by Peña-Becerril et al. (2005).

Experimental management

Soil preparation: 4 kg of river sand were weighed and placed in each pot. For the treatments that included organic matter, 80 g of compost were added per pot, creating a homogeneous mixture.

Cadmium (Cd) contamination: this was carried out using CdCl₂, applying 0.9 meq of Cd·100 g⁻¹. For each treatment, the corresponding amount of contaminant was weighed and dissolved in 100 mL of water. It was then uniformly applied via irrigation, 8 days before sowing.

Application of calcium (Ca) sources: for each treatment, 0.9 meq of Ca·100 g⁻¹ was applied through calcium nitrate and calcium sulfate sources. This was dissolved in 100 mL of water and applied via irrigation 5 days after the cadmium contamination.

Sowing: the seeds were soaked for 24 hours before sowing to ensure their viability. Two seeds were planted per pot, and constant irrigation was maintained to keep the soil moisture at field capacity throughout the study period.

Table 1 shows the soil analysis used in the research, which presented a slightly alkaline pH and low organic matter content. The levels of essential nutrients such as phosphorus, potassium, calcium, and magnesium were also generally low.

Table 1. Physical-chemical analysis of soil.

Texture class	pH	Organic matter (%)	Phosphorus (mg·kg ⁻¹)	Potassium (mq·100 g ⁻¹)	Calcium (mq·100 g ⁻¹)	Magnesium (mq·100 g ⁻¹)
Sandy loam	7.6	0.9	1.3	0.06	1.4	0.24

The chemical analysis of the compost (table 2) showed a pH close to neutrality. Regarding macronutrients, nitrogen was found to be at adequate levels, while phosphorus (0.84%) and potassium (1.04%) presented moderate concentrations. Calcium, with 3.43%, stood out for its high concentration, and magnesium, at 0.82%, was found in appropriate amounts for plant development.

Table 2. Chemical analysis of the compost.

pH	Organic matter (%)	Nitrogen (%)	Phosphorus (%)	Potassium (%)	Calcium (%)	Magnesium (%)
7.47	42.68	1.98	0.84	1.04	3.43	0.82

The plant material used was the Trueno maize hybrid. To examine the impact of the application of Ca and organic matter (OM) on mitigating cadmium (Cd) toxicity in maize, the plants were kept in pots for a period of 60 days. During this period, the following variables were recorded and analyzed:

Dry biomass of root and stem: the complete plants were extracted, separating the aerial parts from the roots. Ten maize plants were collected per experimental unit (considering the border effect) and dried in an oven at 70°C until a constant biomass was obtained, which indicated that the sample was completely dry (Andrade et al., 2023).

Cd Concentration in Root, Stem, and Soil: Once the plant samples were dried, they underwent a digestion process to obtain a solution for Cd concentration analysis. The concentration of cadmium was measured using an atomic absorption spectrophotometer (Pedraza & Rojas, 2017).

Statistical Analysis: Data were analyzed using ANOVA, and for variables with significant differences, Tukey's post-hoc test was applied with a significance level (alpha) of 0.05, along with correlation analyses. All statistical processes were performed using the InfoStat 2018 software.

Results and discussion

In Figure 1, the results obtained after the application of organic matter (OM) are shown, with statistically significant differences ($P < 0.0001$) in the dry biomass of leaves (BSF) and roots (BSR) of maize. In the treatment without OM application (0% OM), the BSF and BSR were 9.79 g and 7.20 g, respectively. These values were significantly lower compared to the treatment with 2% OM, where the BSF and BSR reached 46.21 g and 21.6 g, respectively.

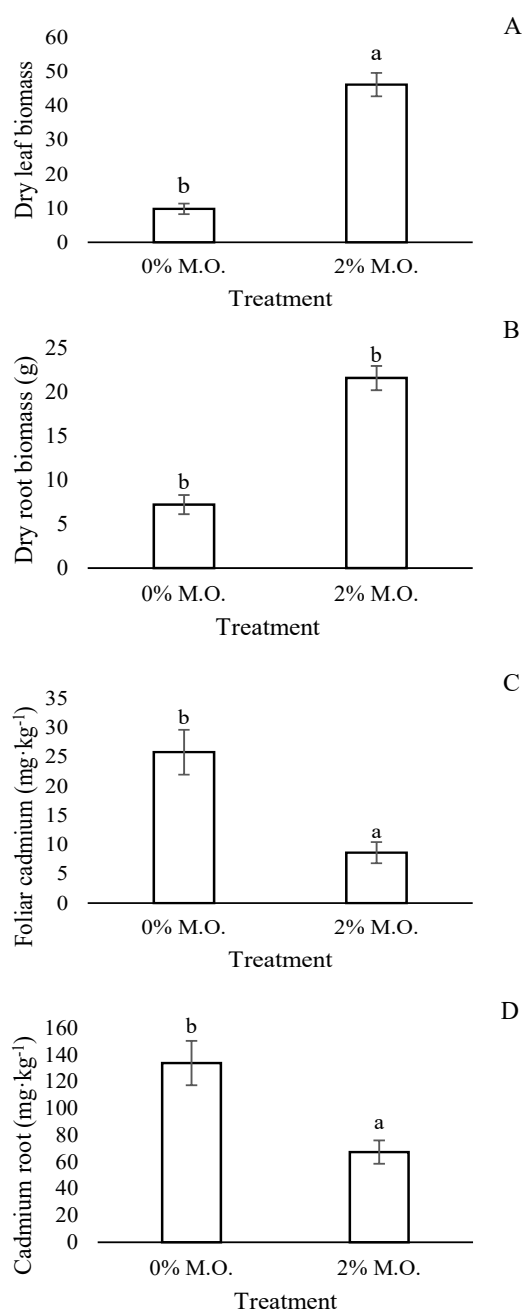


Figura 1. Dry biomass (A and B), and Cd concentration (C and D) in the stem and root of maize under Cd contamination conditions after the application of organic matter.

Furthermore, the cadmium concentration (Cd) in the foliage (CdF) and roots (CdR) also presented statistically significant differences ($P < 0.0001$). In the treatment without OM, the CdF and CdR concentrations were 25.8 mg·kg⁻¹ and 133.7 mg·kg⁻¹, respectively. This suggests that the application of OM improved plant biomass and reduced cadmium accumulation in the plant organs, indicating a potential mitigating effect on Cd toxicity. These values were considerably higher than in the treatment

with 2% OM, where the CdF and CdR concentrations were $8.6 \text{ mg} \cdot \text{kg}^{-1}$ and $67.2 \text{ mg} \cdot \text{kg}^{-1}$, respectively.

These results suggested that the application of OM at a 2% concentration significantly improved maize growth, increasing both foliar and root dry biomass, and reducing Cd accumulation in the plant. OM has been widely recognized for its ability to enhance the retention capacity of nutrients and heavy metals in the soil, as well as reduce their bioavailability and mobility (Li et al., 2019). This may explain the positive effect observed in biomass production in maize plants exposed to Cd. In similar studies, the application of amendments such as biochar has shown a significant reduction in the mobility of Cd and its absorption by plants, which is consistent with the findings of this study (Anwar et al., 2024).

Figure 2 shows the results obtained from the different Ca treatments, which displayed significant variations in plant dry biomass and Cd concentration in the maize stem and root. In the treatment without Ca addition, the foliar dry biomass (FDM) and

root dry biomass (RDM) were 20.1 and 10.8 g, respectively. The Cd concentration in the foliage (CdF) and root (CdR) were 25.4 and $135.8 \text{ mg} \cdot \text{kg}^{-1}$, respectively. These values serve as a reference to compare the effectiveness of treatments with different forms of Ca.

On the other hand, $\text{Ca}(\text{NO}_3)_2$ may play a crucial role in mitigating the negative effects of Cd on maize plants. Calcium has been shown to compete with Cd for absorption sites in the roots, thus reducing its entry into plant tissues and limiting its toxicity (Varma et al., 2017). Additionally, Ca can activate stress tolerance mechanisms in plants and regulate ionic homeostasis (Choudhary et al., 2010). These studies helped to provide an explanation for the results obtained in this research.

Table 3 shows that the foliar dry biomass (FDM) varied significantly among treatments, as indicated by the ANOVA probability ($P < 0.0001$), suggesting a clear effect of OM application and the different sources of Ca.

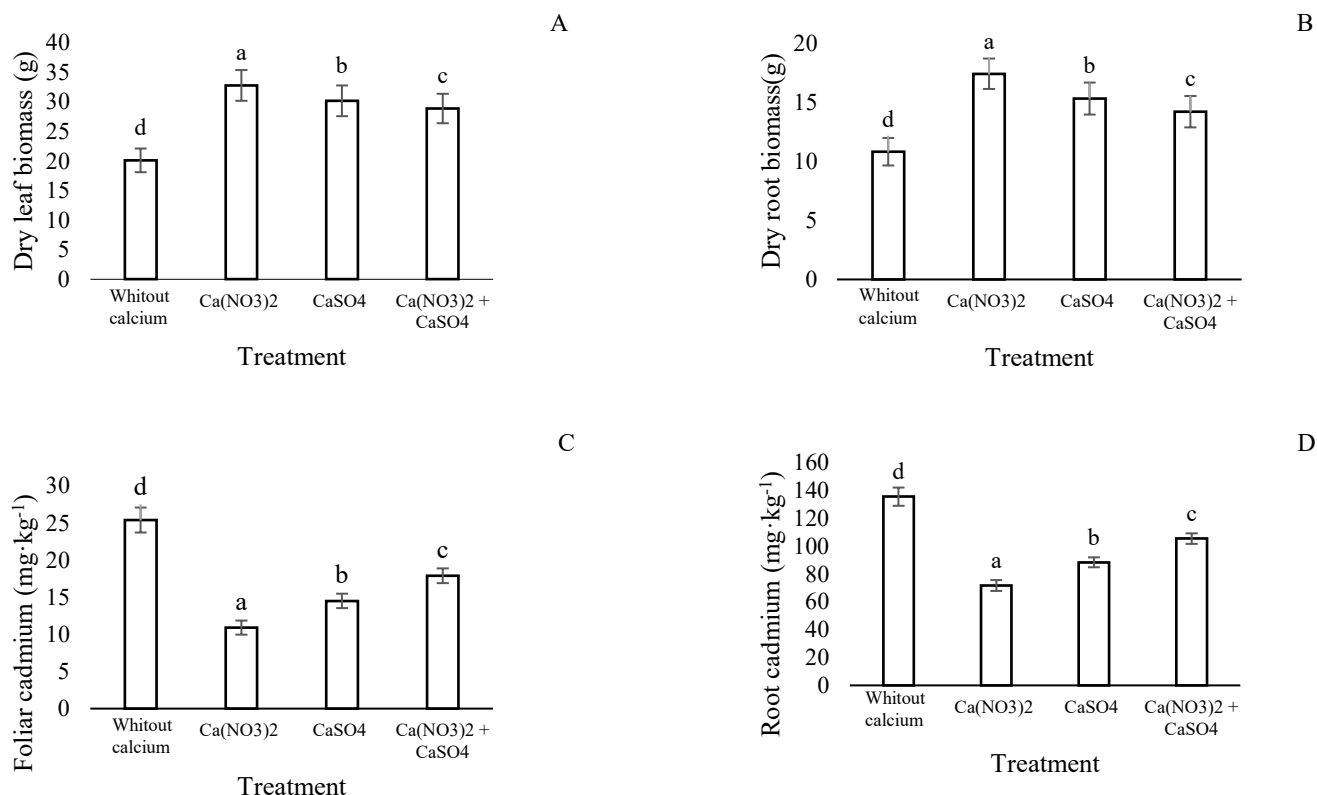


Figure 2. Dry biomass (A and B) and cadmium concentration (C and D) in maize stem and root under cadmium contamination conditions after the application of different calcium sources.

Table 3. Evaluation of leaf and root dry biomass and leaf and root cadmium concentration in maize plants exposed to cadmium contamination under potted conditions after application of organic matter and different sources of calcium.

Tratamiento	LDB (g)	RDB (g)	Cd foliar (mg·kg ⁻¹)	Cd root (mg·kg ⁻¹)
0% OM + No Calcium	5.1 ± 0.21 g	4.3 ± 0.12 h	37.8 ± 0.53 h	184.4 ± 5.04 h
0% OM + Ca(NO ₃) ₂	13.2 ± 0.18 e	10.2 ± 0.08 e	18.0 ± 0.33 e	100.9 ± 1.85 e
0% MO + CaSO ₄	10.7 ± 0.26 f	7.7 ± 0.14 f	21.8 ± 0.35 f	115.4 ± 1.79 f
0% MO + Ca(NO ₃) ₂ + CaSO ₄	10.2 ± 0.31 f	6.9 ± 0.09 g	25.5 ± 0.44 g	133.9 ± 1.39 g
2% OM + No Calcium	35.2 ± 0.36 d	17.4 ± 0.34 d	12.9 ± 0.66 d	87.2 ± 1.96 d
2% MO + Ca(NO ₃) ₂	52.4 ± 0.21 a	24.6 ± 0.19 a	3.8 ± 0.27 a	42.8 ± 0.76 a
2% MO + CaSO ₄	49.6 ± 0.54 b	22.9 ± 0.26 b	7.3 ± 0.24 b	61.6 ± 0.92 b
2% MO + Ca(NO ₃) ₂ + CaSO ₄	47.6 ± 0.31 c	21.7 ± 0.09 c	10.5 ± 0.35 c	77.2 ± 0.56 c
CV (%)	2.19	2.23	4.9	3.52
ANOVA probability	P<0.0001	P<0.0001	P<0.0001	P<0.0001

Means with common letters were not significantly different among treatments for each variable ($P>0.05$). Abbreviations: LDB = leaf dry biomass, RDB = root dry biomass, CV = coefficient of variation.

The application of calcium had a positive impact on the dry biomass of the foliar part (FDM), with Ca(NO₃)₂ standing out, showing the greatest increase compared to other treatments. Although the combination of Ca(NO₃)₂ and CaSO₄ also increased FDM, it did not surpass the efficacy of calcium nitrate alone, suggesting that using a single calcium source may be more efficient. Additionally, the incorporation of organic matter showed a significant influence on improving FDM across all treatments, especially when combined with Ca(NO₃)₂, reaching the maximum FDM value.

In the case of root dry biomass (RDM), a similar trend was observed: treatments with Ca(NO₃)₂ achieved the highest increase compared to treatments without calcium. The combination of calcium with organic matter also enhanced RDM, supporting the importance of organic matter as a complement. These results indicated that both the type of calcium source and the addition of organic matter were determining factors for optimizing FDM and RDM in maize, with Ca(NO₃)₂ being the most effective source for both variables.

The concentration of Cd in the leaves showed highly significant differences between treatments ($P<0.0001$). The application of calcium and organic matter showed significant effects in mitigating cadmium absorption in maize plants, highlighting their potential as phytoremediation strategies in contaminated soils. The absence of Ca resulted in the highest Cd concentration in the leaves, suggesting that, without a stabilizing agent, Cd freely accumulated in plant

tissues. The addition of Ca(NO₃)₂ was particularly effective, reducing this concentration by half, indicating a notable protective effect. The application of CaSO₄ and the combination of Ca(NO₃)₂ + CaSO₄ also reduced Cd levels, albeit less effectively than Ca(NO₃)₂. These results emphasized the role of calcium in reducing Cd absorption and suggested that the method of application may influence the mitigation efficiency.

The presence of OM enhanced these effects, contributing to an overall decrease in Cd absorption, likely due to its ability to improve soil properties and promote metal immobilization. The treatment with OM and Ca(NO₃)₂ achieved the lowest Cd concentration in both leaves and roots, highlighting the synergy between OM and calcium in mitigating Cd toxicity. These results were consistent with previous research, such as that by Kaleem et al. (2022), which underscored the critical role of calcium in regulating oxidative stress induced by heavy metals in plants. For example, Zhang et al. (2020) demonstrated that the application of calcium reduced Cd absorption by competing with the metal at root absorption sites, limiting its entry into the plant. Similarly, Choong et al. (2014) found that calcium reduced Cd absorption by competing for cellular transport channels due to their physicochemical similarities, thus blocking Cd entry into cells. Additionally, calcium can displace Cd from critical binding sites in cellular proteins, reducing toxic effects and Cd accumulation in plant tissues.

Furthermore, the results of this study demonstrated that the combined application of calcium (Ca) and organic matter (OM) at 2% in contaminated soils was an effective strategy to reduce cadmium (Cd) absorption in maize crops. This combination not only minimized Cd accumulation in plant tissues but also increased biomass production, a key indicator for maintaining agricultural productivity in soils affected by heavy metals. These findings suggested that the use of OM and Ca in contaminated soils could be a sustainable practice to mitigate Cd toxicity, addressing both environmental and food safety risks posed by this heavy metal. Cd, a toxic contaminant associated with industrial and agricultural activities, can accumulate in soils, posing a significant threat to human health and the environment (Alloway, 2012).

The notable reduction in Cd concentration in the leaves and roots with the application of Ca, especially when combined with OM, highlights the synergy between these treatments in limiting Cd bioaccumulation. This effect can be explained by the ability of Ca to form stable complexes with Cd, thereby decreasing its mobility and absorption by the plant roots (Mazhar et al., 2023). Additionally, OM improves the soil's nutrient retention capacity, increasing the availability of Ca and other essential nutrients for the plants, which further reinforced the mitigation of Cd toxicity (Fang et al., 2024). Furthermore, Cai et al. (2021)

and Safari et al. (2024) suggested that the combination of OM and $\text{Ca}(\text{NO}_3)_2$ seems to be more effective in reducing Cd absorption by maize, possibly due to the improvement in soil structure and nutrient retention promoted by these amendments.

Figure 3 shows a strong positive correlation ($r = 0.954$) between foliar dry biomass and root dry biomass, indicating that as biomass increased in the leaves, root biomass also increased. This relationship is expected, as greater growth of the aerial part is generally accompanied by proportional development in the root system, reflecting an overall healthy and vigorous state of the plant (Bektas et al., 2023).

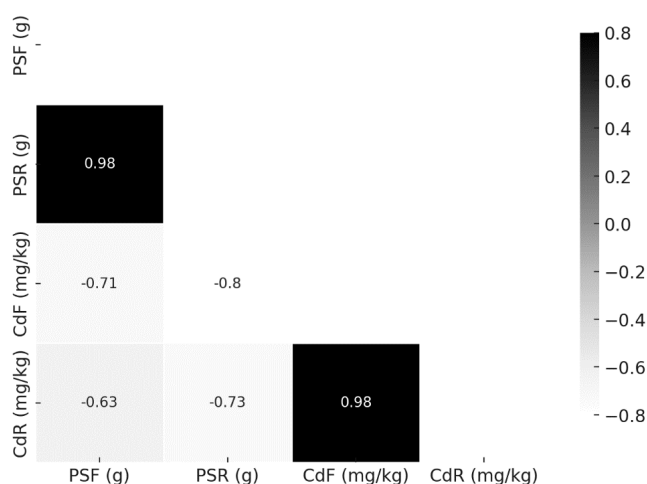


Figure 3. Correlation matrix between dry biomass variables and cadmium concentration. BSF: leaf dry biomass; BSR: root dry biomass; CdF: foliar cadmium; CdR: cadmium in the root."

On the other hand, there was a moderate negative correlation between foliar dry biomass and Cd concentration in the leaves ($r = -0.746$). This implied that as the concentration of Cd in the leaves increased, foliar dry biomass tended to decrease. This relationship was consistent with the toxic effect of Cd, which inhibited plant growth and development by interfering with photosynthesis, respiration, and other key metabolic processes. Therefore, higher Cd absorption was associated with a reduction in foliar growth (Aslam et al., 2023).

Similar to the previous relationship, the negative correlation between foliar dry biomass and Cd concentration in the roots ($r = -0.709$) indicated that a higher Cd content in the roots was also associated with a reduction in foliar dry biomass. The negative correlation between root dry biomass and Cd concentration in the leaves ($r = -0.8$) suggested that an increase

in Cd absorption in the leaves was associated with a reduction in root development. Since the root system is essential for water and nutrient uptake, the presence of Cd can damage the roots, thus reducing their biomass and affecting the plant's ability to sustain healthy growth (Kumar et al., 2024).

A moderate negative correlation was observed between root dry biomass and Cd concentration in the roots ($r = -0.73$). This indicated that higher Cd content in the roots was associated with a decrease in root biomass. This result was consistent with Cd toxicity, which can directly damage the roots, impairing their development and function (Mongkhonsin et al., 2018).

The very strong positive correlation ($r = 0.98$) between Cd concentration in the leaves and roots indicated that, in general, as Cd accumulation increased in the roots, it also increased in the leaves. This suggested that plants with a high capacity for Cd absorption tended to translocate this metal from the roots to the leaves, which could be a mechanism to minimize root damage but simultaneously exposed the foliar tissues to Cd-induced stress (Zhang et al., 2024).

Conclusion

It is evident that calcium and organic matter play a key role in reducing the availability of cadmium in the soil, as calcium directly competes as a cation and organic matter promotes the formation of insoluble complexes with metals. However, the influence of pH, which is inversely related to the solubility of heavy metals, is a critical factor that must also be considered in the availability of these elements.

Conflict of interest

The authors declare that they have no conflicts of interest in this publication at any stage.

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Author contributions according to CRediT

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