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Research Article

Cadmium Stress Responses in Medicinal and Oilseed Plants: Safflower as a Promising Candidate for Soil Cadmium Remediation

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Abstract: This study investigated the growth responses of five economically important plants—chamomile (*Matricaria recutita*), oregano (*Origanum vulgare*), safflower (*Carthamus tinctorius*), sage (*Salvia officinalis*), and wormwood (*Artemisia absinthium*) exposed to increasing Cd levels. Over 60 days, plants were subjected to Cd concentrations of 0, 1, 2, 3, and 4 mg per pot. Growth parameters and Cd accumulation were then assessed. At the highest Cd concentration (4 mg Cd pot^{-1}), the species were categorized into: (i) chamomile and safflower, which were identified as Cd accumulators due to their Cd tolerance and significant shoot Cd accumulation, and (ii) oregano, sage, and wormwood, which behaved as non-accumulator species, forming a spectrum ranging from indicators to excluders. Across species, shoot Cd concentration had a significant negative correlation with biomass production and growth indices, suggesting that higher shoot Cd levels reduce biomass in non-accumulators, likely due to dilution or concentration effects. Species with high biomass typically had low Cd accumulation in their shoots and exhibited strong Cd tolerance. We conclude that safflower, an oilseed crop, is a strong candidate for remediating Cd-contaminated soils, with potential for biodiesel production and revenue generation.

Keywords: Phytomanagement, Renewable Energy, Soil Pollution, Cd Accumulation

Introduction

Contamination in soil can be derived from heavy metals like cadmium (Cd). Such mobile elements possess the ability of being relocated from soil to plant or moved downwards to ground water. Pollution of soil Cd has increased over recent years. For instance, a recent study, which collected data on soil contamination across Europe, reported that 31% of European soils, approximately 600,000 km², are affected by heavy metal contamination (Pravalie *et al*., 2024). Additionally, 21% of the EU's topsoils are contaminated with high levels of Cd (Kafouris *et al*. 2024). Although Cd can be naturally present in soil, high concentrations are typically attributed to mining activities, industrial operations, and intensive agriculture (Li *et al*., 2024; Grammenou *et al*., 2024). This level of exposure has been linked to serious health issues, including various types of cancer (Derkacz *et al*., 2024).

Phytoremediation is widely regarded as a sustainable, eco-friendly, and economically viable solution, for mitigating Cd soil contamination. However, many studies have utilized agro-food crops for remediation. This approach presents drawbacks, such as the risk of contaminant transfer into the food chain. Furthermore, phytoremediation requires multiple growing cycles, which can be time-consuming and may not generate profit (Madhav *et al*., 2024). To overcome such limitations, phytomanagement has emerged. Phytomanagement utilizes non-edible plants for remediation purposes and energy production (biofuels) (Saran *et al*., 2024). This approach can lead to soil restoration and the generation of profit to soil end-users.

Recent studies have evaluated metal tolerance of industrial plant species with significant global market value (Jia *et al*., 2023; Saran *et al*., 2024). The ability of some medicinal plants to thrive under Cd contamination is shown by many studies, including chamomile (*Matricaria recutita*) (Bagheri *et al*., 2021; Ait Elallem *et al*., 2021; Colak *et al*., 2023), oregano (*Origanum vulgare*) (Kulbat-Warycha *et al*., 2020; Colak *et al*., 2023; Thalassinos *et al*., 2024), and sage (*Salvia officinalis*) (Al-Solaimani *et al*., 2024; Koohi *et al*., 2024; Mhadhbi *et al*., 2024). However, few investigations have assessed their Cd-tolerance and remediation potential under standardized conditions.

This investigation includes also wormwood (*Artemisia absinthium*) and safflower (*Carthamus tinctorius*). Wormwood has the ability to tolerate heavy metal contamination (Qader *et al*., 2024; Chaplygin *et al*., 2024). Its tolerance and adaptability make it a strong candidate for phytomanagement applications. Safflower is notable for its significant biomass production alongside its status as a commercially valuable oilseed crop (Diquattro *et al*. 2024; Ahmad *et al*., 2024; Amoozad *et al*., 2024; Kikis *et al*., 2025). As non-food crops, wormwood and safflower reduce the risk of introducing Cd into the food chain. This diversity also facilitates a comprehensive assessment of how varying plant types respond to Cd stress.

While phytomanagement studies have increased in the last years, limited research has been conducted on polluted fields using aromatic plants. These species allow for an exploration of the effects of Cd stress on medicinal and industrial crops, bridging the gap between agricultural sustainability and soil remediation research, and contribute to the development of sustainable strategies for managing Cd-contaminated soils while simultaneously creating economic value. This study aimed to (i) evaluate the accumulation of Cd and growth response of medicinal and industrial plant species under Cd exposure, (ii) assess their ability to remove Cd and ensure remediation, and (iii) investigate the hypothesis that plants with rapid growth concentrate low amounts of Cd due to the dilution effect.

Materials and Methods

Experimental Design

A 60-day pot experiment was conducted in a greenhouse under natural sunlight conditions at the University of Thessaly in Volos, Greece. The experiment involved chamomile, oregano, sage, wormwood, and safflower. Seeds of all five plant species were purchased from Pharmasaat Gmbh, Germany. Commercial horticultural-grade perlite (2–5 mm particle size) was used as substrate, due to its zero interaction and inability to retain metals, permitting high metal uptake. Seeds were directly sown into perlite-filled pots and germinated under the same greenhouse conditions without pre-treatment. Each plant was cultivated in individual plastic pots (15 cm diameter, 20 cm depth) containing approximately 1.5 L of perlite. The experiment lasted 8 weeks with the first 4-week period being dedicated to the initial plant development. In the second half of the 8-week period there was a spiking with 1 mg of Cd per week, by adding 10 mL per pot of a solution containing 100 mg Cd L^{-1} as $CdCl_2 \cdot 2.5\ H_2O$ (pH = 7.10±0.15, EC = 1.8 μS cm^{-1}). Pots were grouped into those of the 1st, 2nd, 3rd, and 4th week, and arranged in a completely randomized design to minimize edge effects and environmental gradients within the greenhouse. Thus, there was a total of 120 pots (5 plant species × 4 weeks × 6 replicates). Out of these, 30 [5 plant species × 6 replicates (3 treated plus 3 control)] were dedicated for the 1st week, which were treated with only 1 mg Cd per pot. The same occurred with the 30 pots of the weeks 2 (2 mg Cd pot^{-1}), 3 (3 mg Cd pot^{-1}) and 4 (4 mg Cd pot^{-1}). To sustain optimal growth, plants were daily fertilized by adding 100 mL of a solution consisting of 6 mM KNO_3, 4 mM Ca $(NO_3)_2$, 2 mM KH_2PO_4, 1 mM $MgSO_4$, 50 μM H_3BO_3, 4 μM $ZnSO_4$, 4 μM $MnCl_2$, 0.3 μM $CuSO_4$, and 50 μM FeEDTA. In addition to daily fertilization with 100 mL of nutrient solution, plants were irrigated with 100 mL of deionized water every two days to maintain consistent moisture levels.

Plant Growth Parameters and Cd Accumulation

During the second 4-week period, on the last day of each week, dedicated plants were harvested and carefully separated to shoots and roots. Shoot and root biomass and Cd concentrations within roots and aboveground biomass were measured. Plants were dried in an oven at 70^{0}C for a period of 2-3 days until no further weight loss was observed for the assessment of dry biomass. For the determination of Cd within the dried tissues, 0.5 g of each sample was dry ashed at 500^{0}C for five hours and recovered with 10 mL of 20% HCl. Cd concentration of all samples was determined by flame atomic absorption spectrometry (Perkin Elmer A3300).

Indices of Plant Growth and Cd Translocation

In order to assess how plants reacted to the presence of Cd, the Absolute Growth Rate (AGR) was determined. As proposed by Lommen *et al*. (2024), this is defined as:

$$AGR = Mt - M_{t-\Delta t}/_{\Delta t},$$

Where:

M is the biomass at two successive time points, divided by the time interval between these points.

In addition, to evaluate the resistance of plants to Cd, the Growth Ratio (GR) was measured, defined as the ratio of plant biomass (shoot or root) when exposed to Cd compared to its control biomass. Values of GR exceeding the value of 1 indicate that the presence of Cd has little to no effect on plant growth.

To evaluate the effectiveness of plants extracting Cd from perlite, total Cd uptake in shoots was calculated as indicated by Liu *et al*. (2024); Ma *et al*., (2024): Total Cd in shoots = $[Cd]_{shoots} \times M_{shoot}$, where M_{shoot} is shoot biomass. As for Cd movement within the plant, the Translocation Factor (TF) was measured as proposed by Kikis *et al*. (2024). Specifically, TF is defined as: TF= Cd concentration in the aboveground biomass / Cd concentration in the root system. Additionally, TF values exceeding the value of 1, indicate easy movement of the contaminant from the roots to the upper parts of the plant.

Statistical Analysis and Quality Control

Blanks were included in all extraction batches to account for potential in-lab contamination. Reference material was also utilized to ensure the accuracy of the analyses. For the analysis of data end extraction of results, the SPSS software (IBM SPSS Statistics v.29) was used. The data underwent analysis of variance (ANOVA) to assess the impact of plant species and Cd treatments on shoot and root biomass, AGR, GR, and the accumulation of Cd by the plant species. In order to identify significant differences between means ($p<0.05$) Duncan's test was employed.

Results

Plant Growth and Cd Tolerance

Plant species exhibited significant variations in their growth (Fig. 1a-c). Safflower and wormwood exhibited superior biomass production relative to the other species. Additionally, they demonstrated higher AGR, underscoring their robust growth performance. As the concentration of Cd in perlite increased, there was a significant decrease in biomass production and AGR in all plants. In fact, in the Cd concentration of 4 mg Cd per pot, shoot biomass decreased by a rate of 3.4% to 35.2% and root from 5.5% to 32% among the species compared to the control. Out of the five plants, chamomile and safflower demonstrated the least reduction in biomass production in the highest added Cd (4 mg pot^{-1}), indicating a notable tolerance to Cd-induced stress.

In addition, Growth Rate (GR) significantly decreased among plant species as Cd concentrations increased in perlite (Fig. 2a-b). Notably, interactions between species and Cd treatments were observed, indicating that a plant's Cd tolerance depends on the exposed Cd concentration. For instance, oregano exhibited the highest GR_{shoot} and GR_{root} values at the lowest Cd treatment (1 mg pot^{-1}). In contrast, chamomile and safflower demonstrated the highest GR_{shoot} and GR_{root} values at the highest Cd treatment (4 mg pot^{-1}). Moreover, chamomile and safflower experienced the smallest decreases in GR_{shoot} and GR_{root} among the five plant species at Cd concentrations of 2, 3, and 4 mg pot^{-1}.

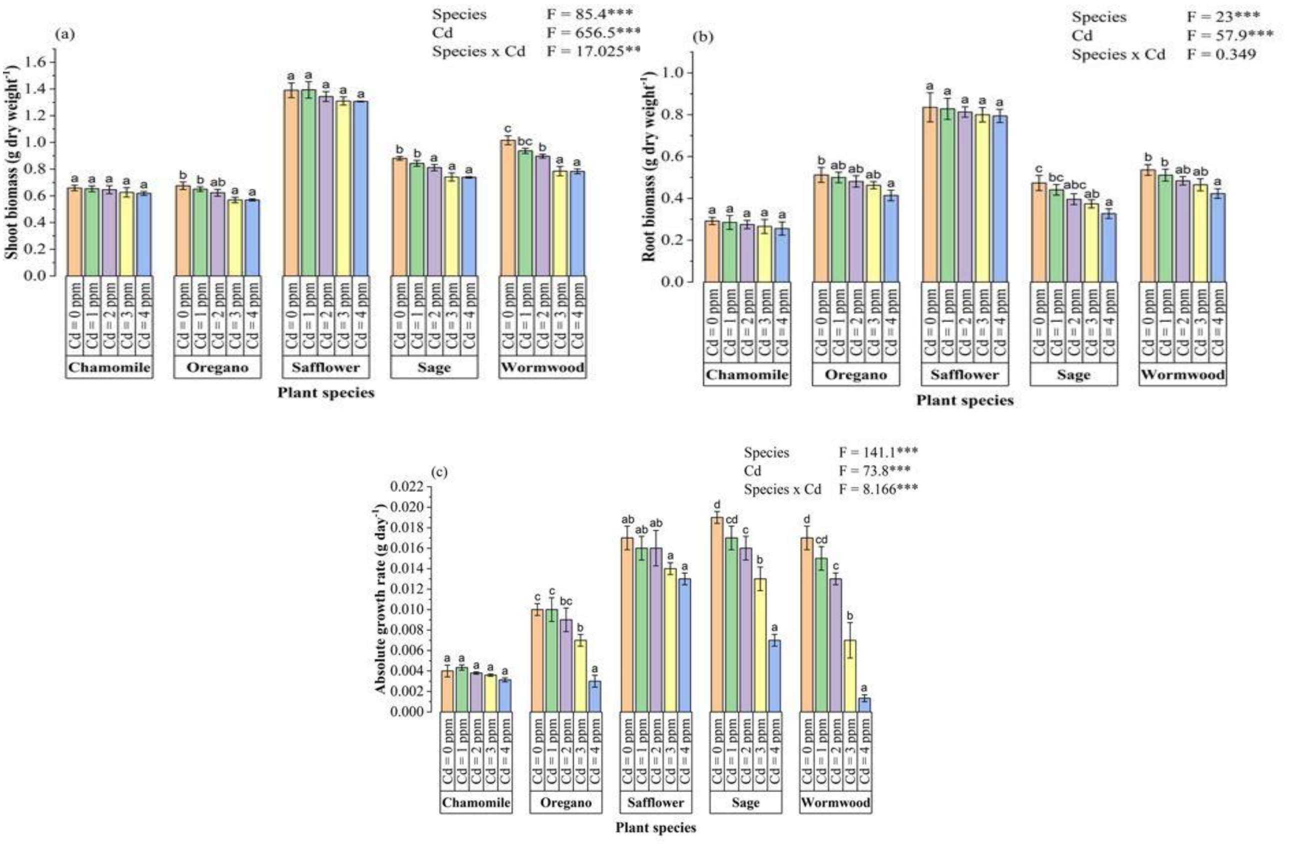

Fig. 1: Shoot (a) and root (b) production and absolute rate growth (c) of the 5 tested plants, chamomile (*Matricaria recutita*), oregano (*Origanum vulgare*), safflower (*Carthamus tinctorius*), sage (*Salvia officinalis*), and wormwood (*Artemisia absinthium*) at different added Cd levels in perlite. Different letters within species indicate significant differences at $p<0.05$. Values are mean ± SE (n = 3); *** indicate significance at p 0.001

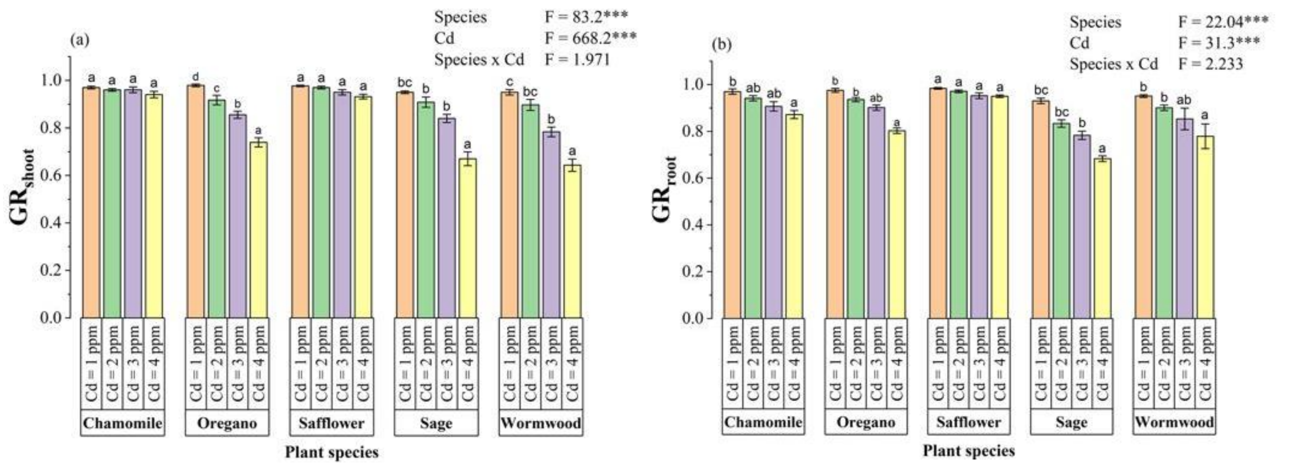

Fig. 2: Shoot (a) and root (b) growth ratio of the five tested plant species, chamomile (*Matricaria recutita*), oregano (*Origanum vulgare*), safflower (*Carthamus tinctorius*), sage (*Salvia officinalis*), and wormwood (*Artemisia absinthium*) at different added Cd levels in perlite. Different letters within species indicate significant differences at $p < 0.05$. Values are mean ± SE ($n = 3$); *** indicate significance at $p<0.001$

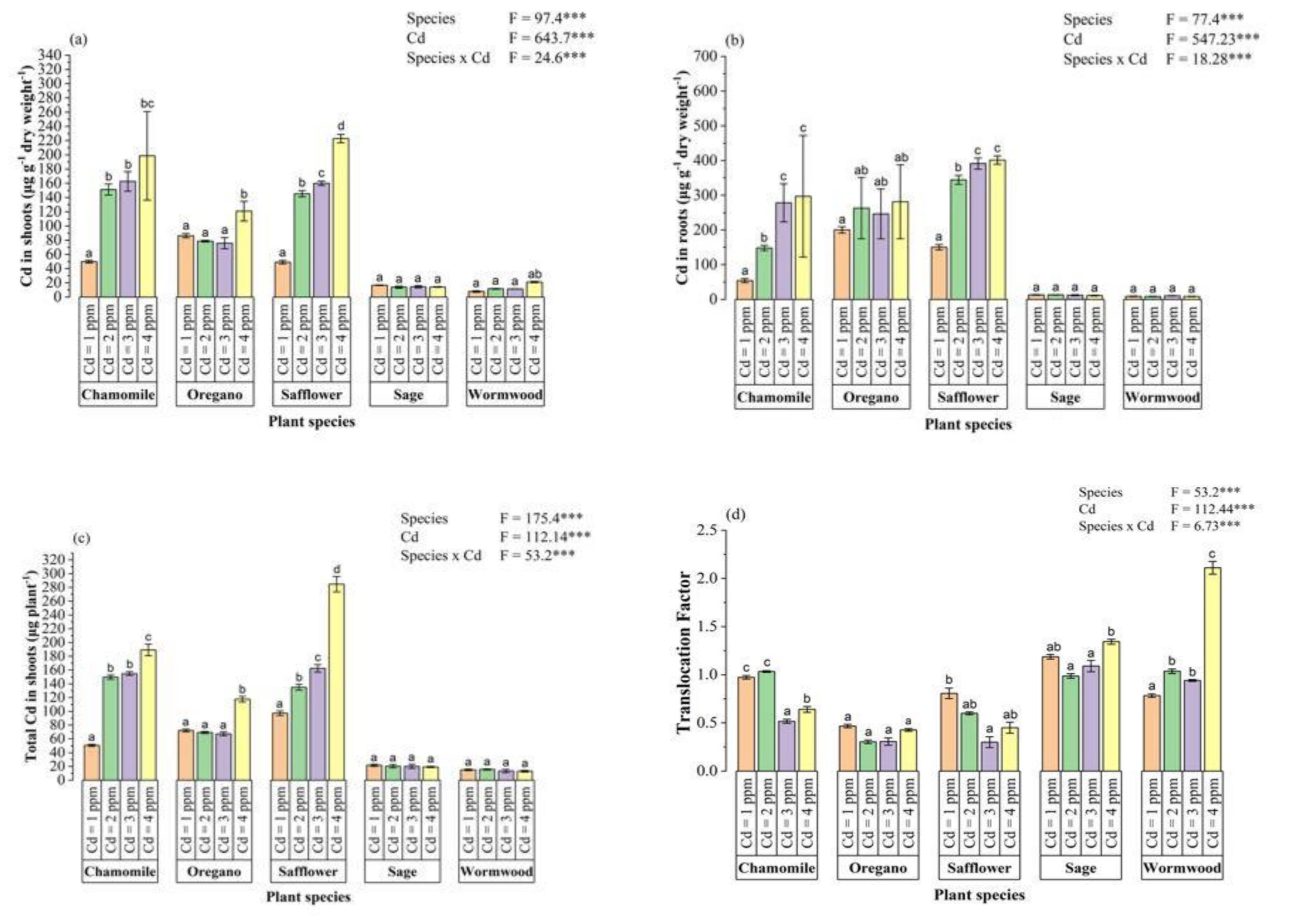

Fig. 3: Cd in shoots (a), Cd in roots (b), total Cd (c), and translocation factor (d) of Cd in chamomile (*Matricaria recutita*), oregano (*Origanum vulgare*), safflower (*Carthamus tinctorius*), sage (*Salvia officinalis*), and wormwood (*Artemisia absinthium*) at different added Cd levels in perlite. Different letters within species indicate significant differences at $p<0.05$. Values are mean ± SE ($n=3$); *** indicate significance at $p<0.001$

Cd Accumulation in Plants

Cd concentrations in both shoots and roots were significantly influenced by both plant types ($p < 0.001$) and Cd doses ($p < 0.001$), as well as the interaction between them ($p < 0.001$). The accumulation of Cd within shoots and roots increased with the increase of Cd dosage in perlite for the majority of the plants (Fig. 3a- b). Notably, shoot and root Cd accumulation was decreased in the case of sage, while wormwood displayed a decrease only in the accumulation of Cd in the roots. The Cd concentration in plant shoots ranged from 7.93 to 97.45 mg kg^{-1} for the lowest Cd treatment (1 mg pot^{-1}), and from 14.15 to 198.6 mg kg^{-1} for the highest Cd treatment (4 mg pot^{-1}) among the species. Chamomile exhibited the highest shoot Cd concentration, followed by safflower and oregano, while wormwood and sage showed the lowest Cd accumulation. For root accumulation, Cd concentrations varied from 8.80 to 200.21 mg kg^{-1} for the 1 mg pot^{-1} treatment, and from 8.3 to 443.12 mg kg^{-1} for the 4 mg pot^{-1} treatment across the species. The concentrations of Cd in safflower exceeded by far those of the rest of the species and was followed by chamomile and oregano. Sage and wormwood displayed the lowest Cd accumulation in both shoots and roots at all treatments. Furthermore, safflower exhibited the highest total Cd concentration in its shoots, followed by chamomile. Specifically, the Cd concentration in safflower shoots was on average 19-fold higher than the lowest concentration observed in plants treated with 1 mg pot^{-1} of Cd, and 25-fold higher than the lowest concentration in plants treated with 4 mg pot^{-1} of Cd. Both safflower and chamomile exceeded the Cd hyperaccumulation threshold of 100 mg Cd kg^{-1}, indicating promising potential for remediating Cd-contaminated soils. The TF was also significantly influenced by the plant species and Cd concentrations as well as the interactions between them. Chamomile (only in the 2 mg pot^{-1} treatment), as well as sage and wormwood, they all surpassed the critical value of the translocation factor (TF > 1).

Correlation Between Cd Accumulation and Plant Growth

For all species except safflower, there was a significant negative correlation between Cd shoot concentration vs. shoot biomass (Fig. 4a; $p < 0.01$), root biomass (Fig. 4b; $p<0.05$), and AGR (Fig. 4c; $p < 0.05$). As for safflower, a negative non-significant correlation between shoot Cd concentration vs. shoot biomass production ($r = 0.762$), root biomass ($r = 0.713$), and AGR ($r = 0.842$) were observed. Safflower was isolated from the analysis due to the observed dilution effect. If safflower was included in the dataset, the trend lines would be significantly flattened. This suggests that safflower exhibits a fundamentally different response pattern compared to the other plant species.

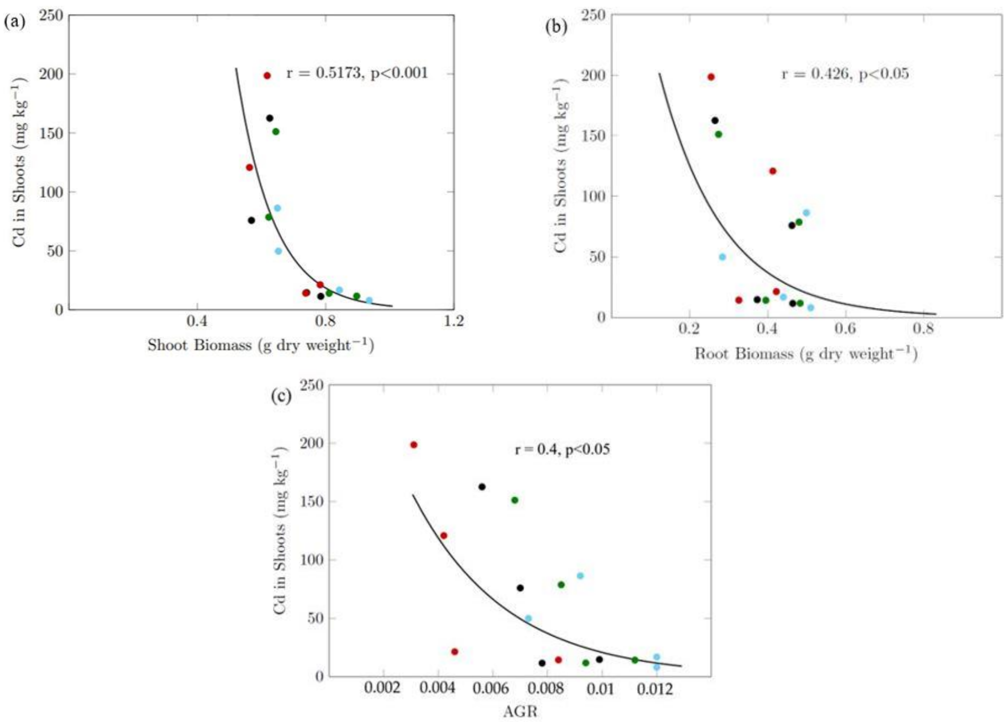

Fig. 4: Correlation between Cd concentrations in the shoots and (a) shoot biomass, (b) root biomass, and (c) absolute growth rate (AGR) of the plant species exposed to 1 mg kg^{-1} (light blue circles), 2 mg kg^{-1} (green circles), 3 mg kg^{-1} (black circles) and 4 mg kg^{-1} (red circles) Cd in the perlite

Discussion

Our results reveal that chamomile and safflower had a different behavior in terms of Cd accumulation (Fig. 3). When exposed to 4 mg Cd pot^{-1}, chamomile accumulated Cd concentrations as high as 198.6, safflower 193.41, and oregano 120.7 mg kg^{-1}—approximately 49 and 30 times higher than the added Cd concentration (Fig. 3). It is important to note that this study was conducted in perlite as a growth medium rather than soil. Such experiments often yield more pronounced results concerning plant accumulation due to the enhanced mobility of metals to plants, and do not represent field conditions. In this case, it was decided to opt for a non-reactive growth medium in order to elucidate more clearly the effects of Cd to plants as an initial assessment before moving to a study under more realistic conditions, i.e., soil.

Safflower exhibited robust biomass production (Fig. 1), demonstrated Cd tolerance (Fig. 2), and achieved substantial Cd accumulation in its tissues (Fig. 3d). Total Cd in shoots (Fig. 3c) exhibited the efficiency of Cd extraction, with safflower demonstrating the highest Cd ability among the species. Safflower is easily cultivated as a prevalent oilseed crop which can be used for biodiesel and energy production. In this case it could be a feasible option for remediating contaminated soils, while returning profits to landowners (Isık *et al.*, 2024; Pannacci *et al.*, 2024). Once the clean-up is completed, these lands could resume food production.

In general, plants employ the strategy of exclusion to cope with Cd contamination by developing mechanisms to mitigate toxicity (Li *et al*., 2024). Our findings indicate that chamomile and safflower exhibited strong resistance capabilities against Cd stress. Despite their ability to tolerate Cd, these plants typically show lower Cd concentrations in their shoots, resulting in low TF values (as indicated in Fig. 3d). This could be the result of the "growth dilution effect," where plant biomass increases at a higher pace than Cd uptake increase, thereby diluting the Cd concentration in its tissues (Li *et al*., 2024; Huang *et al*., 2024). This strategy falls under the "avoidance" mechanism of dealing with Cd contamination, a characteristic of excluders. Thus, chamomile and safflower in our study demonstrated the ability to mitigate Cd stress either by diluting Cd within their biomass or by immobilizing it in the roots.

Several studies observed a significant increase in Cd accumulation in safflower treated with 4.5 mg kg^{-1}, accompanied by TF values below 1 (Kovacik *et al*. 2020; Kovacik *et al*., 2022). Additionally, their study also reported a significant reduction in growth parameters in the 4.5 mg kg^{-1} treatment in a hydroponic experiment. In contrast, our study found only a 6% overall decrease in growth in the 4 mg per pot treatment. Amjadi *et al*. (2021) strongly advocated for safflower use in remediating Cd-contaminated soils. Our study's results align with other works which strongly support safflower as a suitable candidate for remediating Cd-contaminated sites (Namdjoyan *et al*., 2011; Pourghasemian *et al*., 2013). Additionally, its oil content can be used for biodiesel production. This dual-use capability makes safflower a perfect fit for phytomanagement scenarios (Beyyavas *et al*., 2024).

Sage and wormwood were sensitive to Cd and primarily accumulated Cd in their aboveground biomass. Wormwood exhibited the highest TF levels, reaching up to 2.2 in the 4 mg per pot treatment. This elevated TF can be attributed to the use of perlite as a substrate. Equally, high Cd concentrations in the aboveground parts of wormwood plants have also been observed (Chaplygin *et al*. 2024). Sage exhibited a high translocation ability, relocating Cd to the upper parts of the plant. In the same way Salvia species have been reported to accumulate Cd primarily in leaves, followed by roots and stems (Lone & Gaffar 2021). These species can be classified as bioindicators of high Cd levels in the environment. On the other hand, oregano exhibited a maximum TF value of 0.48 and a biomass decrease of approximately 25% at the highest Cd dose. Similar results found maximum TF values ranging from 0.04 to 0.2 in soil spiked with 40 mg Cd kg^{-1} (Thalassinos *et al*. 2023). However, in such cases, minimal Cd uptake of 0.006 mg kg^{-1} was reported (Marinescu *et al*. 2020). These studies were conducted in contaminated soils, whereas our study used perlite as a substrate, which allows for immediate metal availability.

Conclusion

Studied plants exhibited varying Cd accumulation and tolerance to Cd stress. Based on that, species could be categorized into two main classes: (1) chamomile and safflower, which demonstrated significant Cd resistance and accumulation in shoots, thus being considered as Cd accumulators. Safflower was able to maintain plant growth under Cd stress, with lower Cd concentrations in shoots, due to the growth dilution effect. Future experiments incorporating metal absorption enhancers could increase Cd phytoextraction, potentially leading to hyperaccumulation of Cd by safflower. (2) Species, classified as non-accumulators, ranged from Cd-sensitive species (indicators) to Cd excluders. This study concludes that safflower is a promising crop for remediating Cd-contaminated soils. Furthermore, its potential for biodiesel production makes safflower an ideal candidate for phytomanagement strategies.

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved. We declare compliance with institutional guidelines for plant research.

Author's Contributions

Christos Kikis: Conducted the experiment and collected the data; performed the statistical analyses; interpreted the results and wrote the manuscript.

Giorgos Thalassinos and Vasileios Antoniadis: Conceived and designed the study; reviewed and supervised the experiment. All authors approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest related to this study.

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