



## Integrated phytoremediation of lead and cadmium-contaminated soils using *Helianthus annuus*: A pathway to green remediation

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### ABSTRACT

Phytoremediation is an eco-friendly and cost-effective approach that utilizes plants to remediate heavy metal contamination in urban and rural environments. A greenhouse study was conducted to evaluate the ability of sunflowers (*Helianthus annuus L.*) to absorb heavy metals such as lead (Pb) and cadmium (Cd) from contaminated soils. The soil was treated with varying concentrations of Pb (100 and 150 mg/kg) and Cd (20 and 30 mg/kg), as well as combinations of Pb and Cd (10+50 and 15+75 mg/kg). Fertilizers including urea, di-ammonium phosphate (DAP), and potassium sulfate (SOP) were applied to the treatments. The collected soil was sterilized using 10% formalin, and its physical properties—such as water content, organic matter, pH, and particle size—were assessed following ASTM standards. Results indicated that the sunflower's ability to remediate individual metals was more effective than for combined metal treatments. The efficiency of phytoremediation increased with higher concentrations of Pb and Cd. Statistical analysis using Tukey's HSD test further highlighted sunflower's significant potential as a phytoremediator, alongside its role in food and energy production.

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**Capsule Summary:** A greenhouse study evaluated sunflower's ability for phytoremediation of lead (Pb) and cadmium (Cd) from contaminated soils, demonstrating higher efficiency for individual metals than combined treatments. The study highlights sunflowers' dual role in environmental remediation and sustainable production.

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### INTRODUCTION

Most of the time, the term "heavy metal" (HM) refers to metals and metalloids that are linked to environmental pollution, toxicity and negative effects on living things like plants (Ayejoto and Egbueri, 2024; Zhao et al., 2024). Heavy metals are naturally occurring metals with an elemental density of more than 5 g/cm<sup>3</sup> and an atomic number greater

than 20 (Ali and Khan, 2018). Due to contrived activities like mining, burning fossil fuels and electroplating, the ongoing plundering of natural resources causes the release of HM into the environment with the rapid development of the global economy (Ali et al., 2019).

Cadmium (Cd) is a major HM pollutant that is highly toxic, persistent in soil and relatively easily absorbed by plant roots (Reyes-Calderón et al., 2022). As a result, it can disrupt the food chain and then bioaccumulate in people's bodies,

manifesting its toxic effects (Suhani et al., 2021). Several variables can affect Cd absorption, but pH is among the most significant factors because a rise in pH for a certain period multiplies the amount of Cd that soils can absorb (Zhai et al., 2018). The usage of Cd-containing fertilizers, atmospheric deposition of combustion emissions and mining are a few significant anthropogenic sources of Cd (Kubier et al., 2019). Due to its same ionic radius, equal charge and similar chemical behavior, Cd is one of the most hazardous and mobile elements in the environment, and may replace calcium in minerals. As a result, Cd can enter the body of a human and build up to high levels in various organs. Cd enters the human diet mostly through terrestrial channels, such as through vegetables, in contrast to other harmful elements like mercury and arsenic (Chen et al., 2018).

Lead (Pb) is the core and most toxic heavy metal present in the contaminated soils. Photo extraction and phytostabilization are two separate techniques used in the phytoremediation of Pb-contaminated soil (Espada et al., 2022). Pb has no biological purpose in plants, despite the possibility that it could cause biochemical and physiological problems (Collin et al., 2022). Additionally, it is a hazardous heavy metal that negatively impacts several physiological plant systems, inhibiting growth and ultimately reducing crop yields (Zulfiqar et al., 2019).

Phytoremediation, sometimes known as "green remediation," is thought of as an environmentally beneficial method of restoring soil (Tiwari and Pal, 2021). Phytoremediation is an eco-friendly, cost-effective, and sustainable approach that utilizes plants to remove, degrade, or stabilize contaminants from soil, water, and air. This green technology is gaining attention as an alternative to conventional remediation methods due to its minimal environmental impact and ability to restore polluted ecosystems. Plants used in phytoremediation employ various mechanisms to absorb heavy metals pollutants, and other hazardous substances. With advancements in biotechnology and plant genetics, phytoremediation is becoming a promising solution for mitigating environmental pollution while maintaining soil fertility and ecosystem balance (Kavusi et al., 2022). Plants must be used to clean up pollution in the environment to get the ecology and ecosystems back in balance (Awa and Hadibarata, 2020). A cost-effective and environmentally friendly technology called Phytoremediation uses plants to remove pollutants from both urban and rural environments (Ashraf et al., 2019).

Ornamental plants contribute to preserving biodiversity, providing various environmental services and enhancing human wellbeing (Rocha et al., 2021). Sunflower (*Helianthus annuus* L.), one of the world's most significant crops, can be used for phytoremediation and providing food and energy (Mathur et al., 2022). It has been examined for its growth on contaminated ground for simultaneous remediation and additional energy generation because it is a known metal accumulator (Wang et al., 2021). It is a crop with a large

biomass, and has additional benefits of oil and biomass that make it useful for phytoremediation of areas polluted with HM (Edgar et al., 2021).

One of the most environmentally friendly crops that are used in a variety of situations for environmental clean-up is sunflower which has important agronomic traits like tolerance to high and low temperatures and adaptation to different soil and climate conditions (Haq et al., 2020). It grows quickly, has a large biomass and can hyper-accumulate HM. The amount of HM removed from the polluted soil varied depending on how long the sunflower plant was grown (Al-Jobori and Kadhim, 2019).

The term "hyperaccumulator" refers to plants that can thrive on soil that is polluted with metals, and can collect large levels of metals in their organs far more quickly than other plants. Hyper accumulator plants have a high aboveground/root concentration ratio because the majority of the heavy metals they absorb are dispersed in the aboveground sections of the plant (Cheng et al., 2021).

Through several phytoremediation processes, sunflowers can purify soil that has been contaminated with Cd and Pb. The primary entry point for HM into sunflowers is through their roots. Concerning soil characteristics and sunflower varieties, it absorbs HM differently. The main element influencing how readily HM is absorbed by plants is their concentration in soil solutions (Kaninga et al., 2020). According to reports, in environments with mixed metal contamination, sunflowers experience simultaneous hyperaccumulation of several HM. Sunflowers' ability to absorb metals is significantly influenced by the duration of exposure. After planting, sunflowers can absorb Cd and Pb in their buds for up to 8 weeks. The most crucial elements in the phytoremediation of contaminated soil utilizing sunflower plants are the type of metal and the timing of exposure (Genchi et al., 2020).

## MATERIAL AND METHODS

### Sample collection

In the present study, the soil samples were collected from locales close to Lahore in August 2022, and transferred to the lab immediately. For the establishment of the nursery, Hysun-33 sunflower hybrid seeds were used.

### Sunflower seeds

Like many seeds with hard shells, require a little assistance to help them sprout. A small beaker was used and seeds were placed within it with water covering them. Before sowing, seeds were submerged in water for about 8 to 10 hrs. After being removed from the beaker, the sunflower seeds were wrapped in wet tissue paper. The seeds were then covered in an airtight zip bag. It was placed outside in the morning sun for a couple of hrs. After a few days, seeds were removed from the bag and sprouting was observed. The seeds

were then covered in an airtight zip bag. It was placed outside in the morning sun for a couple of hrs. After a few days, seeds were removed from the bag and sprouting was observed. Seed trays were used to develop seedlings that were healthier, more consistent and vigorous without disrupting the root system. In the soil, sunflower seeds were sown one inch deep.

### Experimental Design

An experiment was conducted to assess the effects of sunflower plant-based phytoremediation on lead and cadmium-contaminated soil. In 2022, during the Kharif season, the experiment was conducted. The following treatments were used in three replications of the study, which was conducted using a completely randomized design (CRD).

### Experimental treatments

The study comprised seven treatments with three replicates of each concentration i.e. Control (zero Cd and Pb) Cd 20 mgkg<sup>-1</sup> soil, Cd 30 mgkg<sup>-1</sup> soil, Pb 100 mgkg<sup>-1</sup> soil, Pb 150 mgkg<sup>-1</sup> soil, Cd 10 + Pb 50 mgkg<sup>-1</sup> soil and Cd 15 + Pb 75 mgkg<sup>-1</sup> soil. To prevent deficits of other important nutrients, potassium (K) was applied at the prescribed rate as sulphate of potash, nitrogen (N) was applied as urea fertilizer and phosphorus(P) was applied as di-ammonium phosphate (DAP). Soil weighing 500 g was taken and in labeled plastic containers. Three replicates of the control were then given 100 mL solutions containing water and supplementary nutrients. It is required that the poured solution reach the bottom of plastic containers uniformly before planting seeds in the soil. Sunflower seedlings from a seed tray were then placed in pots with soil that had the appropriate concentrations of heavy metal solutions. For proper growth, pots received 10 mL of water for a few days. Pots received enough sunlight to promote rapid growth and also to stave against fungal infection. After the seedlings had developed, the amount of water provided was raised to 30 or 40 mL, depending on how much water was required to moisten the top of the pots.

### Analysis of plant samples

The plant samples were collected after 14 days, when the foliage began to turn yellow and the biomass yield was determined by weighing the entire plant. For physical inspection, the plant samples were carefully removed from plastic containers. Throughout the test, the plant's height, and total plant weight was observed.

### Cd uptake by Plant

It was calculated using the relation shown in Eq. 1. By utilizing an acid digestion reagent combination; including nitric acid and hydrogen peroxide, 0.20 g of each biological

sample was digested. Cadmium in plants was examined using MP-AES, or microwave plasma-atomic emission spectroscopy (Oliva et al., 2019).

$$Cd \text{ uptake } \left( \frac{g}{pot} \right) = \frac{Biomass \left( \frac{g}{pot} \right) plant \text{ Cd}(\%)}{100} \quad (1)$$

### Pb uptake by Plant

It was calculated by using Eq. 2. Lead Concentration in the plant was determined using an acid-digestion extraction process. This extraction process utilized aqua regia which was created by combining strong concentrated hydrochloric acid (HCl) and concentrated nitric acid (HNO<sub>3</sub>) in a ratio of 3:1. The extracted samples are ready to be used for further laboratory examination after the extraction procedure is finished. The analysis was conducted utilizing the AAS (Atomic Absorption Spectrophotometry) measurement technique (Maulana et al., 2019).

$$Pb \text{ uptake } \left( \frac{g}{pot} \right) = \frac{Biomass \left( \frac{g}{pot} \right) plant \text{ Pb}(\%)}{100} \quad (2)$$

### Cd and Pb concentration in Soil

The soil sample was first homogenized and dried at 105 °C for the lead and cadmium assays. A 300 mL beaker containing 1.0 g of the sieved sample was then added and 15 mL of nitric acid (HNO<sub>3</sub>, 69%) and 25 mL of perchloric acid (HClO<sub>4</sub>, 58%) were heated at 230 °C. The digested solution was filtered after being reduced to ash and increased in volume to 50 mL in a volumetric flask. A spectrophotometer that measures atomic absorption was used to calculate the metal concentrations (Sisay et al., 2019).

### Soil texture

The hydrometer and USDA triangle procedures were used to determine the soil texture. The pipette or hydrometer technique is used to compute the proportion of sand, silt and clay, which provides an understanding of the texture of the soil. Utilizing USDA standards, the final categorization is made. As it indicates the percentage of sand, silt and clay contained in a soil sample, the hydrometer method was chosen by the proposed system as the laboratory way of classifying soil texture. Using the triangle technique developed by the United States Department of Agriculture (USDA), the soil sample's textures are determined (Barman and Choudhury, 2020).

### Availability of phosphorus in soil

The Bray method was used to assess the availability of P from a soil sample. For this, 20 mL of the extraction solution (0.025 M HCl and 0.03 M NH<sub>4</sub>F) and 2 g of soil in 50 mL flasks were used to calculate to P contents in the soil samples. After

**Table 1:** Tukey HSD all-pairwise comparisons test of Cd and Cd by treatment

Treatments	Significant rate mg/kg	Cadmium concentration (Cdcn)	Cadmium uptake (CdU)
Control	0	0.0000 e	0.0000 d
	20	0.4600 b	1.6067 b
Cd	30	0.5700 a	1.7200 a
	100	0.0000 e	0.0000 d
Pb	150	0.0000 e	0.0000 d
	10+50	0.2033 d	0.4633 c
Cd+Pb	15+75	0.2700 c	0.5133 c
	HSD	0.0304	0.1033

Cd = Cadmium, Pb = Lead, HSD = Honest Significant Difference

Numbers in columns sharing the same letter(s) do not differ significantly at  $p < 0.05$

**Table 2:** Tukey HSD all-pairwise comparisons test of Pb and Pb by treatment

Treatments	Significant rate mg/kg	Lead concentration (Pbcn)	Lead uptake (PbU)
Control	0	0.0000 e	0.0000 e
	20	0.0000 e	0.0000 e
Cd	30	0.0000 e	0.0000 e
	100	1.0700 b	1.0700 b
Pb	150	1.5667 a	1.5667 a
	10+50	0.5267 d	0.5267 d
Cd+Pb	15+75	0.7600 c	0.7600 c
	HSD	0.0417	0.0919

Cd = Cadmium, Pb = Lead, HSD = Honest Significant Difference

Numbers in columns sharing the same letter(s) do not differ significantly at  $p < 0.05$

being shaken for five minutes, the sample in the extraction solvent was filtered. The colorimetric ascorbic acid technique was used to determine the P content (Elbasiouny et al., 2020).

### Saturation percentage

It is calculated as the mass of totally dried soil divided by the volume of water applied to saturate dry soil samples. It takes time and is somewhat costly to assess saturation percentage directly. The traditional method involves first drying soil samples, then soaking them in deionized water and drying them in an oven for 24 hrs at 105 °C. The saturation percentage (SP) is calculated by dividing the weight of dry soil by the amount of water needed to completely saturate the pore space (Zaman et al., 2018).

### Field capacity

A pot was used and its surfaces were coated with a thin plastic sheet that had many holes in it to drain any excess water (with drain water, soil particles must not be removed). A known volume of soil was added to the container. The top of the pot was covered to prevent evaporation after the soil in the pot had been soaked with extra water for several hours, filling up all of the soil's micropores with water. The net weight of the damp soil was measured at the time the gravitational water seized, which likely occurred during the night. Following dry-down, the quantity of water retained by the soil was determined, using 100% FC (Michael et al., 2017).

### Statistical analysis

The data were analyzed using ANOVA and Statisticix 8.1 and Tukey's HSD test was performed to evaluate mean differences across treatments.

## RESULTS AND DISCUSSION

The purpose of the experiment is to evaluate how well sunflower plants absorb Pb and Cd from contaminated soil. To achieve this, different fertilizers, including urea, DAP and SOP are given to soil containing varying amounts of lead and cadmium and responses are presented in Table 1-3 and discussed in the following sections.

### Shoot length

It is one of the most important plant traits that may be evaluated to ascertain how various treatments affect plant growth. In the early phases of sunflowers, the shoot grows to its maximum length; beyond that, the length does not significantly expand. After harvesting, a scale was used to measure the length of the shoots. The length of a plant shoot is influenced by environmental characteristics. The analysis of variance shows that the result is very significant under the rate of fertilizer applied, combined with different amounts of heavy metals and their interaction. Seven treatments control, sole Cd 20 mgkg<sup>-1</sup>, sole Cd 30 mgkg<sup>-1</sup>, sole Pb 100 mgkg<sup>-1</sup>, sole Pb 150 mgkg<sup>-1</sup>, Cd+Pb 10+50 mgkg<sup>-1</sup> and Cd+Pb 15+75 mgkg<sup>-1</sup>. The Highest shoot length was obtained at control where we do not apply any treatment. The lowest one was observed in Cd+Pb at a rate of 15+75 mgkg<sup>-1</sup>. These results align with the work of (Tan, Zhang, Zhang, & Cui, 2023). Among sole applications of Cd, the highest shoot length was at a rate of 20 mgkg<sup>-1</sup> and the lowest at a rate of 30 mgkg<sup>-1</sup>. In the case of Sole Pb treatment, the shoot length was highest at a rate of 100 mgkg<sup>-1</sup> and lowest at a rate of 150 mgkg<sup>-1</sup> respectively.

### Root length

The lowest root length estimate was made when Cd and Pb were combined at a rate of 15+75 and the greatest value was noted in the control. The solitary Cd treatments showed that sole Cd 20 mgkg<sup>-1</sup> had the longest roots and single Cd 30 mgkg<sup>-1</sup> had the shortest. When solitary Pb fertilizer treatment outcomes were compared, solo 150 mgkg<sup>-1</sup> treatment produced the lowest results and alone 100 mgkg<sup>-1</sup> treatment produced the highest results. The outcomes are consistent with the findings of (uz Zaman et al., 2021).

### Shoot weight

Shoot dry weight is one of the allowed measurements when analyzing a plant's biomass. This technique is often used to calculate the yield of a plant, but it is also a reliable indicator of plant biomass. The lowest value obtained with

the Cd+Pb combination at a rate of 15+75 is 1.1633. Shoot weight for the control group is at its highest, 3.7267. In Cd-only treatments, the greatest value, 2.2700, was noted for Cd20 and the lowest, 2.0733, was noted for Cd30 concentration. The greatest and lowest Pb single treatment doses, respectively, were 100 mgkg<sup>-1</sup> and 150 mgkg<sup>-1</sup>. These outcomes mirror those of (Mousavi Kouhi et al., 2019).

### Root weight

At 20 mg/kg and 30 mg/kg, the results of single Cd treatment were considerably dissimilar. Cd at a dosage of 20 mg/kg set a high bar when compared to therapy at 30 mg/kg. Overall, the Cd+Pb treatment at rates of 10+50 mg/kg and 15+75 mg/kg correspondingly had the highest root weight value, whereas the control group had the lowest value. These outcomes are consistent with those of (Dinu et al., 2021).

### Total plant weight

The order of values of total plant weight is controlled > sole Pb 100 mg/kg > sole Cd 20 mg/kg > sole Pb 150 mg/kg > sole Cd 30 mg/kg > Cd+Pb 10+50 mg/kg > Cd+Pb 15+75 mg/kg. Plants, which are sessile organisms, are unable to resist undesirable environmental changes. Plants must develop and/or adopt several strategies to be able to deal with the negative effects of heavy metal toxicity since exposure to heavy metals results in a wide variety of physiological and biochemical abnormalities (Singh et al., 2016).

### Cadmium concentration

Data shows seven treatments based on the amount of heavy metal added to the soil that showed the greatest and lowest levels of cadmium concentration. While there is no cadmium in this protocol, the lowest value was obtained with Pb treatments at rates of 100 mg/kg and 150 mg/kg. The treatment at 30 mg/kg resulted in the largest amount of cadmium, with a value of 0.5700. The lowest value for Cd20 observed in Cdonly treatments is 0.4600. The maximum value in the combined treatment of Cd+Pb was seen at a rate of 15+75 mg/kg with a value of 0.2700 and the lowest value was at a rate of 10+50 mg/kg with a value of 0.2033, respectively. The lowest uptake was shown in samples that had Pb treatment at rates of both 100 mg/kg and 150 mg/kg, with the Cd uptake value being respectively 0.0000 and 1.7200. The maximum value of cadmium uptake was obtained in the solo Cd treatment at a rate of 30 mg/kg. When Cd concentrations were raised, a pattern of increasing metal uptake by roots in single treatments was visible (Niu et al., 2023).

### Lead concentration



**Table 3:** Tukey HSD all-pairwise comparisons test of growth parameters by treatment

Treatments	Significant (mg/kg)	rate Shoot (cm)	length root (cm)	length shoot weight (g)	Root weight (g)	Total weight (TPW)	Plant root ratio (RSR)	shoot
Control	0	24.200 a	17.533 a	3.7267 a	1.8100 a	5.5367 a	0.4867 b	
	20	19.000 c	13.367 c	2.2700 c	1.2167 c	3.4867 c	0.5367 b	
Sole Cd	30	16.467 e	11.567 d	2.0733 d	0.9433 e	3.0167 e	0.4533 b	
	100	22.433 b	14.500 b	2.9700 b	1.4333 b	4.4033 b	0.4833 b	
Sole Pb	150	17.533 d	11.333 d	2.0500 d	1.1067 d	3.1567 d	0.5400 b	
	10+50	13.333 f	9.4667 e	1.4767 e	0.7900 f	2.2667 f	0.5333 b	
Cd+Pb	15+75	10.033 g	8.2667 f	1.1633 f	0.7367 f	1.9000 g	0.6333 a	
	HSD	0.9292	0.6994	0.1190	0.1031	0.0907	0.0869	

Cd = Cadmium, Pb = Lead, HSD = Honest Significant Difference

Numbers in columns sharing the same letter(s) do not differ significantly at  $p < 0.05$

Data presents the seven treatments with the highest and lowest concentrations of lead. The lowest value was obtained with Cd treatments at rates of 20 mg/kg and 30 mg/kg even though this protocol does not contain any lead. At a value of 1.5667, the treatment at 150 mg/kg produced the highest lead concentration. The control treatment produced the lowest value for Pb, which is 0.0000. Under the combination treatment of Cd+Pb, the highest value was observed at a rate of 15+75 mg/kg with a value of 0.7600 and the lowest value was observed at a rate of 10+50 mg/kg with a value of 0.5267.

The lowest uptake was shown in control along with samples that had Cd treatment at rates of both 20 mg/kg and 30 mg/kg, with the Cd uptake value being respectively 0.0000. The maximum value of Pb uptake was obtained in the solo Pb treatment at a rate of 150 mg/kg with a value of 1.5667. A pattern of increased metal uptake by roots in single treatments was noticeable when Pb concentrations were enhanced (Kalyvas et al., 2022). In combined concentration, the highest value was observed in the treatment of Cd+Pb 15+75 mg/kg with a value of 0.7600 and the lowest Pb uptake was observed in combined Cd+Pb at a rate of 10+50 mg/kg with a value of 0.5267.

## CONCLUSION

The findings of the study revealed that the application of cadmium (Cd) and lead (Pb) as individual treatments in contaminated soil resulted in significantly higher absorption by sunflower plants compared to their combined application. While sunflowers were able to absorb both heavy metals when applied together, the efficiency of absorption was notably reduced in comparison to sole treatments. Additionally, the study demonstrated a positive correlation between the concentration of heavy metals in the soil and their uptake by

the sunflower plants. Higher concentrations of Cd and Pb in the soil led to a more pronounced accumulation of these metals within the plant tissues. Among the individual treatments, the absorption of Cd and Pb was significantly greater at their highest respective concentrations compared to treatments with lower concentrations. This suggests that sunflowers exhibit an enhanced phytoremediation capacity when exposed to elevated levels of these contaminants. The results underline the potential of sunflower (*Helianthus annuus* L.) as an effective phytoremediant, particularly in soils with high levels of individual heavy metals like Cd or Pb. The study highlights the importance of optimizing treatment strategies to maximize the efficiency of phytoremediation, emphasizing the suitability of sunflowers for mitigating heavy metal pollution in both urban and rural environments.

## DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interest.

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