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PHYTOREMEDIATION OF PB AND CD CONTAMINATED SOILS USING *MEDICAGO SATIVA*

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ABSTRACT

Heavy metal contamination of soils represents a significant environmental and public health concern due to the inherent toxicity, persistence, and non-biodegradable nature of metals such as lead (Pb) and cadmium (Cd). These elements accumulate in terrestrial ecosystems, disrupt soil biochemical processes, and pose serious risks to food safety through plant uptake and trophic transfer. In this context, phytoremediation has emerged as an environmentally sustainable and cost-effective approach for the management of metal-contaminated soils. The present study investigated the phytoremediation potential of *Medicago sativa* for soils co-contaminated with Pb and Cd, with particular emphasis on the role of soil chemical modification, nutrient amendments, and chelating agent application in regulating metal bioavailability and plant uptake. Controlled pot experiments, complemented by field trials, were conducted using Pb-contaminated soil amended with cadmium to simulate multi-metal contamination scenarios. Soil pH was adjusted to 5.5, 6.5, and 7.0, while nitrogen, phosphorus, and potassium were applied at agronomically relevant rates to assess their influence on plant growth and metal accumulation. To enhance metal mobility, chelating agents including ethylenediaminetetraacetic acid (EDTA) and citric acid were applied. Bioavailable fractions of Pb and Cd were quantified using diethylenetriaminepentaacetic acid (DTPA) extraction, and metal concentrations in plant tissues were determined by atomic absorption spectrometry. The results revealed a pronounced influence of soil pH on metal uptake, with acidic conditions (pH 5.5) significantly enhancing the bioavailability and accumulation of Pb and Cd by approximately 30% and 45%, respectively. Nitrogen supplementation promoted vegetative growth and biomass production, resulting in increased total metal uptake despite relatively modest changes in tissue metal concentrations. Among the chelating agents, EDTA induced the greatest enhancement of Pb and Cd uptake, although its application was associated with observable phytotoxic effects, likely due to excessive metal mobilization. In contrast, citric acid produced moderate increases in metal uptake while maintaining normal plant growth, highlighting its potential as a more environmentally benign chelating agent. Across all treatments, Cd uptake consistently exceeded Pb uptake, reflecting its greater mobility and weaker sorption to soil constituents. This study provides a robust scientific basis for the development of effective and environmentally sustainable phytoremediation strategies targeting multi-metal contaminated soils.

KEYWORDS: Phytoremediation, Lead, Cadmium, *Medicago sativa*, Soil pH, Chelating agents.

INTRODUCTION

Heavy metal contamination of soils represents a significant environmental challenge owing to the toxicity, persistence, and non-biodegradable nature of these pollutants. Among various heavy metals, liken lead (Pb) and Cadmium (Cd) are of particular concern due to its strong tendency to bioaccumulate and bio magnify, resulting in severe ecological disturbances and adverse human health effects. Soil Pb and Cd contamination primarily originates from anthropogenic activities, including mining and smelting operations, battery manufacturing, textile dyeing industries, and the extensive use of lead-based paints and phosphate fertilizers,

Conventional remediation strategies, such as soil excavation, solidification, and chemical treatments, have proven effective in reducing Pb and Cd concentrations; however, these approaches are often economically intensive and environmentally intrusive**,** limiting their applicability on a large scale **(Nedjimi, 2021).** Consequently, phytoremediation has emerged as a sustainable and environmentally benign alternative. This green remediation technology utilizes plants to extract, immobilize, or detoxify contaminants from soil, offering advantages such as cost-effectiveness, minimal site disturbance, and ecological compatibility **(Chen *et al.*, 2022; Mathur & Panwar, 2024).**

Sources of Heavy Metal Contamination

The sources of heavy metals in soils are broadly categorized into natural (geogenic) and anthropogenic origins. Natural sources include parent rock weathering, volcanic activity, and soil-forming processes, which contribute background levels of metals. However, anthropogenic activities are the dominant contributors to elevated soil metal concentrations. These include mining and smelting operations, industrial effluents, coal combustion, waste disposal, traffic emissions, application of phosphate fertilizers, sewage sludge amendments, and pesticide use. Numerous studies have documented significantly higher metal concentrations in soils surrounding industrial zones, mining areas, and urban centers compared to uncontaminated reference sites.

Soil Chemistry and Metal Behavior

The behavior, mobility, and bioavailability of heavy metals in soil are governed by soil physicochemical properties such as pH, organic matter content, cation exchange capacity (CEC), redox potential, and clay mineralogy. **(Gul *et al.*, 2021).** Soil pH is widely recognized as a primary controlling factor; acidic conditions generally enhance metal solubility and mobility, increasing bioavailability, whereas alkaline conditions promote metal precipitation and adsorption. Organic matter plays a dual role by forming stable complexes that may either immobilize metals or, under certain conditions, enhance their transport through dissolved organic carbon complexes.

***Medicago sativa* L.: Botanical and Environmental Significance**

Medicago sativa L., commonly known as alfalfa, is a perennial leguminous plant belonging to the family Fabaceae. It is widely cultivated across temperate and semi-arid regions due to its high adaptability, rapid growth, and substantial biomass production. Owing to these characteristics, *M. sativa* has received considerable attention not only as a forage crop but also as a model species in environmental and ecological studies.

Heavy Metal Tolerance and Accumulation

Several studies have demonstrated that *M. sativa* possesses moderate tolerance to heavy metals such as lead (Pb), cadmium (Cd), zinc (Zn), and copper (Cu). Although it is not classified as a hyperaccumulator, its high biomass yield compensates for relatively lower metal concentration in tissues, resulting in substantial total metal removal from contaminated soils. Lead uptake in *M. sativa* is primarily influenced by soil physicochemical conditions, particularly pH, organic matter content, and metal bioavailability.

Role in Phytoremediation

Medicago sativa is widely investigated for its role in phytoremediation strategies, including phytoextraction and phytostabilization **(Chemosphere. (2024).** Its tolerance to metal stress, combined with rapid growth and ease of cultivation, makes it suitable for remediation of moderately contaminated agricultural and industrial soils. Moreover, its dense root network contributes to soil stabilization, reducing erosion and limiting metal dispersion.

Physiological and Biochemical Responses to Metal Stress

Exposure to heavy metals induces a range of physiological and biochemical responses in *M. sativa*, including alterations in antioxidant enzyme activity, metal sequestration in root tissues, and complexation with organic ligands. These adaptive mechanisms enable the plant to mitigate metal toxicity while maintaining growth under contaminated conditions.

MATERIALS AND METHODS

- **Experimental Design**

Both controlled pot experiments and complementary field trials were conducted to evaluate the influence of soil amendments on lead (Pb) bioavailability and uptake by *Medicago sativa* L. All the experiments were carried out in triplicates.

- **Plant Material and Soil Preparation**

Certified seeds of *Medicago sativa* were used as the test plant species. Soil samples were collected from a Pb-contaminated site, air-dried, sieved (<2 mm), and thoroughly homogenized prior to treatment application to ensure uniformity.

- **Soil Treatments**

Soil pH was adjusted using sulfuric acid to decrease pH and agricultural lime to increase pH, establishing target pH levels of 5.5, 6.5, and 7.0. Macronutrient amendments, including nitrogen (N), phosphorus (P), and potassium (K), were applied at varying concentrations to assess their effects on plant growth and Pb uptake dynamics. In addition, chelating agents—ethylenediaminetetraacetic acid (EDTA) and citric acid—were applied at rates of 5 and 10 mmol kg^{-1} soil to evaluate their influence on Pb solubility and plant absorption.

➢ **Assessment of Pb Bioavailability and Plant Growth**

Soil Pb bioavailability was determined using diethylenetriaminepentaacetic acid (DTPA) extraction. Above-ground plant biomass was harvested at maturity, oven-dried to a constant weight, and weighed to quantify biomass production. Lead concentrations in plant tissues were determined using atomic absorption spectrometry (AAS).

➢ **Statistical Analysis**

Experimental data were subjected to analysis of variance (ANOVA) to identify statistically significant differences among treatments at an appropriate confidence level.

RESULTS

Figure 1: Comparative growth response of *Medicago sativa* under different chelating agent and soil pH treatments.

Table 1: Comparative influence of soil pH modification, nutrient amendment, and chelating agents on Pb and Cd uptake by *Medicago sativa*.

Factor	**Increase in Pb uptake (%)**	**Increase in Cd uptake (%)**	**Comparative interpretation**
Soil pH adjustment (5.5 vs. 7.0)	30	45	Cadmium uptake exhibited greater sensitivity to acidic conditions, reflecting its higher intrinsic mobility and weaker sorption to soil constituents compared to lead.
Nitrogen amendment (100 mg kg⁻¹ soil)	20	18	Enhanced biomass production contributed to increased metal accumulation in both cases, with a marginally greater effect observed for Pb.
EDTA application (10 mmol kg⁻¹ soil)	40	50	EDTA promoted substantial mobilization of both metals; however, stronger soluble complex formation with Cd resulted in comparatively higher Cd uptake.
Citric acid application (10 mmol kg⁻¹ soil)	25	30	Citric acid moderately enhanced metal bioavailability, with Cd showing a greater response, while maintaining low phytotoxicity and environmental compatibility.

Statistical Interpretation

Statistical analysis indicated that soil pH adjustment, nutrient amendment, and chelating agent application exerted significant effects on Pb and Cd uptake by *Medicago sativa* ($p < 0.05$). Among the tested factors, soil acidification (pH 5.5) and EDTA application produced the most pronounced and statistically significant increases in metal uptake for both Pb and Cd, confirming their dominant role in controlling metal bioavailability at the soil–plant interface.

Comparative analysis revealed that Cd uptake was significantly higher than Pb uptake across all treatments ($p < 0.05$), particularly under acidic conditions and EDTA application. The greater responsiveness of Cd to soil pH modification was statistically supported by a stronger treatment × metal interaction effect, reflecting Cd's higher solubility and weaker adsorption to soil particles relative to Pb. Nitrogen amendment resulted in moderate but statistically significant increases in metal accumulation ($p < 0.05$), primarily driven by enhanced biomass production rather than changes in tissue metal concentration, with no significant difference between Pb and Cd responses.

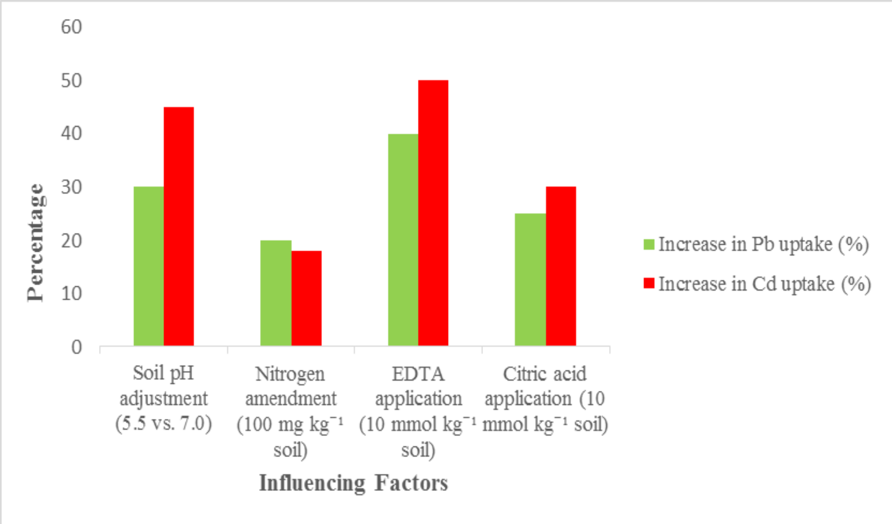

Figure 2: **Influence of various factors on Pb and Cd uptake by *Medicago sativa*.**

Chelating agent treatments showed distinct statistical trends. EDTA application significantly increased Pb and Cd uptake compared to both the control and citric acid treatments ($p < 0.01$); however, variability in plant response was also higher under EDTA treatment, consistent with observed phytotoxic effects. In contrast, citric acid induced a statistically significant but comparatively lower increase in metal uptake ($p < 0.05$), with reduced variance and no detectable adverse effects on plant growth. This suggests a more stable and environmentally compatible chelation mechanism.

Overall, the statistical outcomes demonstrate that treatment effects were metal-specific and factor-dependent, with Cd exhibiting consistently higher sensitivity to soil chemical modifications than Pb. These findings emphasize the importance of optimizing soil pH and selecting appropriate chelating agents to maximize phytoextraction efficiency while minimizing ecological risk.

DISCUSSION

The present study clearly demonstrates that soil pH, nutrient amendments, and chelating agents exert decisive control over the phytoavailability and uptake of lead (Pb) and cadmium (Cd) by *Medicago sativa*. The observed uptake patterns are consistent with established soil–plant–metal interaction mechanisms reported in contemporary phytoremediation literature, confirming the pivotal role of soil chemical conditions in regulating metal mobility and plant acquisition. **(Nouri & Hashempour, 2021).**

Soil pH emerged as one of the most influential determinants of metal uptake. Adjustment of soil pH to mildly acidic conditions (pH 5.5) significantly enhanced the accumulation of both Pb and Cd, with a markedly greater response observed for Cd. This differential behavior is attributable to the inherently higher mobility and weaker adsorption affinity of Cd in soils relative to Pb. Acidification reduces metal sorption to soil colloids and promotes the formation of soluble metal species, thereby increasing their availability for root uptake. Previous studies have consistently reported a sharp increase in Cd solubility with decreasing pH, whereas Pb tends to form comparatively stable complexes or precipitates at neutral to alkaline pH, limiting its mobility.

Nitrogen amendment significantly enhanced plant biomass, which indirectly increased total metal uptake. This biomass-driven effect was more pronounced for Pb than for Cd, suggesting that Pb accumulation in *Medicago sativa* is more strongly influenced by root surface area expansion and transpiration-driven mass flow than by changes in soil solution chemistry alone. Similar observations have been reported in studies where improved nutrient availability enhanced plant vigor and metal accumulation without substantially altering soil metal solubility **(Jyoti Mathur and Ritu Panwar, 2024).**

In contrast, phosphorus and potassium amendments did not significantly affect Pb or Cd solubility but contributed to improved physiological performance and stress tolerance under metal exposure, indicating their indirect role in supporting plant resilience rather than promoting metal mobilization.

Chelating agents substantially enhanced the uptake of both metals, with EDTA exhibiting the highest efficiency. **(Islam *et al.*, 2024)**. EDTA application resulted in pronounced increases in Pb and Cd accumulation due to the formation of highly soluble metal–EDTA complexes that facilitate metal desorption from soil matrices and subsequent uptake by plant roots. The stronger response observed for Cd reflects its higher affinity for EDTA and greater intrinsic mobility. However, EDTA-induced enhancement was accompanied by signs of phytotoxicity at elevated concentrations, likely resulting from excessive metal influx and disruption of cellular homeostasis. These findings corroborate earlier reports highlighting the trade-off between increased phytoextraction efficiency and ecological risk associated with persistent synthetic chelators.

In contrast, citric acid produced moderate yet consistent increases in Pb and Cd uptake without inducing visible phytotoxic effects. Although less effective than EDTA, citric acid offers clear advantages in terms of biodegradability, lower persistence in soil, and reduced risk of metal leaching into groundwater. Recent studies increasingly support the use of low-molecular-weight organic acids as environmentally compatible alternatives for chelate-assisted phytoremediation, particularly under field conditions where long term ecological impacts must be minimized.

Comparative evaluation of Pb and Cd uptake revealed consistently higher accumulation of Cd across all treatments **(Ritu *et al.*, 2021).** This behavior reflects fundamental differences in soil chemistry and plant uptake pathways between the two metals. Cadmium is readily transported through calcium and zinc uptake channels due to its chemical similarity, whereas Pb uptake is more restricted and largely confined to apoplastic pathways. The slightly reduced uptake efficiency observed under combined Pb–Cd exposure suggests competitive interactions at the root interface, a phenomenon commonly reported in multi-metal contamination scenarios.

Overall, the results confirm that *Medicago sativa* exhibits substantial tolerance and accumulation capacity for both Pb and Cd, particularly when soil chemical conditions are appropriately managed. The integrated manipulation of soil pH, nutrient availability, and chelating agent application represents a viable strategy for enhancing phytoremediation efficiency in co-contaminated soils.

CONCLUSION

The present investigation demonstrates that *Medicago sativa* is an effective phytoremediator for soils contaminated with lead and cadmium, both individually and in combination. Soil pH adjustment to mildly acidic conditions significantly increased the bioavailability and uptake of both metals, with cadmium exhibiting a stronger response than lead due to its greater mobility. Nitrogen supplementation enhanced metal accumulation indirectly through increased biomass production, while phosphorus and potassium primarily contributed to improved plant stress tolerance rather than direct metal mobilization.

Chelate-assisted phytoextraction proved to be a powerful approach for enhancing Pb and Cd uptake, with EDTA showing the highest extraction efficiency. However, the associated phytotoxicity and potential environmental risks necessitate cautious and controlled application. In contrast, citric acid emerged as a safer and more environmentally sustainable alternative, offering moderate enhancement of metal uptake with minimal adverse effects on plant growth.

The consistently higher uptake of Cd relative to Pb reflects inherent differences in metal chemistry and plant uptake mechanisms, while competitive interactions under combined metal exposure underscore the importance of considering multi-metal dynamics in phytoremediation design. In conclusion, the combined application of soil pH optimization, balanced nutrient management, and biodegradable chelating agents can substantially enhance the phytoremediation potential of *Medicago sativa*. These findings provide a robust scientific foundation for developing cost-effective and environmentally sustainable remediation strategies, particularly for large-scale and low-to-moderate heavy metal contamination scenarios.

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