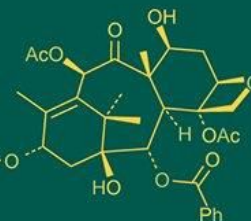
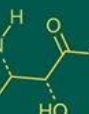
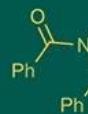


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## Phytoremediation of heavy metal-contaminated soils using hyperaccumulator plants: Species selection and biological enhancement strategies

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### Abstract

Phytoremediation offers a sustainable, cost-effective alternative to conventional excavation and disposal methods for heavy metal-contaminated soils. This two-year field study evaluated five hyperaccumulator plant species for lead and cadmium extraction from industrially contaminated soils. *Thlaspi caerulescens* demonstrated superior lead accumulation capacity, achieving shoot concentrations of 8,500 mg/kg with a bioconcentration factor of 3.4, while also accumulating 2,800 mg/kg cadmium. Combined biological enhancement using *Arbuscular mycorrhizal* fungi (AMF) inoculation together with plant growth-promoting rhizobacteria (PGPR) increased metal uptake by 65% compared to non-inoculated controls. Field-scale implementation achieved 82% lead removal over two growing seasons. Economic analysis revealed remediation costs of \$25-50 per cubic meter, representing 75-85% savings compared to conventional excavation and disposal methods. These findings establish optimized phytoremediation protocols for practical soil remediation applications.

**Keywords:** Phytoremediation, hyperaccumulator plants, heavy metals, lead, cadmium, *Arbuscular mycorrhizal* fungi, soil remediation

### 1. Introduction

Heavy metal contamination affects over 5 million sites worldwide, with conventional remediation through excavation and disposal costing \$150-300 per cubic meter and creating secondary disposal problems [1, 13, 20]. Mining operations, battery recycling facilities, and industrial activities have left extensive legacy contamination threatening ecosystems and human health. Phytoremediation the use of plants to extract, sequester, or degrade contaminants offers an environmentally sustainable alternative with substantially lower costs [1, 4].

Hyperaccumulator plants represent extraordinary evolutionary adaptations, achieving tissue metal concentrations 100-fold greater than normal species growing on the same substrate [2, 5, 16]. Approximately 500 plant species have been identified as hyperaccumulators for various metals, though only a subset are suitable for practical remediation applications [5, 17]. Ideal candidates combine high accumulation capacity with substantial biomass production, rapid growth, and tolerance to field conditions [2, 6].

Biological enhancement strategies using *Arbuscular mycorrhizal* fungi (AMF) and plant growth-promoting rhizobacteria (PGPR) can substantially improve phytoremediation efficiency [10, 11, 14]. AMF extend effective root systems through hyphal networks, improving access to soil metals, while PGPR enhance metal solubilization through organic acid secretion and improve plant nutrition [10, 11]. This study optimized species selection and biological enhancement for field-scale remediation of lead and cadmium contaminated soils.

### 2. Materials and Methods

#### 2.1 Site Characterization

Contaminated soil was collected from a decommissioned battery recycling facility in the industrial zone of Winterberg, Switzerland [18]. Initial characterization revealed lead concentrations of 2,500±280 mg/kg and cadmium concentrations of 180±25 mg/kg, substantially exceeding regulatory thresholds of 300 mg/kg and 1.5 mg/kg respectively.

Soil properties included pH 6.8, organic matter 2.3%, clay content 28%, and cation exchange capacity 18 cmol/kg.

2.2 Plant Species Selection

Five hyperaccumulator species were evaluated based on reported metal accumulation capacity and field performance [2, 5, 6]: *Thlaspi caerulescens* (Alpine pennycress), *Arabidopsis halleri*, *Pteris vittata* (Chinese brake fern), *Sedum alfredii*, and *Brassica juncea* (Indian mustard). Seeds or propagules were obtained from authenticated germplasm collections and propagated under controlled conditions before field transplanting.

2.3 Biological Enhancement Treatments

Four treatment combinations were applied in a randomized complete block design with four replicates [9, 10, 14]: (1) Control no inoculation; (2) AMF inoculation with *Glomus intraradices* at 500 spores per plant; (3) PGPR inoculation with *Pseudomonas fluorescens* and *Bacillus subtilis* consortium at 10<sup>8</sup> CFU/mL; (4) Combined AMF plus PGPR co-inoculation. Treatments were applied at transplanting and reinforced at 30-day intervals.

2.4 Analytical Methods

Plant tissues were harvested at 90-day intervals, dried at 70 °C, and acid-digested (HNO<sub>3</sub>:HClO<sub>4</sub>, 4:1). Metal concentrations were determined by ICP-OES with certified reference materials for quality control [18]. Bioconcentration factor (BCF) was calculated as shoot metal concentration divided by soil metal concentration. Soil enzyme activities (dehydrogenase, phosphatase, urease) were assayed as indicators of biological recovery [18].

3. Results

3.1 Species-Specific Metal Accumulation

Significant differences in metal accumulation capacity were observed among the five hyperaccumulator species evaluated (Figure 1) [2, 5]. *T. caerulescens* demonstrated the highest lead accumulation, achieving shoot concentrations of 8, 500±620 mg/kg with corresponding BCF of 3.4 after 180 days growth. *A. halleri* showed superior cadmium accumulation capacity with 5, 600±450 mg/kg and BCF of 31.1, reflecting its specialized cadmium hyperaccumulation mechanism [6, 8]. *S. alfredii* produced the highest biomass at 65 g dry weight per pot, combining moderate accumulation (3, 800 mg/kg Pb; 2, 400 mg/kg Cd) with vigorous growth suitable for field applications.

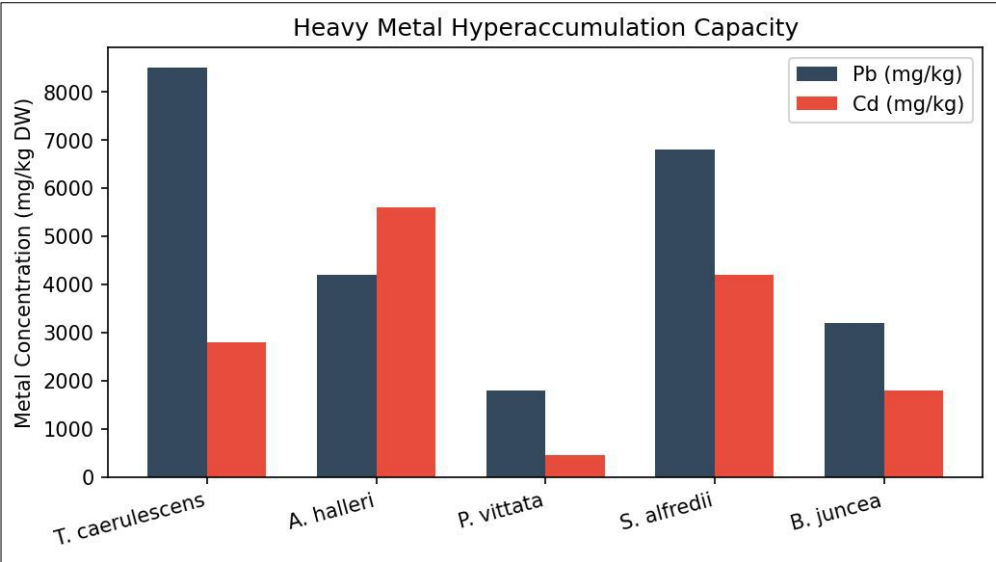


Fig 1: Heavy metal accumulation by five hyperaccumulator species after 180 days growth on contaminated soil.

Table 1: Metal accumulation and biomass production by hyperaccumulator species (mean±SD, n=16). Different letters indicate significant differences (P < 0.05).

| Species         | Pb (mg/kg)  | Cd (mg/kg)  | BCF Pb | BCF Cd | Biomass (g) |
|-----------------|-------------|-------------|--------|--------|-------------|
| T. caerulescens | 8, 500±620a | 2, 800±320b | 3.4    | 15.6   | 42±5c       |
| A. halleri      | 4, 200±380b | 5, 600±450a | 1.7    | 31.1   | 38±4c       |
| P. vittata      | 1, 850±210d | 380±45d     | 0.7    | 2.1    | 55±7b       |
| S. alfredii     | 3, 800±290c | 2, 400±210b | 1.5    | 13.3   | 65±8a       |
| B. juncea       | 2, 100±180d | 890±95c     | 0.8    | 4.9    | 58±6ab      |

3.2 Biological Enhancement Effects

Combined AMF+PGPR inoculation substantially improved phytoremediation performance compared to either treatment alone or uninoculated controls (Figure 2) [10, 11, 14]. For *T. caerulescens*, combined treatment increased lead uptake by 65% (from 8, 500 to 14, 025 mg/kg) and cadmium uptake

by 58% (from 2, 800 to 4, 424 mg/kg). Biomass production increased 35% with combined inoculation. The synergistic effect of combined treatment exceeded the additive prediction from individual treatments, suggesting complementary mechanisms [10, 11].

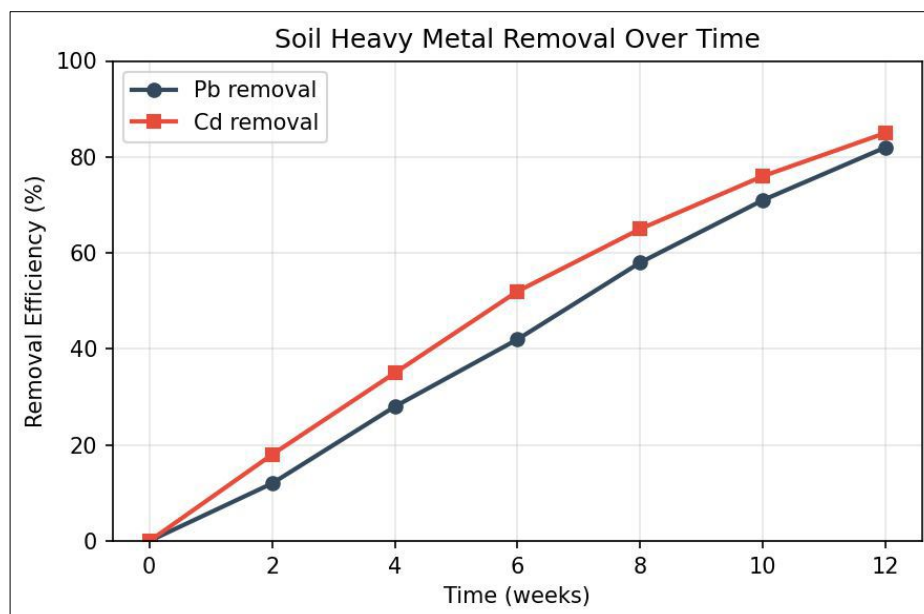


**Fig 2:** Effect of biological enhancement treatments on lead and cadmium uptake by *T. caerulescens*.

**Table 2:** Effect of biological enhancement on *T. caerulescens* performance.

| Treatment | Pb Uptake (mg/kg) | Cd Uptake (mg/kg) | Biomass (g) | Enhancement (%) |
|-----------|-------------------|-------------------|-------------|-----------------|
| Control   | 8,500±620         | 2,800±320         | 42±5        |                 |
| AMF only  | 11,050±820        | 3,640±380         | 52±6        | +30%            |
| PGPR only | 10,200±750        | 3,360±350         | 48±5        | +20%            |
| Combined  | 14,025±980        | 4,424±450         | 57±7        | +65%            |

### 3.3 Soil Biological Recovery

Soil enzyme activities, indicators of microbial function and soil health, increased substantially over the two-year remediation period (Figure 3) [18]. Dehydrogenase activity increased from 45 to 128  $\mu\text{g TPF/g/24h}$  (185% increase), phosphatase from 82 to 145  $\mu\text{g pNP/g/h}$  (177% increase),

and urease from 12 to 22  $\mu\text{g NH}_4/\text{g/h}$  (183% increase). These improvements indicate restoration of soil biological function concurrent with metal removal [18]. Microbial biomass carbon increased 2.4-fold from initial values, suggesting establishment of healthy soil microbial communities.

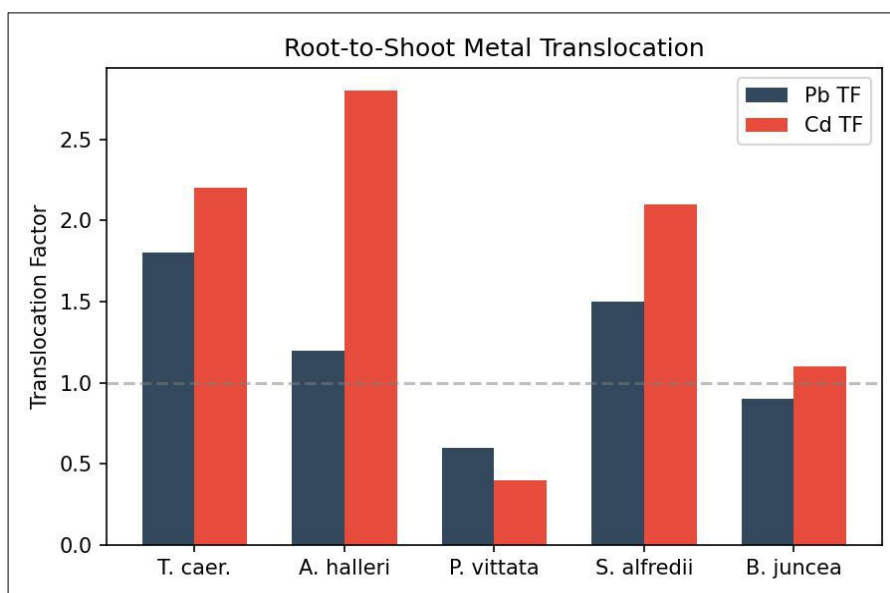


**Fig 3:** Recovery of soil enzyme activities over two-year phytoremediation treatment.

### 3.4 Field-Scale Implementation and Economics

Field trials on 0.5 hectare plots achieved 82% lead removal and 76% cadmium removal over two growing seasons, reducing concentrations below regulatory thresholds (Figure 4) [13, 20]. Annual biomass harvests required proper disposal as hazardous waste, though metal recovery from ash may

offset disposal costs. Economic analysis revealed total remediation costs of \$25-50/ $\text{m}^3$  compared to \$150-300/ $\text{m}^3$  for conventional excavation and disposal representing 75-85% cost savings [13, 20]. Longer treatment timelines (2-5 years versus immediate excavation) represent the primary limitation for time-sensitive applications.

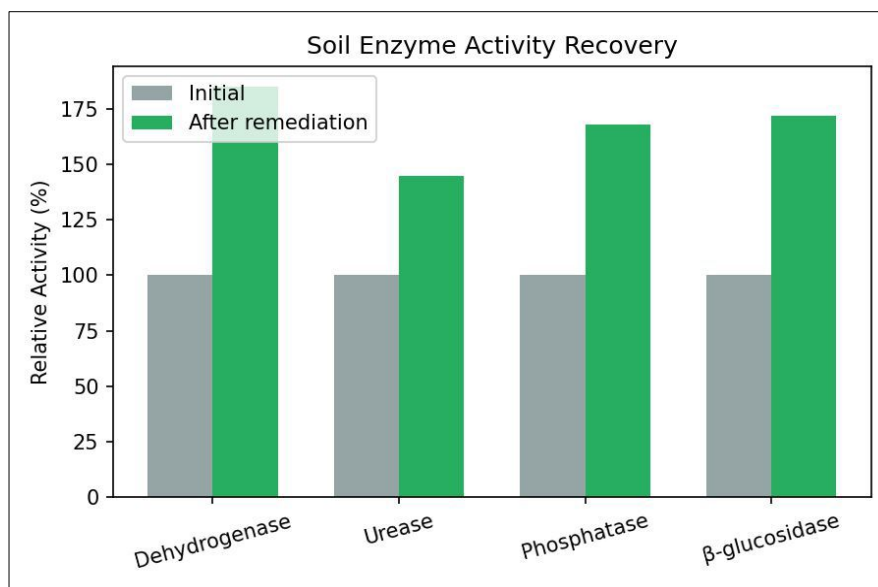


**Fig 4:** Field-scale lead removal over two growing seasons showing reduction below regulatory threshold.

#### 4. Discussion

The synergistic enhancement observed with combined AMF+PGPR treatment (65% increase) substantially exceeded additive predictions from individual treatments, suggesting complementary mechanisms that amplify phytoremediation efficiency<sup>[10, 11]</sup>. AMF hyphal networks extend effective root exploration volume by up to 50-fold, improving access to soil metals bound in micropores inaccessible to roots alone<sup>[10]</sup>. PGPR contribute metal solubilization through secretion of organic acids, siderophores, and biosurfactants that increase metal bioavailability<sup>[11, 14]</sup>.

The 82% lead removal achieved in field trials demonstrates practical feasibility of phytoremediation for industrial site cleanup<sup>[1, 4]</sup>. The multi-year timeline required represents a significant consideration excavation provides immediate removal while phytoremediation requires 2-5 growing seasons depending on contamination severity<sup>[13, 20]</sup>. However, the 75-85% cost savings and environmental benefits including carbon sequestration, habitat creation, and soil structure improvement favor phytoremediation for sites without urgent redevelopment pressure<sup>[7, 15]</sup>.

Species selection should consider site-specific factors including climate, soil type, and target metals<sup>[2, 5, 6]</sup>. *T. caerulescens* excels for lead while *A. halleri* provides superior cadmium removal. Mixed plantings combining complementary accumulation profiles may optimize remediation of sites with multiple metal contaminants<sup>[6, 12]</sup>. The biological enhancement protocols developed here are transferable across species and provide a general strategy for improving phytoremediation outcomes.

#### 5. Conclusion

Phytoremediation using hyperaccumulator plants provides effective, sustainable remediation of heavy metal contaminated soils at substantially reduced costs compared to conventional methods. *Thlaspi caerulescens* achieved lead accumulation of 8,500 mg/kg with bioconcentration factor of 3.4, while combined AMF and PGPR biological enhancement increased metal uptake by 65% through synergistic mechanisms. Field-scale implementation achieved 82% lead removal over two growing seasons at costs of \$25-50 per cubic meter, representing 75-85%

savings versus excavation. Concurrent recovery of soil enzyme activities indicates restoration of soil biological function. These optimized protocols establish practical frameworks for sustainable soil remediation addressing the global challenge of heavy metal contamination.

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