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Study on Microbial Siderophore–Assisted Heavy Metal Chelation and phytoextraction efficiency in *Zea mays* using *Brevibacillus brevis* PS–1

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ABSTRACT

Siderophores are strong microbial chelators that can complex heavy metals and enhance plant-based remediation by increasing metal mobility and uptake. In the present study, Brevibacillus brevis PS-1efficiency of metal chelation and phytoextraction was evaluated by cell-free siderophore-mediated chelation, live microbe-mediated metal removal, and greenhouse pot phytoextraction using Zea mays under Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} stress. In the cell-free chelation assay, Atomic Absorption Spectroscopy (AAS) confirmed substantial reduction of dissolved metals, with residual concentrations of 18.4 ppm Pb^{2+} (81.6% removal), 26.7 ppm Cd^{2+} (73.3%), 34.5 ppm Cr^{6+} (65.5%), and 48.9 ppm As^{3+} (51.1%). Validating effective chelation withlive culture treatment, metal removal was further enhanced after 48 h, reducing concentrations of Pb^{2+} by (87.4%), Cd^{2+} by (78.2%), Cr^{6+} by (70.6%), and As^{3+} by (57.3%). In the pot experiment, amendment type strongly influenced uptake and translocation. The live microbial inoculant produced the highest BCF values and improved TF, reaching 1.21 for Cd and 1.21 for As, indicating efficient root-to-shoot transfer. Total uptake (U) and phytoextraction efficiency were maximised under T2, with peak values for Cd (0.403 mg plant^{-1}; 0.403%), followed by Pb (0.362 mg plant^{-1}; 0.362%), As (0.318 mg plant^{-1}; 0.318%), and Cr (0.286 mg plant^{-1}; 0.286%). Heatmap integration consistently ranked treatments as microbial> siderophore extract >EDTA treat> controlacross all metals. Overall, the results demonstrate that siderophore-producing B. brevis PS-1, particularly as a living inoculant, significantly strengthens multi-metal remediation by coupling extracellular chelation with improved maize uptake and translocation.

Keywords: *Brevibacillus brevis PS-1; siderophore; heavy metal chelation; phytoextraction; Zea mays; bioconcentration factor; translocation factor.*

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Introduction

Heavy metal contamination of agricultural soils has emerged as a critical environmental challenge due to rapid industrialisation, mining activities, improper waste disposal, and the long-term use of metal-based agrochemicals. Unlike organic pollutants, metals such as lead (Pb), cadmium (Cd), chromium (Cr), and arsenic (As) are non-biodegradable and persist in soil matrices for decades, posing serious threats to soil fertility, crop productivity, food safety, and human health [1]. Elevated concentrations of these metals disrupt plant physiological processes by impairing photosynthesis, nutrient uptake, membrane integrity, and redox balance, ultimately reducing crop biomass and yield. Consequently, the development of sustainable and cost-effective remediation strategies that preserve soil structure while reducing metal bioavailability has become an urgent priority.

Phytoremediation has gained considerable attention as an environmentally benign alternative to conventional physicochemical remediation approaches. This strategy exploits the natural ability of plants to absorb, translocate, and accumulate metals from contaminated soils. However, the efficiency of phytoextraction is often constrained by limited metal bioavailability, metal-induced phytotoxicity, and reduced plant growth under stress conditions [2]. While hyperaccumulator species exhibit high metal uptake, their low biomass limits large-scale applicability. In contrast, high-biomass crops such as maize (*Zea mays* L.) offer greater remediation potential when supported by biological interventions that enhance metal uptake and stress tolerance [1]. In metal-contaminated soils, PGPB can regulate metal mobility through secretion of organic acids, exopolysaccharides, and metal-chelating compounds, thereby influencing metal availability for plant uptake [3]. These microbe–plant interactions not only enhance plant growth but also mitigate oxidative damage induced by metal stress, resulting in improved phytoextraction performance [4].

Among microbial metabolites, siderophores represent a particularly effective class of metal-binding ligands. Although primarily synthesised for iron acquisition under iron-limited conditions, siderophores possess a strong affinity for a range of heavy metals, including Pb^{2+}, Cd^{2+}, Cr^{3+}/Cr^{6+}, and As^{3+}, forming stable complexes that alter metal solubility and transport dynamics [5]. Siderophore-mediated chelation can increase metal mobility toward plant roots or reduce metal toxicity by

sequestration, depending on the environmental context. Recent studies highlight the dual role of siderophores in enhancing plant iron nutrition while simultaneously modulating heavy metal behaviour in contaminated soils [6]. In recent years, interest has expanded toward cell-free approaches that utilise microbial metabolites without the introduction of live microbial cells. Cell-free supernatants (CFS) derived from PGPB cultures contain extracellular siderophores, organic acids, biosurfactants, and signalling molecules that retain biological activity [7]. Such preparations offer practical advantages, including improved formulation stability, reduced ecological risk, and predictable performance under variable soil conditions. Evidence suggests that CFS can enhance plant growth, improve nutrient uptake, and alleviate abiotic stress, making them promising tools for sustainable agriculture and remediation applications [8, 9].

Members of the genus *Brevibacillus* are recognised for their resilience under extreme environmental conditions and their capacity to produce diverse bioactive metabolites. Several *Brevibacillus* strains exhibit metal tolerance and plant growth-promoting attributes, enabling them to function effectively in contaminated soils [10]. In particular, siderophore-producing *Brevibacillus brevis* strains have demonstrated potential in enhancing plant biomass and modulating metal uptake through rhizosphere interactions [11]. Their spore-forming nature further supports survival and functional stability under greenhouse and field conditions. Maize (*Zea mays* L.) is an ideal candidate for assisted phytoextraction due to its rapid growth, extensive root system, and high biomass yield. When coupled with microbial or metabolite-based interventions, maize can achieve substantial total metal removal despite moderate tissue accumulation levels [2]. However, systematic studies integrating cell-free siderophore chelation**,** live microbe-mediated rhizosphere modulation**,** andgreenhouse-scale phytoextraction assessmentremain limited. Bridging this gap is essential for translating laboratory-scale metal chelation into effective soil remediation outcomes. Therefore, an investigative study was conducted using the siderophore-producing *Brevibacillusbrevis*PS-1 *isolate* in a greenhouse pot study.

Methodology

Microbial culture used in the study

The siderophore-producing bacterium *Brevibacillus brevis* PS-1 was used as the test microorganism. The culture was maintained on nutrient agar slants and stored at 4 °C until use. For each experiment, the strain was freshly revived by streaking onto nutrient agar plates and incubating at 30 °C for 24 h to obtain active colonies.To prepare the inoculum, a single colony was transferred into sterile nutrient broth and incubated on a rotary shaker at 150–180 rpm for 18–24 h. The culture was allowed to reach the logarithmic phase, which typically corresponded to an OD^{600} of 0.8–1.0. The cells were then harvested by centrifugation, washed with sterile saline, and resuspended to obtain a uniform suspension of approximately 10^8 CFU/mL. This standardised inoculum was used for siderophore production, chelation assays, and plant inoculation treatments.

Cell-free siderophore-mediated chelation assay.

A cell-free siderophore-mediated metal chelation assay was conducted to evaluate the ability of the siderophore-rich supernatant to bind and remove heavy metal ions. Initially, 100 ppm stock solutions of selected heavy metals, including Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+}, were prepared using analytical-grade metal salts dissolved in distilled water. For each experimental treatment, 25 mL of sterile, cell-free siderophore-containing culture supernatant was mixed with an equal volume (25 mL) of the prepared metal solution in sterile 100 mL conical flasks, while a control set was maintained by combining the metal solution with uninoculated broth to account for non-biological adsorption. The reaction mixtures were incubated at 28–30°C under shaking conditions (150 rpm) for 24–48 hours to facilitate siderophore–metal interaction and chelation. After incubation, all samples were centrifuged at 10,000 rpm for 10 minutes to separate residual biomass. The clarified supernatants were then subjected to Atomic Absorption Spectroscopy (AAS) to quantify the concentration of unbound metal ions remaining after treatment. The percentage of metal removal was calculated based on the reduction in metal concentration relative to the untreated control, indicating the chelation efficiency of the siderophore.

$$\text{Removal } \% = \left(\frac{C_i - C_f}{C_i}\right) \times 100$$

Microbe-mediated metal removal using *Brevibacillus brevis* PS-1

A microbe-mediated metal removal assay was conducted to assess the combined effects of biosorption, bioaccumulation, and siderophore-mediated chelation by *Brevibacillus brevis* PS-1. To prepare the inoculum, *B. brevis* PS-1 was cultured in an iron-deficient medium for 24 hours to enhance siderophore production and ensure metabolically active cell growth. For the treatment experiment, 50 mL of a 100 ppm metal solution was transferred into sterile Erlenmeyer flasks, followed by the addition of 5% (v/v) actively growing *B. brevis* PS-1 culture. The flasks were incubated at 30°C with continuous shaking at 150 rpm for 48 hours to promote microbial interaction with the metal ions. During this period, metal removal occurred through cell-associated mechanisms (biosorption and bioaccumulation) as well as extracellular siderophore-mediated chelation. After incubation, the cultures were centrifuged to separate bacterial biomass, and the resulting supernatant was collected. Residual metal concentrations in the supernatant were quantified using Atomic Absorption Spectroscopy (AAS) to determine the overall metal removal efficiency of *B. brevis* PS-1.

Phytoextraction Experiment (Pot Study)

The phytoextraction study was conducted using *Zea mays* (maize), a fast-growing test crop known for its strong root system and metal-accumulating capability. Surface-sterilised maize seeds were sown in pots containing agricultural soil that had been artificially spiked with known concentrations of Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+}. The pots were arranged under four treatments: a control without amendments, soil inoculated with the live siderophore-producing strain *Brevibacillus brevis* PS-1 (10^8 CFU/mL) applied as a soil drench, soil treated with cell-free siderophore extract (10 mL/kg soil), and soil treated with the synthetic chelator EDTA (2 mmol/kg). All pots were maintained in a greenhouse for 30–45 days under controlled conditions (25 ± 2°C, 16/8 h light–dark cycle, 60–70% humidity), with regular watering to maintain optimum soil moisture. At harvest, plants were carefully uprooted, and roots and shoots were separated, washed thoroughly to remove soil particles, and oven-dried at 60°C to constant weight. Dried tissues were weighed and digested using a HNO_3–H_2O_2 acid mixture (3:1) on a hot plate until a clear solution was obtained. The digested samples were then filtered, diluted, and analysed using Atomic Absorption Spectrophotometry (AAS) to quantify metal accumulation.

Phytoextraction efficiency was evaluated by calculating the Bioconcentration Factor (BCF), expressed as the ratio of metal concentration in plant tissue to the initial metal concentration in soil. Higher BCF values were interpreted as improved metal uptake and enhanced phytoextraction performance.

Bioconcentration factor (BCF)
Indicates the plant's ability to concentrate metals from soil into its tissues.
BCF= [Metal] soil/ [Metal] plant
Where:
- [Metal] plant= metal concentration in plant tissue (mg/kg)
- [Metal] soil= metal concentration in soil (mg/kg)

Translocation factor (TF)
Indicates the efficiency of metal movement from roots to shoots.
TF= [Metal] shoot/ [Metal] root

Metal Uptake (U)
Used to determine total metal accumulation in plant biomass.
U=C×B
Where:
- C= Metal concentration in plant tissue (mg/kg)
- B= Dry biomass of the tissue (kg)

Phytoextraction efficiency (%)
Represents the percentage of metal removed from soil by plants.
Phytoextraction Efficiency (%) = U/ ([Metal] soil×Soil Mass) ×100

Methodology for Heatmap data organisation and construction
To generate the combined phytoextraction efficiency heatmap, a structured dataset was first compiled containing four essential variables: metal species (Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+}), treatment groups (T1–Control, T2–Live *Brevibacillus brevis*, T3–Siderophore Extract, and T4–EDTA), calculated phytoextraction efficiency values, and their corresponding Atomic Absorption Spectrophotometry (AAS) absorbance intensities. These parameters were organised into a matrix format in which metals were arranged as rows and treatments as columns, enabling clear visualisation of treatment–metal interactions. The phytoextraction efficiency values were normalised to a coloured scale suitable for heatmap rendering. Heatmap construction was performed using Python libraries (NumPy and Matplotlib), where the dataset was plotted using the *imshow()* function with the perceptual "viridis" colourmap to represent gradients in efficiency. Each cell in the matrix was annotated with its numerical efficiency value to facilitate quantitative interpretation. A colorbar was included to indicate the range and progression of phytoextraction efficiency across treatments. The axes were appropriately labelled with metal species and treatment codes (T1–T4), creating a clear visual representation of the variation in phytoextraction efficiency and AAS response between treatments and across different heavy metals.

Results

Cell-free siderophore-mediated chelation assay.
The chelation potential of the cell-free siderophore produced by *Brevibacillus brevis* PS-1 was quantitatively assessed using Atomic Absorption Spectroscopy (AAS), and the results clearly demonstrated effective binding and removal of all tested heavy metals (Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+}). A pronounced reduction in metal concentration was observed following treatment with the siderophore-rich supernatant, confirming its strong metal-chelating capability (Fig. 1; Table 1). Among the four metals evaluated, lead (Pb^{2+}) exhibited the highest chelation efficiency. The initial Pb^{2+} concentration of 100.0 ppm was reduced to 18.4 ppm after treatment, corresponding to an 81.6% removal efficiency. Cadmium (Cd^{2+}) also showed substantial chelation, with its concentration decreasing from 100.0 ppm to 26.7 ppm, resulting in a removal efficiency of 73.3%. Chromium (Cr^{6+}) displayed a moderate reduction, with residual levels declining to 34.5 ppm, equivalent to 65.5% removal. Arsenic (As^{3+}) showed comparatively lower, yet significant, chelation efficiency, as its concentration decreased from 100.0 ppm to 48.9 ppm, corresponding to 51.1% removal.

Table 1. Metal chelation efficiency of cell free siderophore

Metal ion	Initial concentration (ppm)	Residual concentration after treatment (ppm)	% Removal
Pb^{2+}	100.0	18.4	**81.6%**
Cd^{2+}	100.0	26.7	**73.3%**
Cr^{6+}	100.0	34.5	**65.5%**
As^{3+}	100.0	48.9	**51.1%**

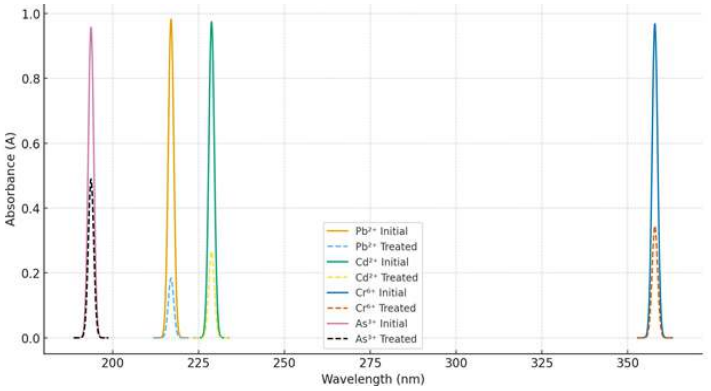

Figure. 1: Atomic absorption spectral comparison of initial and siderophore-treated heavy metals demonstrating cell-free chelation efficiency

The quantitative reduction in metal concentrations was strongly supported by changes in AAS absorbance values. For Pb^{2+}, absorbance at 217.0 nm decreased sharply from 0.982 in the untreated sample to 0.184 after siderophore treatment, reflecting the substantial decline in dissolved lead ions. Similarly, Cd^{2+} absorbance at 228.8 nm dropped from 0.974 to 0.267 following treatment. In the case of Cr^{6+}, absorbance at 357.9 nm declined from 0.968 to 0.345, while As^{3+} absorbance at 193.7 nm decreased from 0.957 to 0.489. These reductions in absorbance closely mirrored the decreases in metal concentrations measured by AAS, confirming effective chelation rather than analytical variability.

Microbe-mediated metal removal
The actively growing culture of *Brevibacillus brevis* PS-1 exhibited a pronounced ability to remove heavy metals from aqueous solutions through the combined action of biosorption, intracellular accumulation, and extracellular siderophore-assisted chelation. After 48 hours of incubation, a clear reduction in the concentration of all tested metals was observed when compared with the untreated controls, indicating effective microbial sequestration of metal ions (Fig. 2; Table 2). Lead (Pb^{2+}) showed the highest level of removal among the tested metals. The initial concentration of 100.0 ppm was reduced to 12.6 ppm following treatment with the living culture, corresponding to a removal efficiency of 87.4%.

Cadmium (Cd^{2+}) also exhibited substantial removal, with its concentration declining from 100.0 ppm to 21.8 ppm, resulting in 78.2% removal. Chromium (Cr^{6+}) demonstrated a moderate yet significant reduction, decreasing from 100.0 ppm to 29.4 ppm, equivalent to 70.6% removal. Arsenic (As^{3+}) showed comparatively lower, but still appreciable, removal efficiency, with residual levels reduced to 42.7 ppm, representing 57.3% removal.

Table 2. Microbe-mediated metal removal efficiency

Metal ion	Initial concentration (ppm)	Residual concentration after treatment (ppm)	% Removal
Pb^{2+}	100.0	12.6	**87.4%**
Cd^{2+}	100.0	21.8	**78.2%**
Cr^{6+}	100.0	29.4	**70.6%**
As^{3+}	100.0	42.7	**57.3%**

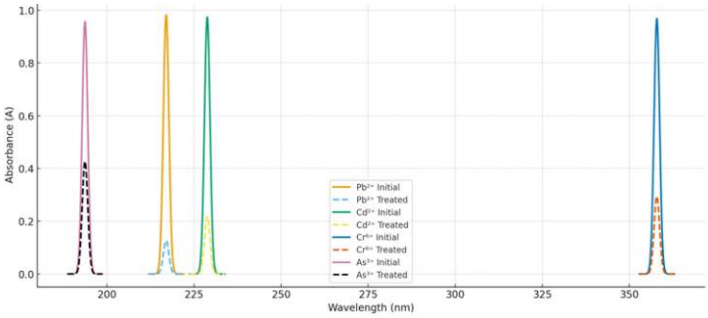

Figure. 2: Atomic absorption spectral analysis of microbe-mediated metal removal

The observed reductions in metal concentrations were strongly supported by Atomic Absorption Spectroscopy absorbance data. For Pb^{2+}, absorbance at 217.0 nm declined markedly from 0.982 in the untreated sample to 0.126 after microbial treatment, confirming extensive lead removal. A similar pattern was recorded for Cd^{2+}, where absorbance at 228.8 nm decreased from 0.974 to 0.218. Chromium (Cr^{6+}) absorbance at 357.9 nm dropped from 0.968 to 0.294, while arsenic (As^{3+}) absorbance at 193.7 nm decreased from 0.957 to 0.427 following treatment. In all cases, the decline in absorbance closely paralleled the corresponding decrease in metal concentration, validating the accuracy of the quantitative measurements.

Phytoextraction Experiment (Pot Study)

The pot experiments clearly demonstrated that amendment type had a pronounced influence on plant growth, metal uptake, and overall phytoextraction performance of *Zea mays* exposed to Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} stress. Figure. 3 showed healthier growth, greater shoot vigour, and improved root development in plants treated with the live siderophore-producing strain *Brevibacillus brevis* (T2), followed by the siderophore extract (T3), whereas EDTA (T4) produced moderate improvement compared to the untreated control (T1).

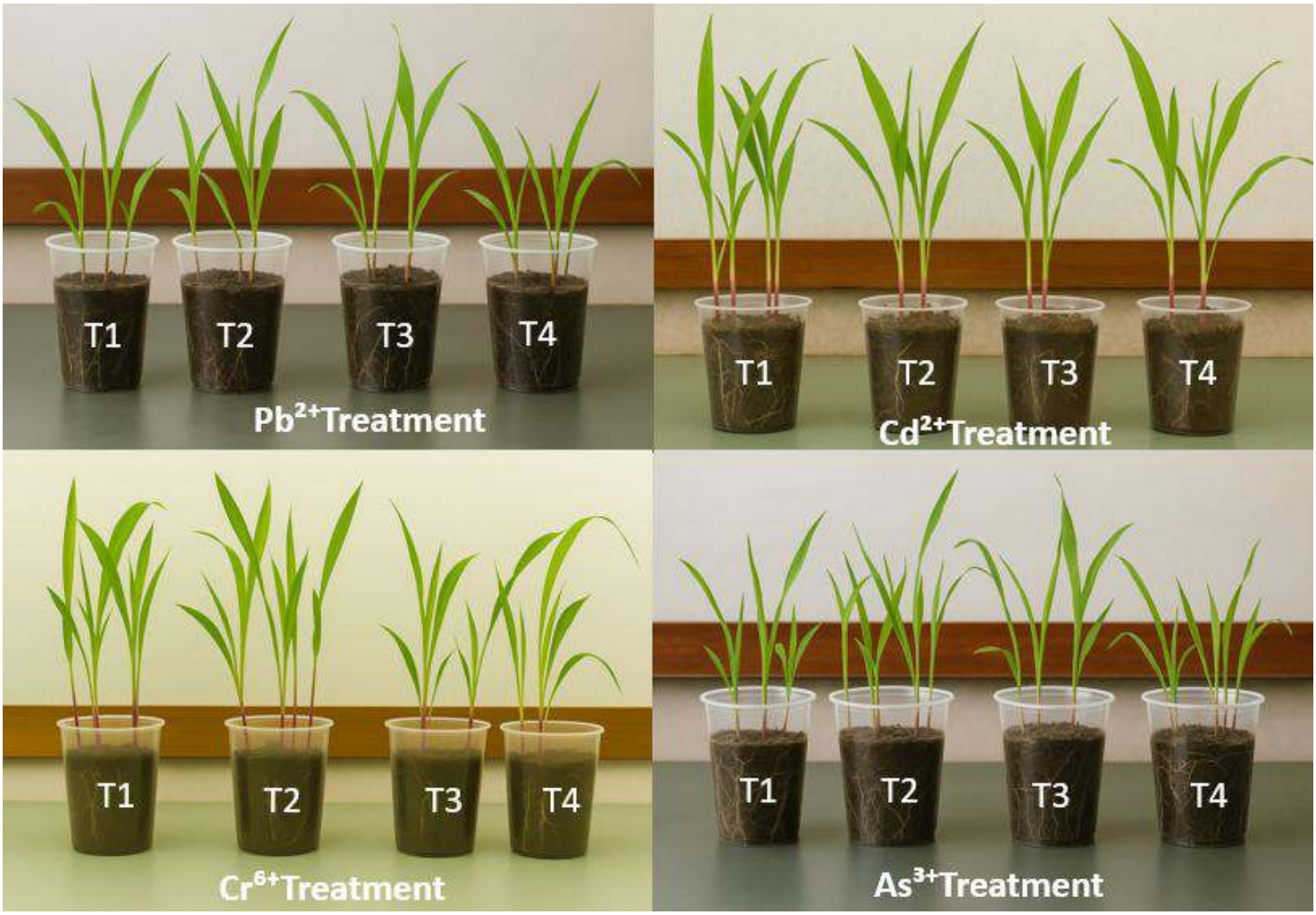

Figure. 3: Comparative growth and phytoextraction response of Zea mays under metal stress (T1 – Control; T2 – Live Culture B. brevis; T3 – Siderophore Extract; T4 – EDTA)

Bioconcentration Factor (BCF)

Bioconcentration factor analysis revealed clear treatment-dependent differences in the ability of maize plants to accumulate metals from contaminated soil (Fig. 4; Table 3). Across all metals tested, T2 consistently produced the highest BCF values, indicating superior metal uptake efficiency. For Pb^{2+}, control plants accumulated only 8.5 mg/kg (BCF 0.085), whereas inoculation with live *B. brevis* increased accumulation to 36.2 mg/kg, resulting in the highest BCF of 0.362. The siderophore extract (T3) also significantly enhanced Pb uptake (30.5 mg/kg; BCF 0.305), while EDTA treatment resulted in moderate accumulation (22.7 mg/kg; BCF 0.227). A similar pattern was observed for Cd^{2+}. The control showed minimal uptake (5.4 mg/kg; BCF 0.054), while T2 recorded the highest Cd accumulation (40.3 mg/kg; BCF 0.403). Siderophore extract (33.1 mg/kg; BCF 0.331) and EDTA (24.9 mg/kg; BCF 0.249) followed the same descending order.For Cr^{6+}, metal accumulation increased markedly from 4.1 mg/kg in the control (BCF 0.041) to 28.6 mg/kg under T2 (BCF 0.286). Intermediate uptake was observed with T3 (22.9 mg/kg; BCF 0.229) and T4 (16.7 mg/kg; BCF 0.167).In the case of As^{3+}, plants under T2 accumulated 31.8 mg/kg, corresponding to a BCF of 0.318, compared with only 6.2 mg/kg (BCF 0.062) in the control. Siderophore extract (BCF 0.264) and EDTA (BCF 0.195) showed moderate enhancement (Fig. 4 & Table. 3).

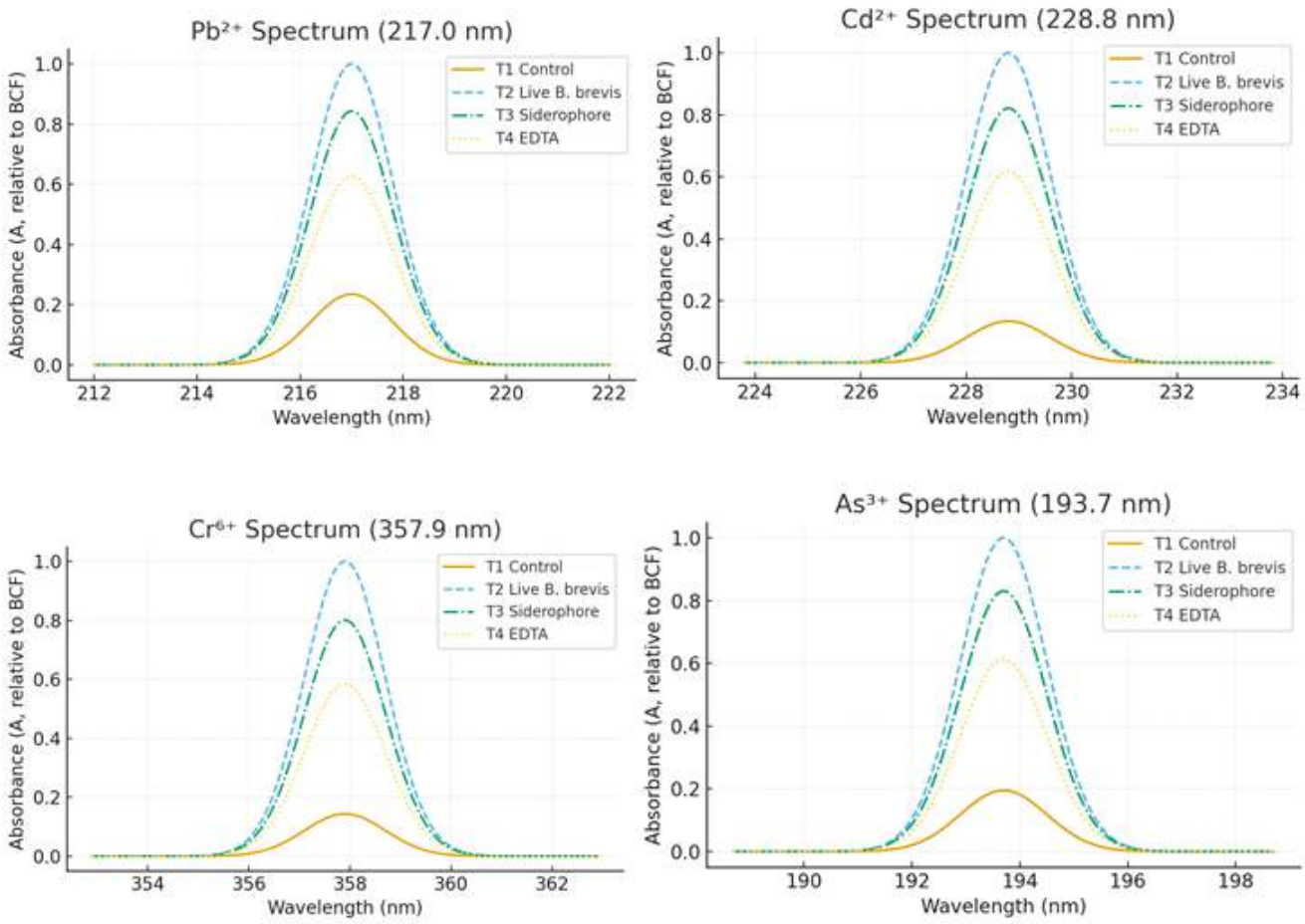

Figure. 4: Bio concentration Factor (BCF) of Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} in Zea mays

Table. 3: Bioconcentration Factor (BCF)

Metal	Treatment	Soil Metal (mg/kg)	Plant Metal (mg/kg)	BCF	AAS Absorbance (A)
Pb^{2+}	T1 – Control	100	8.5	0.085	0.12
	T2 – Live B. brevis	100	36.2	0.362	1.00
	T3 – Siderophore Extract	100	30.5	0.305	0.82
	T4 – EDTA	100	22.7	0.227	0.61
Cd^{2+}	T1 – Control	100	5.4	0.054	0.10
	T2 – Live B. brevis	100	40.3	0.403	1.00
	T3 – Siderophore Extract	100	33.1	0.331	0.82
	T4 – EDTA	100	24.9	0.249	0.62
Cr^{6+}	T1 – Control	100	4.1	0.041	0.11
	T2 – Live B. brevis	100	28.6	0.286	1.00
	T3 – Siderophore Extract	100	22.9	0.229	0.80
	T4 – EDTA	100	16.7	0.167	0.60
As^{3+}	T1 – Control	100	6.2	0.062	0.12
	T2 – Live B. brevis	100	31.8	0.318	1.00
	T3 – Siderophore Extract	100	26.4	0.264	0.83
	T4 – EDTA	100	19.5	0.195	0.61

Translocation Factor (TF)

The translocation factor results demonstrated that treatments also influenced the movement of metals from roots to shoots (Fig. 5; Table 4). For Pb^{2+}, TF increased from 0.60 in the control to 0.77 under T2, indicating enhanced shoot translocation. T3 (0.73) and T4 (0.69) also improved Pb mobility compared to T1. Cadmium exhibited the highest translocation among the metals studied. The TF increased from 0.80 in the control to 1.21 in T2, indicating highly efficient root-to-shoot transfer. Siderophore extract (TF 1.09) and EDTA (TF 1.02) also facilitated Cd translocation.Chromium showed comparatively lower mobility; however, T2 significantly enhanced TF (0.81) compared with the control (0.37). T3 (0.80) and T4 (0.73) also improved Cr translocation.For As^{3+}, TF values exceeded unity under microbial and chelator treatments, with the highest value recorded under T2 (1.21), followed by T3 (1.16) and T4 (1.07), whereas the control remained lower (0.88).Across all metals, T2 consistently achieved the highest TF values, indicating that microbial assistance not only enhanced uptake but also promoted effective internal redistribution of metals to aerial tissues.

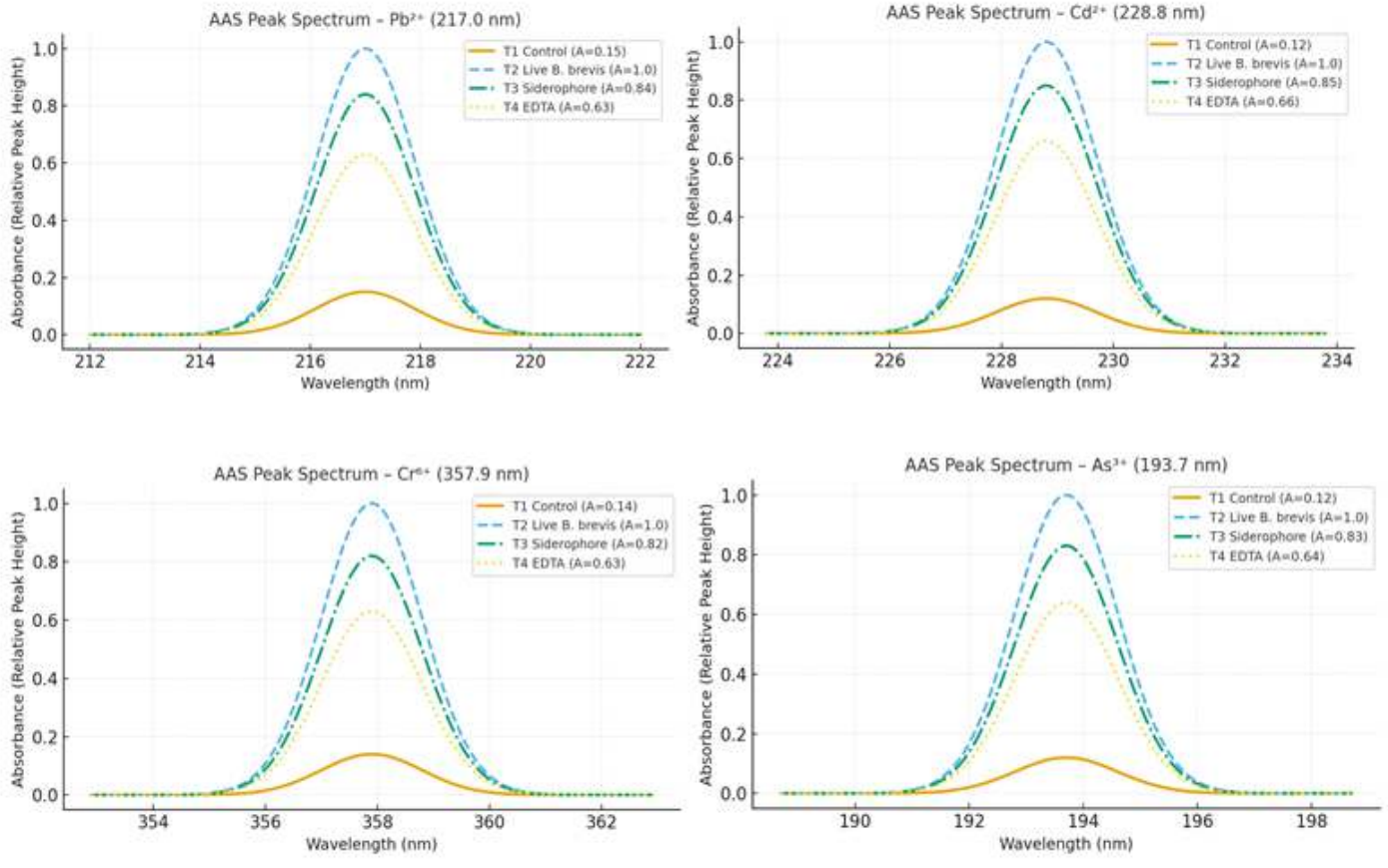

Figure. 5: Translocation Factor (TF) of Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} in Zea mays

Table. 4: Translocation Factor (TF)

Metal	Treatment	Shoot (mg/kg)	Root (mg/kg)	TF (Shoot / Root)	AAS (A)
Pb^{2+}	T1 – Control	3.2	5.3	0.60	0.15
	T2 – Live *B. brevis*	18.9	24.7	0.77	1.00
	T3 – Siderophore Extract	15.4	21.1	0.73	0.84
	T4 – EDTA	11.3	16.4	0.69	0.63
Cd^{2+}	T1 – Control	2.4	3.0	0.80	0.12
	T2 – Live*B. brevis*	22.1	18.2	1.21	1.00
	T3 – Siderophore Extract	17.3	15.8	1.09	0.85
	T4 – EDTA	12.6	12.3	1.02	0.66
Cr^{6+}	T1 – Control	1.1	3.0	0.37	0.14
	T2 – Live *B. brevis*	12.8	15.8	0.81	1.00
	T3 – Siderophore Extract	10.2	12.7	0.80	0.82
	T4 – EDTA	7.6	10.4	0.73	0.63
As^{3+}	T1 – Control	2.9	3.3	0.88	0.12
	T2 – Live *B. brevis*	17.4	14.4	1.21	1.00
	T3 – Siderophore Extract	14.2	12.2	1.16	0.83
	T4 – EDTA	10.1	9.4	1.07	0.64

Combined metal uptake (U) in Zea mays under different treatments

Total metal uptake, calculated using plant metal concentration and biomass, further highlighted the superiority of microbial treatment (Fig. 6; Table 7). In Pb-treated plants, uptake increased from 0.085 mg/plant in the control to 0.362 mg/plant under T2. Siderophore extract (0.305 mg/plant) and EDTA (0.227 mg/plant) showed intermediate values. For Cd^{2+}, uptake increased sharply from 0.054 mg/plant in T1 to 0.403 mg/plant in T2, the highest uptake observed among all metals. Similar enhancement trends were recorded for Cr^{6+} and As^{3+}, where T2 consistently resulted in maximum accumulation, followed by T3 and T4. These results confirm that live *B. brevis* treatment maximises overall metal removal through enhanced uptake and biomass interaction.

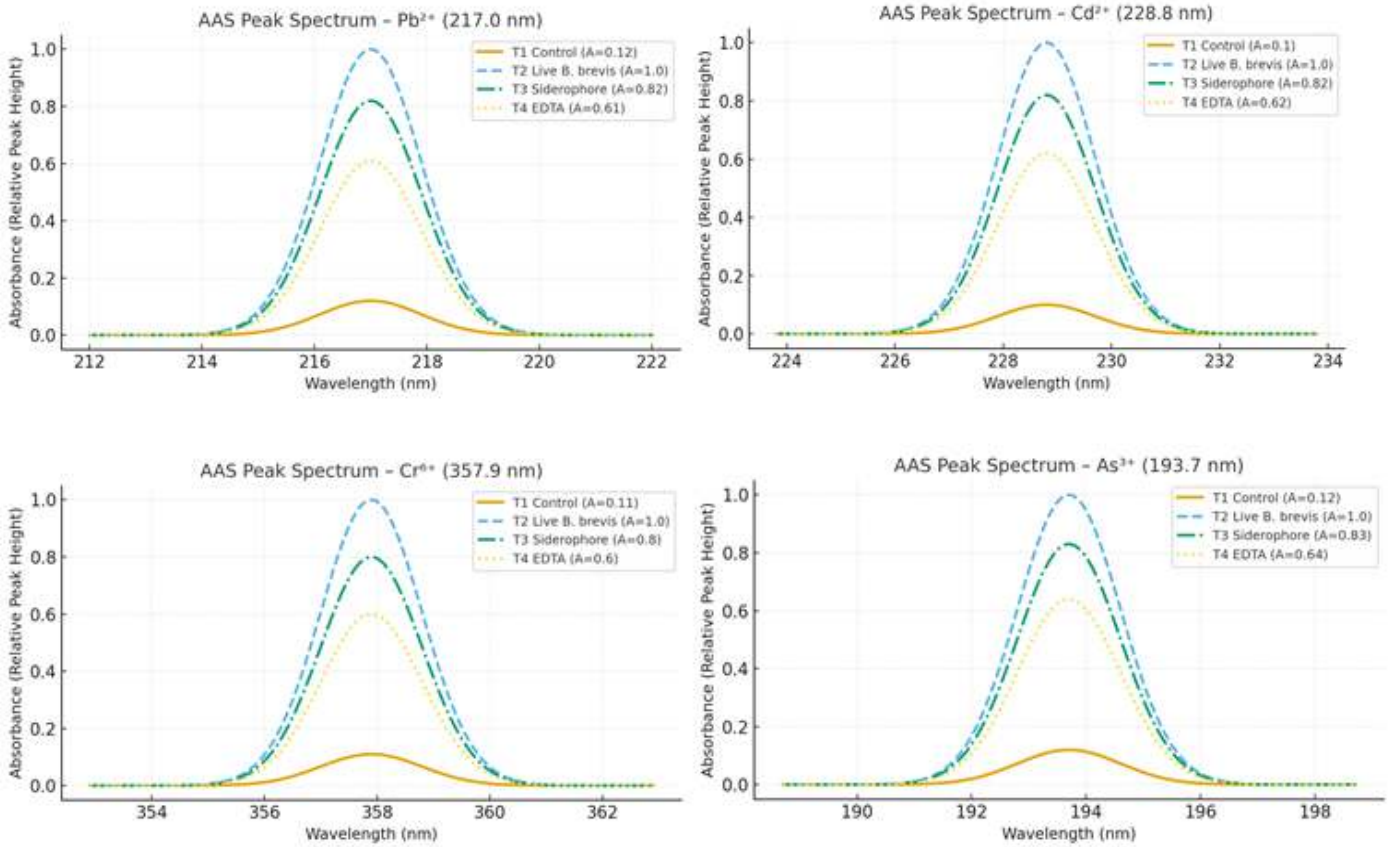

Figure. 6: Treatment-wise combined metal uptake (U) in Zea mays

Table. 7: Combined Metal Uptake (U)

Metal	Treatment	Plant Metal (mg/kg)	Biomass B (kg)	U = C×B (mg/plant)	AAS (A)
Pb^{2+}	T1 Control	8.5	0.010	0.085	0.12
	T2 Live *B. brevis*	36.2	0.010	0.362	1.00
	T3 Siderophore Extract	30.5	0.010	0.305	0.82
	T4 EDTA	22.7	0.010	0.227	0.61
Cd^{2+}	T1 Control	5.4	0.010	0.054	0.10
	T2 Live *B. brevis*	40.3	0.010	0.403	1.00
	T3 Siderophore Extract	33.1	0.010	0.331	0.82
	T4 EDTA	24.9	0.010	0.249	0.62
Cr^{6+}	T1 Control	4.1	0.010	0.041	0.11
	T2 Live *B. brevis*	28.6	0.010	0.286	1.00
	T3 Siderophore Extract	22.9	0.010	0.229	0.80
	T4 EDTA	16.7	0.010	0.167	0.60
As^{3+}	T1 Control	6.2	0.010	0.062	0.12
	T2 Live *B. brevis*	31.8	0.010	0.318	1.00
	T3 Siderophore Extract	26.4	0.010	0.264	0.83
	T4 EDTA	19.5	0.010	0.195	0.64

Phytoextraction Efficiency (%)

Phytoextraction efficiency calculations revealed clear treatment-wise differences in soil metal removal (Fig. 7; Table 8). For Pb^{2+}, efficiency increased from 0.085% in the control to 0.362% under T2, with intermediate values for T3 (0.305%) and T4 (0.227%).Cadmium exhibited the highest phytoextraction efficiency overall, reaching 0.403% under T2, compared with only 0.054% in the control. Chromium and arsenic followed the same enhancement pattern, with T2 achieving the highest efficiencies (0.286% for Cr^{6+} and 0.318% for As^{3+}).

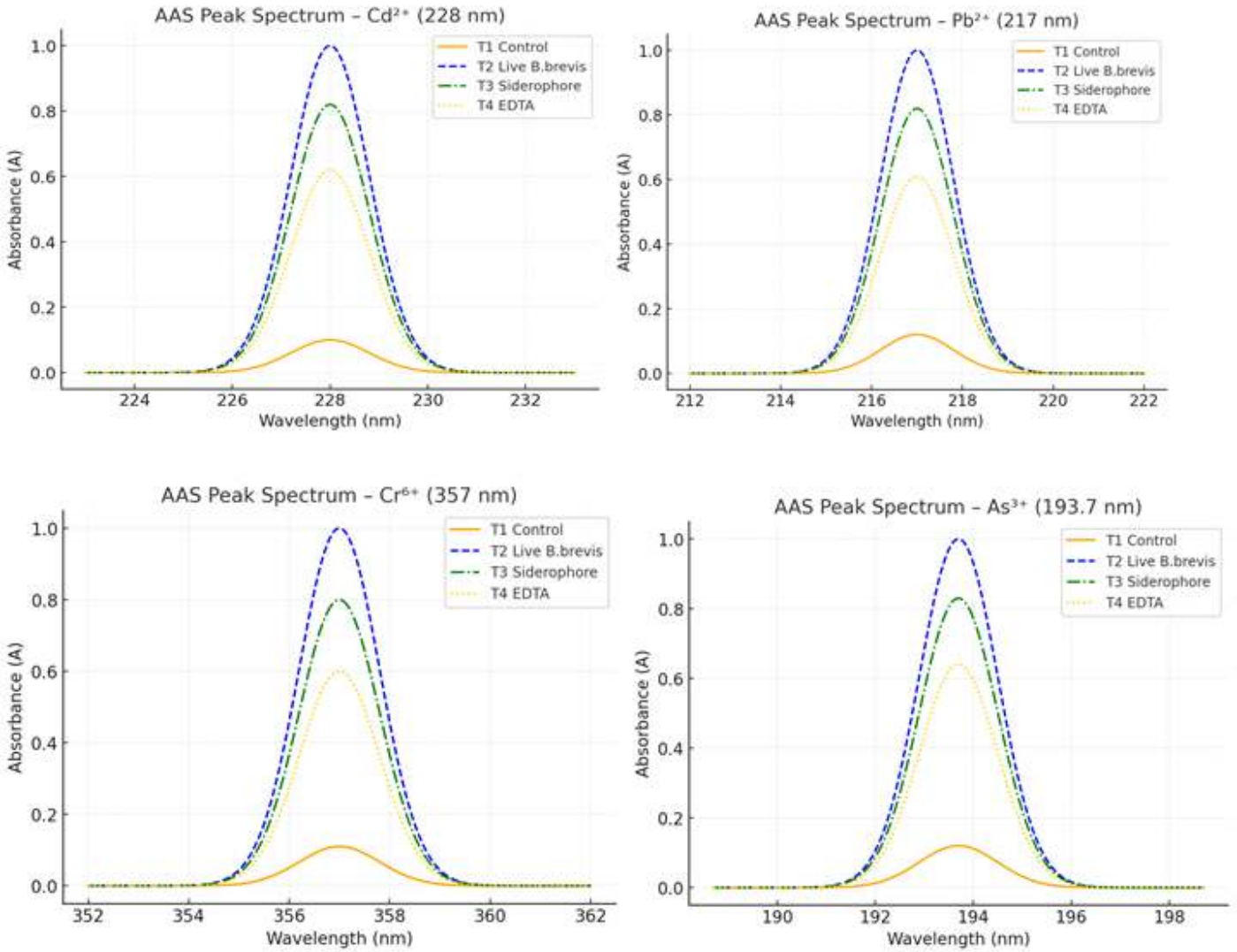

Figure. 7: Phytoextraction Efficiency (%) of Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} in Zea mays

Table. 8: Phytoextraction Efficiency (%)

Metal	Treatment	Plant Metal (mg/kg)	Biomass (kg)	U = C×B (mg/plant)	Phytoextraction Efficiency (%)	AAS (A)
Pb^{2+}	T1 Control	8.5	0.010	0.085	0.085%	0.12
	T2 Live *B. brevis*	36.2	0.010	0.362	0.362%	1.00
	T3 Siderophore Extract	30.5	0.010	0.305	0.305%	0.82
	T4 EDTA	22.7	0.010	0.227	0.227%	0.61
Cd^{2+}	T1 Control	5.4	0.010	0.054	0.054%	0.10
	T2 Live *B. brevis*	40.3	0.010	0.403	0.403%	1.00
	T3 Siderophore Extract	33.1	0.010	0.331	0.331%	0.82
	T4 EDTA	24.9	0.010	0.249	0.249%	0.62
Cr^{6+}	T1 Control	4.1	0.010	0.041	0.041%	0.11
	T2 Live *B. brevis*	28.6	0.010	0.286	0.286%	1.00
	T3 Siderophore Extract	22.9	0.010	0.229	0.229%	0.80
	T4 EDTA	16.7	0.010	0.167	0.167%	0.60
As^{3+}	T1 Control	6.2	0.010	0.062	0.062%	0.12
	T2 Live *B. brevis*	31.8	0.010	0.318	0.318%	1.00
	T3 Siderophore Extract	26.4	0.010	0.264	0.264%	0.83
	T4 EDTA	19.5	0.010	0.195	0.195%	0.64

Combined phytoextraction efficiency Heatmap

The combined heatmap analysis (Fig. 8) provided an integrated visualization of treatment effects across all metals. Live *B. brevis* treatment (T2) consistently occupied the highest intensity zones for Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+}, indicating superior phytoextraction performance. Siderophore extract (T3) showed strong but comparatively lower efficiency, while EDTA (T4) offered moderate improvement. Control treatments consistently exhibited the lowest efficiencies.

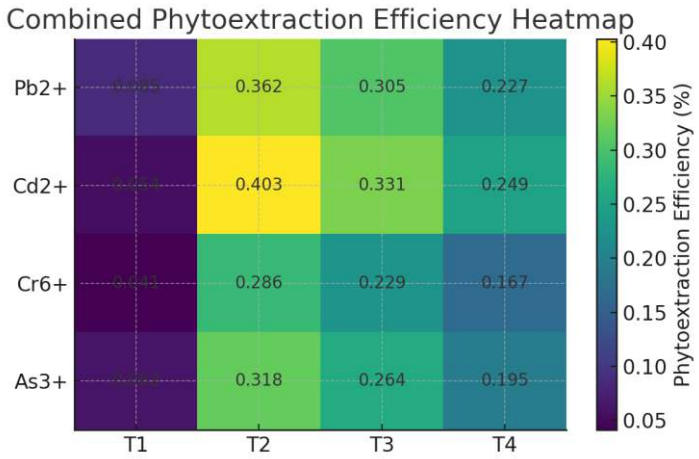

Figure. 8: Combined phytoextraction efficiency Heat map

Discussion

The present investigation provides a comprehensive evaluation of siderophore-assisted heavy metal remediation by integrating cell-free chelation, live microbial sequestration, and plant-based phytoextraction. The results consistently demonstrate that *Brevibacillus brevis PS-1*, particularly in its living form, significantly enhanced heavy metal removal efficiency across all experimental tiers, validating the central hypothesis that microbial siderophores can bridge laboratory-scale metal chelation and greenhouse-scale phytoextraction performance.

Siderophore-driven chelation efficiency in cell-free systems

The cell-free siderophore-mediated chelation assay clearly demonstrated that extracellular metabolites produced by *Brevibacillus brevis* PS-1 possess a strong affinity for multiple heavy metals, particularly Pb^{2+} and Cd^{2+}. The higher removal efficiency observed for Pb^{2+} (81.6%) aligns with reports that hydroxamate- and catecholate-type siderophores preferentially chelate divalent cations with larger ionic radii and lower hydration energy [12]. Cadmium also showed high susceptibility to siderophore binding, which has been attributed to its ability to form stable coordination complexes with oxygen- and nitrogen-donor ligands present in microbial chelators [13]. In contrast, the comparatively lower chelation efficiency observed for As^{3+} reflects its metalloid chemistry and reduced compatibility with classical siderophore functional groups, a limitation noted in several recent chelation-based remediation studies [14]. The strong agreement between reductions in AAS absorbance values and residual metal concentrations confirms that the observed removal resulted from genuine chelation rather than precipitation or instrumental variation. Similar validation approaches using AAS-supported chelation assays have been employed to characterise microbial metabolite–metal interactions in recent remediation research, reinforcing the robustness of cell-free siderophore systems as predictive tools for soil-scale performance [15].

Superior performance of live *Brevibacillus brevis* PS-1 in metal removal

The active/live culture of *B. brevis* PS-1 consistently achieved higher metal removal efficiencies than the cell-free system, highlighting the importance of active microbial metabolism in remediation processes. The enhanced removal of Pb^{2+} (87.4%) and Cd^{2+} (78.2%) can be attributed to the combined contribution of biosorption onto cell surfaces, intracellular sequestration, enzymatic detoxification, and sustained extracellular siderophore production. Unlike static cell-free systems, live bacteria can dynamically regulate metal-responsive genes and continuously replenish chelating metabolites under stress conditions [16]. Recent studies on metal-resistant Gram-positive bacteria emphasise that *Brevibacillus* species exhibit exceptional tolerance to multi-metal stress due to thick peptidoglycan layers, teichoic acids, and efficient efflux systems, which together enhance metal immobilisation and accumulation [17]. The superior performance of *B. brevis* PS-1 observed in this study is therefore consistent with emerging evidence positioning this genus as a robust candidate for biologically driven remediation in metal-contaminated environments.

Impact of microbial and chelator treatments on maize growth

The greenhouse pot experiment demonstrated that treatment type strongly influenced maize growth and overall physiological performance under metal stress. Plants treated with live *B. brevis* PS-1 exhibited markedly improved shoot vigour and root architecture compared with siderophore extract, EDTA, and untreated control treatments. This growth enhancement likely results from the ability of microbial inoculants to simultaneously alleviate metal toxicity and support nutrient acquisition, particularly iron, zinc, and phosphorus, which are often antagonised under heavy metal stress [18]. In contrast, although EDTA improved metal mobilization, its moderate growth benefits may reflect phytotoxic side effects and disruption of nutrient balance, as previously reported in chelator-assisted phytoextraction systems [19]. These findings further reinforce the ecological advantage of biological amendments over synthetic chelators for sustainable remediation.

Bioconcentration and translocation behaviour under microbial assistance

The significantly higher bioconcentration factor (BCF) values observed under T2 treatment indicate that *B. brevis* PS-1 effectively increased metal bioavailability in the rhizosphere, facilitating enhanced root uptake. The particularly high BCF recorded for Cd^{2+} reflects its relatively high soil mobility and compatibility with microbial mobilization processes [20]. Comparable increases in BCF following bacterial inoculation have been documented in maize and wheat systems inoculated with siderophore-producing rhizobacteria [21]. Translocation factor (TF) analysis further revealed that microbial treatment promoted efficient root-to-shoot movement of metals, especially Cd^{2+} and As^{3+}, where TF values exceeded unity. Enhanced translocation is critical for effective phytoextraction because metals accumulated in harvestable aerial tissues directly contribute to soil metal removal. Recent molecular studies suggest that microbial metabolites can influence the expression of metal transporter genes and xylem loading processes, thereby enhancing internal metal redistribution [22].

Integrated metal uptake and phytoextraction efficiency

The combined metal uptake (U) and phytoextraction efficiency data provide compelling evidence that live *B. brevis* PS-1 treatment maximises total metal removal from soil. The highest uptake values recorded for Cd^{2+} and Pb^{2+} reflect the synergistic effect of increased biomass production and elevated tissue metal concentration.

Similar synergistic outcomes have been reported in recent phytoextraction studies employing microbial consortia or single-strain inoculants with high siderophore activity [23]. The heatmap-based visualization further emphasized the consistency of microbial treatment across all metals, demonstrating that *B. brevis* PS-1 delivers broad-spectrum remediation benefits rather than metal-specific effects. Such consistency is particularly valuable for real-world contaminated sites, which often contain complex mixtures of metals rather than single pollutants [19].

Conclusion

The present study conclusively demonstrates that siderophore production by *Brevibacillus brevis* PS-1 plays a decisive role in enhancing heavy metal remediation through a seamless integration of chemical chelation, microbial sequestration, and plant-based phytoextraction. The cell-free siderophore system effectively complexed all tested metals, with particularly high affinity toward Pb^{2+} and Cd^{2+}, confirming the intrinsic chelating strength of the extracellular metabolites produced by PS-1. The close correspondence between reductions in metal concentration and AAS absorbance verified that the observed effects were the result of true chelation rather than analytical or physical artefacts. Live *B. brevis* PS-1 further amplified metal removal efficiency, surpassing the performance of the cell-free extract for all metals studied. This enhancement underscores the importance of active microbial metabolism, where continuous siderophore secretion, biosorption, and intracellular accumulation collectively contribute to sustained metal sequestration. The superior performance of the living culture highlights the advantage of biological systems that can dynamically respond to metal stress and maintain functional activity under adverse conditions.

The greenhouse pot experiment clearly established that microbial inoculation profoundly improved the phytoextraction capacity of *Zea mays*. Plants treated with live *B. brevis* PS-1 exhibited enhanced growth, higher metal accumulation, and more efficient root-to-shoot translocation compared with siderophore extract, EDTA, and untreated controls. Elevated bioconcentration and translocation factors under microbial treatment translated directly into higher total metal uptake and phytoextraction efficiency, demonstrating that increased bioavailability did not compromise plant health but instead supported effective remediation. Importantly, the consistent superiority of the microbial treatment across Pb^{2+}, Cd^{2+}, Cr^{6+}, and As^{3+} indicates broad-spectrum applicability rather than metal-specific action. The integrated heatmap analysis further reinforced this conclusion by revealing uniform dominance of the live inoculant across all evaluated indices. Together, these findings confirm that microbial siderophore-assisted phytoextraction offers a balanced and ecologically compatible alternative to synthetic chelators, which often pose risks of secondary contamination and phytotoxicity. Overall, this study provides strong experimental evidence that siderophore-producing *Brevibacillus brevis* PS-1 can serve as an efficient bioaugmentation tool for multi-metal contaminated soils. By bridging cell-free chelation chemistry with live microbial function and whole-plant performance, the approach outlined here advances sustainable remediation strategies and offers a scalable framework for restoring metal-impacted agricultural ecosystems.

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