International Journal of Recent Advances in Multidisciplinary Topics
Volume 7, Issue 3, March 2026
www.ijramt.com | E-ISSN: 2582-7839 | RESAIM Publishing | www.resaim.com

Bioremediation of Pb, Cd and Cr by Soil Bacteria from Urban-Industrial Transition Zones

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Abstract*: Urban–industrial transition zones are dynamic areas where residential, traffic, and industrial activities coexist, often leading to persistent heavy metal contamination. Metals such as lead (Pb), cadmium (Cd), and chromium (Cr) are of particular concern due to their non-biodegradable nature, long-term persistence in soil, and associated ecological and public health risks. Their accumulation alters soil chemistry and disrupts native microbial communities essential for soil health. This study investigated the resilience of indigenous soil bacteria and their potential role in heavy metal bioremediation. Soil samples were collected from urban gardens and textile-influenced industrial sites and analyzed for physicochemical parameters (pH, moisture, texture) and heavy metal concentrations using standard colorimetric methods. Pb and Cd were estimated by the dithizone method at 520 nm, while Cr was quantified at 540 nm following acid digestion. Industrial soils showed comparatively altered physicochemical properties and higher metal loads. To assess microbial adaptation, samples were enriched in metal-supplemented nutrient broth, and distinct bacterial isolates were purified. Minimum inhibitory concentration (MIC) studies under combined metal stress identified three highly tolerant isolates, which were further tested for compatibility and consortium development. Biosorption potential was evaluated in liquid culture, followed by laboratory-scale soil microcosm experiments using metal-spiked sterile soil. Both individual isolates and the consortium were applied, and metal reduction was monitored over time. The consortium demonstrated enhanced remediation efficiency compared to individual strains. Statistical correlation analysis further examined the relationship between pollutant concentration and removal efficiency. Overall, the findings highlight the adaptive capacity of indigenous soil bacteria and support their potential use as bioindicators and eco-friendly agents for sustainable heavy metal bioremediation.

***Keywords*: Heavy metal bioremediation, Indigenous soil bacteria, Lead, Cadmium, Chromium, Bacterial consortium, Soil microcosm, Metal biosorption, Minimum inhibitory concentration, Urban industrial transition zones.**

1. Introduction

Soil is a dynamic living system that sustains ecosystem functioning through nutrient cycling, organic matter decomposition, and support of terrestrial food webs. However, soil health in urban–industrial transition zones is increasingly threatened by rapid urbanization, industrial discharge, vehicular emissions, construction activities, and waste dumping. These overlapping anthropogenic inputs lead to the accumulation of persistent contaminants, particularly heavy metals such as lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), and zinc (Zn), which do not degrade and progressively accumulate in soils. Beyond altering physicochemical properties, these metals significantly influence soil microbial communities (Hoque et al., 2023).

Urban–industrial ecotones represent complex environments where residential, agricultural, and industrial activities coexist, resulting in continuous pollutant influx. While chemical analyses quantify contaminant concentrations, they provide limited insight into biological impact. Indigenous soil bacteria, due to their rapid growth, metabolic diversity, and sensitivity to environmental change, serve as effective bioindicators of soil health. Changes in bacterial diversity metrics, including Shannon and Simpson indices, often reflect contamination stress, with polluted soils typically exhibiting reduced diversity and evenness Ghosh Roy *et al.* 2022.

Heavy metal contamination consistently drives shifts in microbial community structure. Studies of roadside soils along NH-8A in Gujarat demonstrated reduced microbial diversity alongside dominance of metal-tolerant genera such as *Bacillus*, *Pseudomonas*, and *Arthrobacter* Singh & Hiranmai, 2021. Molecular analyses further revealed the presence of metal-resistance genes, including efflux systems and metal-binding proteins, indicating both adaptive responses and potential functional roles in metal transformation. Controlled microcosm experiments support these findings, showing that pollutant exposure simplifies community structure by suppressing sensitive taxa and favoring tolerant groups Ghosh Roy *et al.* 2022.

Beyond serving as indicators, microbial responses can inform ecological risk assessment. Heavy metal accumulation has been linked to soil degradation and increased ecological risk in river basin agricultural systems Hoque *et al.*2023, with implications for food safety and public health. In densely populated urban–industrial regions, microbial indicators may therefore function as early warning systems for environmental and human health risks.

Although chemical thresholds for soil contamination are well established, microbial indices are not yet standardized within regulatory frameworks. Variability due to soil type, climate, and land-use history further complicates interpretation, underscoring the need for robust study design and localized

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calibration. Nevertheless, integrating indigenous bacterial bioindicators with chemical assessment offers a biologically meaningful and potentially cost-effective approach to soil monitoring. Additionally, metal-tolerant taxa identified in contaminated sites may serve as candidates for future bioremediation strategies.

Collectively, evidence from microcosm experiments, roadside soil studies, and regional assessments indicates that heavy metal pollution reduces bacterial diversity and simplifies microbial communities while selecting for tolerant taxa. Incorporating microbial bioindicators into soil assessment frameworks can enhance our ability to evaluate, interpret, and manage soil health in urban–industrial transition zones.

2. Literature Review

A. Heavy Metal Pollution in Urban–Industrial Soils

Heavy metal contamination is a persistent environmental challenge in urban–industrial transition zones. Rapid urbanization, industrial discharge, vehicular emissions, and mixed land-use activities create spatially heterogeneous pollutant "hotspots." Because heavy metals are non-biodegradable and accumulate over time, they alter both soil physicochemical properties and microbial community structure. Recent literature emphasizes that chemical distribution alone is insufficient for understanding ecological impact; indigenous soil bacteria have emerged as sensitive bioindicators and potential agents of remediation. Across field surveys, controlled experiments, and applied trials, contaminants consistently reshape bacterial community composition, select for tolerant taxa, and influence ecosystem services.

B. Field-Based Assessments and Spatial Variability

Field investigations provide the foundation for linking contamination gradients to ecological risk. Studies such as Parvez *et al*. 2023, demonstrate strong spatial variability in heavy metal loads around industrial zones, reinforcing the need for stratified and hotspot-targeted microbial sampling. In heterogeneous urban–industrial landscapes, microbial responses mirror localized pollutant pressures, highlighting the importance of site-specific calibration in bioindicator studies.

C. Microbial Community Responses to Heavy Metal Stress

Multiple studies report consistent patterns under heavy metal stress: (i) reduced overall microbial diversity and evenness, and (ii) dominance of tolerant or opportunistic taxa Yi *et al.* 2021. These taxonomic shifts are functionally significant, as the loss of sensitive nutrient-cycling microorganisms may impair ecosystem processes such as nitrogen transformation. Such findings strengthen the case for microbial bioindicators, which reveal ecological consequences beyond contaminant presence.

D. Functional and Mechanistic Insights

Mechanistic reviews of contaminated soils, including mining-impacted systems Haghighizadeh *et al.* 2024, show that metal bioavailability depends on interactions among soil matrices, organic matter, and microbial processes. Advances in metagenomics and genome-resolved analyses now link taxonomic identity with resistance genes and metabolic pathways. For example, Li *et al.* 2025 combined community profiling with remediation trials near a lead–zinc mining site, identifying indigenous taxa harboring genes related to metal resistance, sequestration, and transformation. Such functional insights are critical for designing locally adapted bioremediation strategies.

E. Co-Selection of Heavy Metal and Antibiotic Resistance

An emerging concern is the co-selection of antibiotic resistance genes (ARGs) in metal contaminated soils. Heavy metal stress can favor bacteria carrying both metal and antibiotic resistance determinants, raising potential public health risks in densely populated urban–industrial areas. Microbial monitoring therefore serves dual roles in ecological assessment and resistance surveillance.

F. Microbial Indicators in Soil Assessment

Operational use of indigenous bacteria in soil assessment typically involves:

- Diversity indices (e.g., Shannon, Simpson),
- Indicator taxa analysis, and
- Quantification of functional genes via qPCR or metagenomics.

Integrated monitoring frameworks combine chemical speciation with molecular profiling to assess bioavailable metal fractions rather than total concentrations alone. Tiered sampling strategies that incorporate both background and hotspot sites enhance ecological relevance and risk interpretation.

G. Bioremediation Strategies Using Indigenous Microbes

The literature outlines several microbial remediation approaches, including biostimulation, bioaugmentation, rhizosphere-assisted phytoremediation, and engineered microbial consortia. Indigenous strains are particularly valuable due to local adaptation and improved in situ survival. Mechanisms underlying remediation include biosorption, bioaccumulation, biotransformation, immobilization, and enzymatic degradation of organics.

Recent studies demonstrate strong biosorption potential among metal-tolerant isolates. Abbas et al. (2025) reported efficient Pb uptake followed by Cd and Cu, while Kumar *et al.* (2024) described a *Staphylococcus epidermidis* strain removing over 90% of Cd and Pb in solution. *Bacillus* species are frequently identified as effective immobilizers (Ahmady-Asbchin*et al.,* 2024; Liang *et al.,* 2025), and *Pseudomonas* isolates exhibit enhanced Pb removal linked to exopolysaccharide production (Vélez *et al.,* 2021). Soil microcosm studies further validate these findings under realistic conditions, with microbial consortia often outperforming single strains (Pariatamby*et al*., 2024; Gao *et al.,* 2025).

H. Limitations and Methodological Challenges

Despite promising advances, challenges remain. Natural variability in soil properties complicates cross-site comparisons, and molecular data often indicate potential function rather than active metabolism. Complementary

transcriptomic or enzymatic assays may therefore be required. Additionally, concerns about ARG dissemination and long-term ecological stability necessitate cautious implementation and extended monitoring to ensure durable remediation outcomes.

3. Material and Methods

A. Materials

Soil samples were collected from various locations of urban and industrial transition zones from southern Gujarat. Sodium thiosulfate ($Na_2S_2O_3$), Sodium hydroxide (NaOH), Borate buffer, EDTA, Chloroform, Sodium nitrate ($NaNO_3$), Lead Nitrate, Chromium Nitrate, Cadmium chloride were also used for experimental procedure. Nutrient Agar media, Dithizone reagent were obtained from HiMedia laboratories. Pre-sterilized petri plates, Pipettes, Tubes (Dilution, S-line, Suspension), Beaker, Flask, pH paper, Glass rods and transparent storage container were used for the experimental procedure. All chemicals and reagents used in the study were of analytical grade and were utilized without further purification.

B. Methodology

1) Site Selection and Soil Sampling

For this study, a total of twelve soil samples will be systematically collected from selected urban and industrial sites. Sampling was carried out from the surface layer up to a depth of 20 cm, ensuring consistency across all sites. Each sample was approximately 500 g and placed into sterile polyethylene bags or containers, properly sealed, labelled, and subsequently preserved under refrigeration to maintain integrity *Quiñones-Cerna et al. (2024)*. Of the twelve samples, six were urban soils, comprising three garden soil samples and three samples from dumping sites, reflecting common urban land-use conditions. The remaining six samples were drawn from industrial areas, specifically from locations surrounding textile industries, to capture potential contamination arising from industrial effluents and emissions. This design allowed for a comparative assessment of soil health between urban and industrial ecosystems while incorporating adjacent control sites to establish a baseline.

2) Soil Physicochemical Properties Analysis

Soil physicochemical properties will be systematically analyzed to assess both fertility and potential contamination. The analysis will begin with the determination of physical properties.

a) Soil Texture determination:

Soil texture classified via soil texture triangleby *Thien, S. J. (1979)*.

Procedure for this is in the following flowchart:

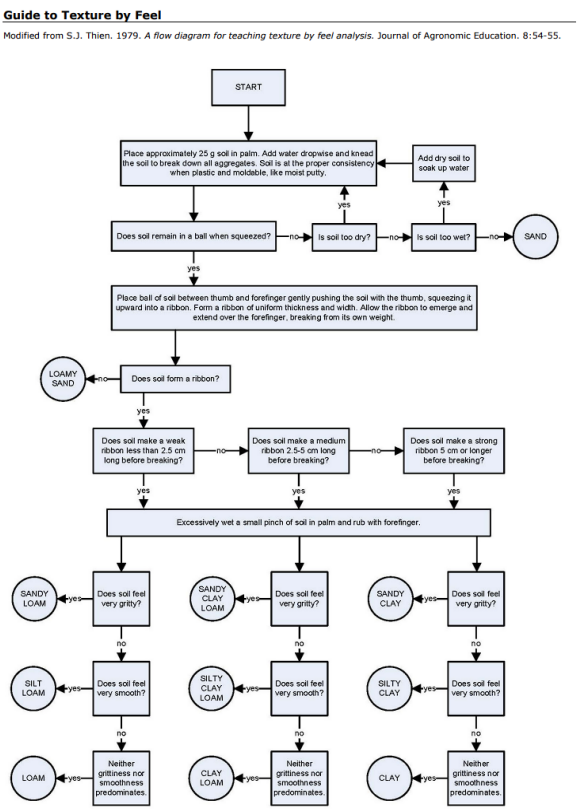

Fig. 1. Flowchart of protocol of soil texture by feel by S.J. Thien (1979)

b) Soil moisture determination:

Moisture content will also be evaluated by gravimetric method by *FAO. 2023. Standard operating procedure for soil moisture content by gravimetric method. Rome.*

The soil samples were reduced in size by quartering. The prepared samples were stored in clearly labelled, airtight containers made of inert material to prevent moisture ingress and contamination.

Each empty container with its lid was weighed on an analytical balance, and the mass (Mc) was recorded. The balance was then tared, and between 5 g moist soil was placed into the container. The mass of the moist soil (Mms) was recorded, and the container was immediately covered with its labelled lid. The uncovered container was placed in a drying oven maintained at 75 °C ± 5 °C, with the corresponding lid placed underneath to maintain identification.

The samples were dried for approximately 24 h, or until constant mass was achieved, defined as less than 0.1 % additional mass loss after an extra hour of heating. After drying, the containers were removed from the oven, immediately covered with their lids, and placed in a desiccator to cool to room temperature for at least 2 h. Once cooled, the containers with the oven-dried soil were weighed promptly to minimize moisture absorption from the atmosphere, and the mass of the oven-dry soil plus container (Mcds) was recorded. Lastly the weight was calculated by equation:

$$W\% = \left(\frac{Mcms - Mcds}{Mcds - Mc}\right) \times 100$$

W%= Water content

Mms= Mass of moist soil, in g

Mcms= Mass of container and moist soil, in g (Mcms = Mc + Mms)

Mcds = mass of container and oven dry sample, in g

Mc = mass of container, in g

c) Soil pH measurement:

Soil pH was measured using litmus paper. The soil samples were firstly weighed about 2gms and kept in separate petri plates with labels on them. Then few drops of distilled water was added to the each of the soil samples to make them wet. pH paper i.e. litmus paper is then made to contact with this soil samples and reading was noted down as per the colour shown from the litmus paper measurement paper.

3) Enrichment of soil samples

8 PTC tubes were taken for the 8 samples collected, these samples were then measured upto 2.5gm and then lept in each of the tubes and labelled accordingly to their samples.

25ml of Sterile nutrient broth spiked with heavy metals (Pb, Cd, Cr) (0.05gm each metal) was added in PTC tubes with soil sample respectively. And the tubes were kept on a shaker for incubation of 7 days at room temperature.

4) Isolation of colonies

Sterile Nutrient Agar plates were prepared with heavy metals (Pb,Cd,Cr)(0.05gm each metal), this provided further isolation so only heavy metal tolerant bacteria could grow. The plates were labelled as A1, A2, A3, A4, I1, I2, I3, I4 respectively and then quadrant streaking method was performed on it, the plates were kept in incubator for 48 hrs under 37°C. And colony characteristics were noted down

5) MIC of soil isolates for tolerance activity determination:

A dilution protocol was followed by 2-fold dilution with range of 3200µg/mLupto 50 µg/mL.

Stock= heavy metal stock prepared of each heavy metals $Pb(NO_3)_2$, $Cr_3(NO_3)_3.9H_2O$, $CdCl_2$. Incorporated to make a single stock containing concentration of each metal of 3200(µg/mL).

Diluent= Sterile Nutrient Broth

Total volume= 2ml

MIC dilution for heavy metal tolerance of Pb, Cd, Cr, of 3200(µg/mL) to 50(µg/mL) with 2-fold dilution.

Key: Mix & transfer 2 ml

Incubation time= 24-48hrs in incubator at 37°C.

6) Preparation of Consortium

For consortium, *Qurbani et al, (2025), Santiago et al., (2017)*. Three of the most promising isolates were picked, a single colony into 10 mL of Sterile Nutrient broth from each isolates individually. Incubate for 24 hours at 37°C.

For compatibility testing was performed cross streaking to detect antagonism: Cross-streak method, on a Nutrient agar plate, I2 isolate was streaked as a straight line from center to edge then on that perpendicularly other tow isolates were streaked which were I4 and A1.

Incubate 24 hrs at room temp. Observation was done at intersection and along streaks.

Once compatibility was observed.

Now, in equal volumes of isolates 100 µL each into 10 mL fresh sterile Nutrient broth and incubate 24 hours at 37°C.

7) Microcosm preparation for bioremediation analysis

Four Soil microcosms flasks were prepared in 250 ml flasks, each containing 75 g of the same pre-characterized sterile soil. Soil sample was autoclaved and then heavy metal determination analysis was done to check the heavy metal concentration present in the soil.

Analysis of lead: Performing Standard assay with dithizone-chloroform.

Once the dilution protocol is performed, one by one each tube is poured into separatory funnel and additional 5ml chloroform is added lastly it is shaken vigorously for reaction to occur. Then let it settle for 15 minutes. Jeffery, G. H. (1989). Funnel out the lower layer containing the green complex of lead-dithizonate and measure O.D at 520nm.

Once the dilution protocol is performed, one by one each tube is poured into separatory funnel and additional 5ml chloroform is added lastly it is shaken vigorously for reaction to occur. Then let it settle for 15 minutes Koroleff, F. *et al.,* 1950; Jeffery, G. H. (1989). Funnel out the lower layer containing the complex and measure O.D at 520nm

Once dilutions are performed then mix and heat it for 15 minutes at 60-70°C.

Let it cool and then measure O.D. at 540nm. Cerar, J. (2015).

After the analysis was done then the 4 microcosm were labelled with C1 (indicating consortium), I2, I4, A1 respectively. In this microcosm flasks, addition of cultures were done, for each microcosm 6ml of enriched nutrient broth was added, i.e. Consoritum 6ml in C1, A1 6ml in A1microcosm, I2 6ml in I2 microcosm andI4 6ml in I4microcosm.

And were kept for incubation for about 7 days in room temperature. Benjelloun, I, *et al.* 2022

With intervals of day3, day 5 and day7 chemical analysis to check the heavy metal concentration decrease is done via the same protocols of the standards.

8) Statistical and Correlational analysis

Statistical and correlation analyses were performed to evaluate the relationship between heavy metal concentration and bacterial degradation efficiency in soil microcosms over time. Standard calibration readings were first used to calculate residual heavy metal concentrations in test samples collected on the 3rd, 5th, and 7th days of incubation. For each isolate, the calculated residual metal concentrations and percentage removal values were paired with corresponding bacterial growth measurements (OD_{600}). The datasets were organized in Microsoft Excel for analysis. Pearson's correlation coefficient (r) was calculated using the built-in CORREL function in Excel to determine the degree of association between bacterial growth and degradation efficiency. One-way ANOVA (single factor) was performed using the Data Analysis ToolPak in Excel to assess the statistical significance of differences in degradation efficiency across the three incubation periods (Day 3, Day 5, and Day 7). A significance level of $p < 0.05$ was used to determine meaningful differences. This combined statistical approach enabled quantitative assessment of the relationship

between pollutant stress, incubation time, and microbial degradation performance, thereby strengthening the reliability of the experimental findings. Asunmo*et. al.*2023.

4. Results

A. Soil Sample Collection

Table 1
Soil samples and their location

Sample no.	Location
A1	Valsad
A2	Valsad
A3	Pardi
A4	Udwada
I1	Vapi
I2	Vapi
I3	Vapi
I4	Vapi

B. Soil Isolation and Enrichment

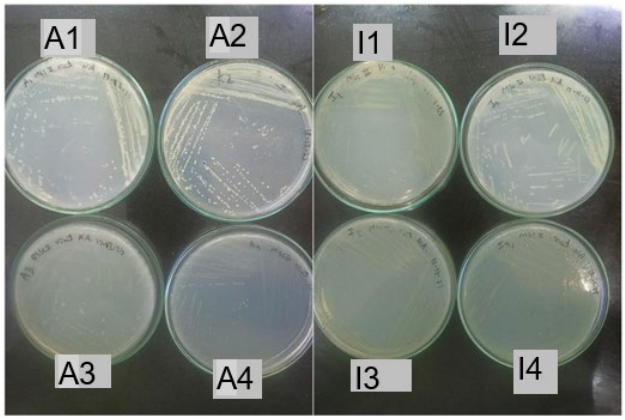

Fig. 2. a) Soil isolates from A1 to A4, b) Soil isolates from I1 to I4

All the isolates were plated via quadrant streaking. And showed visible growth with well isolated colonies.

C. Soil Bacterial Isolates Colony Characteristics

Table 2
Colony characteristics of soil sample isolates

Soil	Shape	Size	Colour	Margin	Elevation	Opacity	Consistency
A1	circular	1mm	white	Entire	Raised	Opaque	Sticky
A2	circular	1mm	white	Entire	Raised	Opaque	Mucoid
A3	circular	2mm	Pale green	Entire	Flat	Opaque	Mucoid
A4	circular	2mm	Pale green	Irregular	Flat	Opaque	Smooth
I1	circular	1mm	Yellowish green	Entire	Raised	Opaque	Smooth
I2	circular	1mm	Off-white	Entire	Raised	Opaque	Slimey
I3	irregular	2mm	Pale yellow	Irregular	Raised	Opaque	Smooth
I4	irregular	1mm	Greenish yellow	Irregular	Raised	Opaque	Mucoid

D. Soil Physiochemical Properties

1) Soil physicochemical properties

The physicochemical characteristics of the eight soil samples (A1–A4: urban sites; I1–I4: industrial sites) are presented in Table 5.4.1. Soil pH ranged from 7.5 to 8.5, indicating slightly alkaline conditions across all sampling locations. Urban soils (A-series) consistently recorded a pH of 7.5, whereas industrial soils showed slightly higher values (8.0–8.5).

In terms of texture, urban soils were predominantly silty clay loam, with one sandy loam sample (A4). In contrast, all industrial soils were classified as clay loam. Moisture content varied substantially among samples, ranging from 10.61% (I2) to 32.27% (I1). Urban soils generally exhibited moderate to high moisture content (12.35–30.54%), while industrial soils displayed broader variability (10.61–32.27%).

These variations reflect differences in soil structure and environmental exposure between urban and industrial sites, potentially influencing heavy metal mobility and microbial adaptation.

Table 3
Physiochemical properties of soil samples

Soil isolates	**pH**	**Soil texture**	**Moisture content(W%)**
A1	7.5	Silty clay loam	30.54
A2	7.5	Silty clay loam	23.76
A3	7.5	Silty clay loam	25.18
A4	7.5	Sandy loam	12.35
I1	8.5	Clay loam	32.27
I2	8	Clay loam	10.61
I3	7.5	Clay loam	22.54
I4	8.5	Clay loam	28.86

2) Screening and selection of heavy metal-tolerant isolates

A total of eight morphologically distinct bacterial isolates were obtained following enrichment under heavy metal stress conditions (Pb, Cd, Cr). Among them, three isolates—A1, I2, and I4—demonstrated comparatively higher tolerance to combined heavy metal exposure. These isolates were therefore selected for further evaluation.

3) Compatibility testing and consortium formulation

The three selected isolates (A1, I2, and I4) were subjected to compatibility testing to assess their suitability for consortium development. No antagonistic interactions were observed among the isolates, confirming their compatibility. Based on these findings, a bacterial consortium was formulated for comparative assessment alongside individual isolates in soil microcosm experiments.

4) Soil microcosm analysis

Sterile soil microcosms artificially spiked with Pb (740 µg/mL), Cd (920 µg/mL), and Cr (500 µg/mL) were incubated and monitored over seven days. Heavy metal concentrations were recorded on Days 3, 5, and 7. Observable reductions in metal concentrations were recorded by Day 7, and percentage reduction was calculated

5) Heavy Metal Reduction Efficiency

a) Lead (Pb)

For Pb (initial concentration: 740 µg/mL), isolates I2 and I4 demonstrated the highest removal efficiency (29.72%), reducing the concentration to 520 µg/mL. The consortium showed moderate reduction (16.21%), lowering Pb concentration to 620 µg/mL. Isolate A1 exhibited no measurable reduction.

These results indicate that Pb remediation was predominantly strain-dependent, with I2 and I4 emerging as superior performers. The consortium did not demonstrate synergistic enhancement for Pb removal.

b) Cadmium (Cd)

For Cd (initial concentration: 920 µg/mL), the consortium exhibited the highest removal efficiency (36.95%), reducing the concentration to 580 µg/mL. Individual isolates I2 and I4 showed moderate and identical reductions (13.04%), while A1 again showed no measurable change.

Unlike the trend observed for Pb, the consortium significantly outperformed the individual isolates for Cd removal, suggesting possible cooperative or synergistic interactions enhancing cadmium remediation.

c) Chromium (Cr)

Chromium reduction displayed marked variability among treatments. With an initial concentration of 500 µg/mL, the consortium achieved the highest removal efficiency (60%), reducing Cr levels to 200 µg/mL. Isolates A1 and I2 each showed moderate reduction (20%), lowering concentrations to 400 µg/mL. In contrast, I4 showed no measurable chromium reduction.

The strong chromium removal observed in the consortium indicates enhanced collective remediation potential, possibly due to complementary detoxification or biosorption mechanisms.

6) Comparative Evaluation

A comparative analysis of heavy metal removal at Day 7 revealed clear strain-dependent and metal-specific differences in remediation performance.

- I2 and I4 were the most effective isolates for Pb removal.
- The consortium demonstrated superior performance for Cd and Cr remediation.
- A1 showed limited effectiveness across all tested metals.
- No single isolate consistently outperformed others across all contaminants.

Overall, these findings demonstrate that bioremediation efficiency depends on both the specific microbial strain and the target heavy metal. The enhanced performance of the consortium for Cd and Cr suggests that microbial interactions may improve remediation outcomes under certain metal stress conditions.

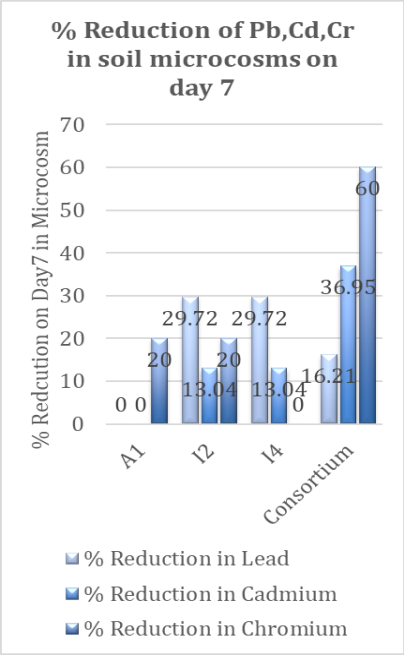

Fig. 3. % Reduction of Lead, Cadmium and Chromium on day-7 of soil microcosms

5. Discussion

A. Interpretation of the Results

The soil microcosm experiments demonstrated measurable removal/immobilization of Pb, Cd and Cr after seven days of incubation with selected indigenous isolates (I2, I4, A1) and the formulated consortium (C1). The starting concentrations in the sterile, spiked microcosm soil were Pb = 740 µg mL^{-1}, Cd = 920 µg mL^{-1}, and Cr = 400–500 µg mL^{-1} (values and absorbance calibration are given in the Results). By day 7, individual treatments produced the following reductions (values read from Table 5.7.1–5.7.3): Pb - A1 & I2 ≈ 520 µg mL^{-1} (≈30% reduction), I4 ≈ 620 µg mL^{-1} (≈16% reduction); Cd - A1 & I2 ≈ 800 µg mL^{-1} (≈13% reduction), I4 ≈ 580 µg mL^{-1} (≈37% reduction); Cr - A1 ≈ 400 µg mL^{-1} (no change), I2 ≈ 500 µg mL^{-1} (showing an apparent increase), I4 ≈ 200 µg mL^{-1} (50% reduction).

Gao et al. (2025) used the BCR sequential extraction approach, Gao and colleagues reported that inoculation with *Bacillus altitudinis* shifted Cd from the exchangeable fraction into less mobile forms, with exchangeable Cd decreasing sharply in the first 0–5 days and achieving ~17–26% reductions in exchangeable Cd by day 15 depending on initial concentration. Microcosms prepared showed Cd reductions (A1/I2 ≈ 13%; I4 ≈ 37% at day 7) are broadly comparable in magnitude to *Gao et al.,* especially noting that I4's ~37% reduction over 7 days matches or exceeds the shorter-term immobilization reported by *Gao et al*. for some conditions. This suggests that I4 is a promising immobilizer of Cd in soil and may operate through mechanisms similar to those described by *Gao et al.*as rapid early sequestration and transformation into less bioavailable fractions.

Liang et al. (2025) reported laboratory adsorption kinetics for strains C9 and C27 with Cd removal rates that increased over time and reached up to ~57% at 48 h (in pure culture/solution conditions). Microcosm in here showed that the Cd data show lower removal than the peak values reported for pure-culture adsorption (Liang et al.). This is expected: adsorption in aqueous systems where contact between metal ions and biomass is maximized typically gives higher short-term removal than soil microcosms because of three reasons which are (i) soil binding phases compete for Cd, (ii) diffusion limitations slow contact, and (iii) background cations/OM reduce uptake. The fact that I4 reached ~37% in a soil matrix within seven days is therefore notable and shows real remediation potential under more realistic conditions.

Cd results fit within the range reported by other studies. The higher performance of I4 suggests stronger biosorption/ bioimmobilization capacity or better survival/activity in soil; follow-up fractionation (BCR) would confirm whether removal reflects transformation to reducible/oxidizable/residual forms as in Gao et al.

Vélez et al. (2021) showed >80% lead removal in liquid contact at 40 h for some *Pseudomonas* isolates (both live and dead biomass), with live biomass often performing slightly better. Compared with these high aqueous-phase numbers, your Pb reductions in soil microcosms (≈16–30%) are modest.

Again, different matrices explain most of the difference: wastewater/solution experiments eliminate soil sorption competition and make metal–biomass contact direct, producing higher apparent removal.

Nonetheless, a ~30% reduction (A1, I2) in a sterile soil matrix over seven days is meaningful at the microcosm scale and indicates potential for further optimization longer incubation, increased inoculum, amendments such as carbon substrates or biochar, etc. to approach higher removal percentages.

Pb removal in soils tends to be lower than in aqueous assays; results found align qualitatively with the literature and justify further optimization and mechanistic probing (e.g., EPS production, cell-surface binding, precipitation).

Cr behavior in soils is complex because of its toxicity and mobility depend on speciation (Cr(III) vs Cr(VI)). Microcosm responses were variable: I4 produced a substantial decrease (≈50% reduction to 200 μg mL^{-1}), A1 resulted in no change, and I2 showed an apparent increase (500 μg mL^{-1}). Such variability can occur when redox transformations or analytical artefacts change detectable species (e.g., reduction of Cr(VI) to Cr(III) that is not equally measured by the assay, or desorption from soil particles during incubation). The increase observed with I2 likely reflects experimental variability or solubilization/desorption of soil-bound Cr rather than net mobilization by microbes, but it must be further investigated.

Cr results are promising for I4 but inconsistent across isolates; this indicates isolate-specific pathways of biosorption and suggesting the need for speciation measurements.

B. Community-level Findings (Comparison with Yi et. al.)

Yi et al. (2021) observed temporal shifts in sediment bacterial communities, where certain phyla increased or decreased in relative abundance alongside changes in heavy metal concentrations. However, they also reported that minor variations in metal levels were not always sufficient to significantly alter overall microbial community composition. In contrast, the present microcosm study focused on the performance of selected individual isolates and their ability to reduce extractable metal concentrations, rather than evaluating shifts in the broader microbial community.

This comparison highlights two important points. (i) targeted bioaugmentation using specific isolates can modify local metal availability without necessarily causing immediate, detectable changes in overall community structure. (ii) microbial responses under real field conditions are highly context-dependent. Therefore, if these isolates are applied in situ, it will be essential to monitor indigenous microbial communities to assess long-term ecological impacts, including possible co-selection of metal and antibiotic resistance or unintended disturbance of native taxa, as emphasized by *Yi et al.*

C. Mechanistic Considerations and Likely Processes

Based on the observed removal patterns and comparison with previous studies, several mechanisms are likely contributing to metal reduction in the soil microcosms.

The most immediate mechanism is biosorption, involving rapid and reversible binding of metal ions to functional groups (e.g., carboxyl, hydroxyl, phosphate, amino groups) present on the bacterial cell wall and extracellular polymeric substances (EPS). This process often dominates short-term uptake and has been widely reported in controlled adsorption studies *(Liang et al.; Vélez et al.)*.

A second mechanism is bioaccumulation, where metal ions are transported into the cell and sequestered intracellularly. This is typically slower and growth-dependent, requiring active metabolism.

In addition, biotransformation or microbially mediated precipitation may occur. For example, Gao et al. demonstrated that bacterial inoculation converted exchangeable Cd into less bioavailable reducible, oxidizable, and residual fractions, indicating immobilization rather than simple surface binding.

Finally, abiotic soil factors play a significant modulatory role. The soils in this study had a pH range of approximately 7.5–8.5 and predominantly clay loam texture. Under slightly alkaline conditions, metal cations may exhibit reduced mobility due to increased adsorption onto clay minerals and organic matter. Competition from background cations such as Ca^{2+} and Mg^{2+} can also influence microbial binding efficiency. Thus, microbial and physicochemical processes likely acted simultaneously to produce the observed reductions.

The comparatively strong performance of isolate I4, particularly for Cd and Cr, suggests enhanced surface-binding capacity (possibly higher EPS production), more efficient metabolic transformation pathways, or improved survival and activity under sterile microcosm conditions. Targeted mechanistic assays will be required to distinguish between these possibilities and confirm the dominant removal pathways.

D. BiostatisticalInterpretations

The ANOVA results indicate significant differences across groups. However, ANOVA alone does not identify which groups (isolates/consortium vs control or each other) differ. A post-hoc multiple comparison test should be performed on the microcosm data to specify pairwise differences (e.g., I4 vs control, consortium vs single isolates). Additionally, the planned Pearson correlation between % removal and OD_{600} is an appropriate test to explore whether removal is growth-linked (active processes) or independent (passive sorption).

6. Conclusion

This study assessed the potential of indigenous bacterial isolates (I2, I4, A1) and a formulated consortium (C1) to reduce Pb, Cd, and Cr concentrations in sterile soil microcosm systems. Within just seven days, noticeable reductions in extractable metal concentrations were observed, although the extent of removal varied depending on the metal and the isolate involved. Among the tested strains, I4 showed particularly strong performance for Cd and Cr, whereas A1 and I2 were more effective in reducing Pb. Statistical analysis confirmed that these differences were significant ($p < 0.05$), demonstrating that microbial inoculation had a measurable impact on metal levels in soil.

While the removal efficiencies were lower than those

typically reported in aqueous adsorption studies, the results obtained under soil conditions are more representative of real environmental systems, where metal availability is strongly influenced by soil–metal interactions. Comparison with previous literature suggests that the observed reductions likely reflect microbial immobilization processes rather than surface adsorption alone. Although sequential fractionation was not performed, the consistent decline in extractable metal concentrations indicates a probable shift toward less bioavailable forms.

The mechanisms responsible for metal reduction are likely a combination of processes. Rapid biosorption to cell wall functional groups and extracellular polymeric substances (EPS) may have contributed to early-stage binding, while intracellular bioaccumulation and potential microbial transformation or precipitation may have supported longer-term immobilization. Soil properties, including the slightly alkaline pH (7.5–8.5) and clay loam texture, likely enhanced these effects by promoting adsorption and limiting metal mobility. Overall, microbial and physicochemical factors appear to have acted together to achieve the observed reductions.

Despite these encouraging findings, further work is needed to clarify the underlying mechanisms and determine practical applicability. Future research should include sequential extraction (e.g., BCR fractionation) and metal speciation analysis particularly for chromium to verify whether metals are being converted into more stable fractions. Additional mechanistic studies, such as EPS quantification, surface functional group analysis (FTIR), and comparisons between live and inactivated biomass, would help distinguish between biosorption, bioaccumulation, and metabolic transformation. Molecular identification of the isolates and screening for metal-resistance genes would further strengthen understanding of their functional capabilities.

Longer-term microcosm and mesocosm studies are also necessary to evaluate the stability of immobilization under changing environmental conditions. For potential field application, monitoring indigenous microbial communities will be important to assess ecological balance and avoid unintended consequences such as co-selection of resistance traits. Optimization strategies such as adjusting inoculum density, using carrier materials (e.g., biochar or compost), nutrient supplementation, or developing synergistic microbial consortia may further improve remediation efficiency.

In conclusion, this study provides evidence that locally isolated bacterial strains can meaningfully reduce the bioavailability of heavy metals in contaminated soils. Although additional mechanistic and field-scale validation is required, microbial immobilization shows strong promise as a sustainable and environmentally compatible approach for managing heavy metal contamination in urban–industrial environments.

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