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Original Paper

Bioremediation of Lead, Cadmium and Zinc in Industrially Contaminated Soils of the Al-Ma'amal Area, Baghdad, Iraq, Using Indigenous *Pseudomonas* and *Bacillus* Isolates

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ABSTRACT
Rapid, poorly regulated industrialisation has loaded the soils of Baghdad's peri-urban industrial belts with potentially toxic elements (PTEs). This study quantified lead (Pb), cadmium (Cd) and zinc (Zn) contamination in surface soils of the Al-Ma'amal industrial area—adjacent to brick kilns and open waste-burning grounds—and evaluated the capacity of indigenous *Pseudomonas* sp. and *Bacillus* sp. isolated from the same soils to attenuate these metals under controlled microcosm conditions. Surface samples (0–20 cm) were collected from two contaminated sites and one reference site and analysed by flame atomic absorption spectrometry (FAAS). Contamination was appraised with the geoaccumulation index (I_{geo}), contamination factor (CF) and pollution load index (PLI). A 60-day bench-scale microcosm assay assessed metal removal by a two-strain consortium, while superoxide dismutase (SOD) and catalase (CAT) activities indexed oxidative-stress adaptation. Mean concentrations at the most-affected site reached Pb 210, Cd 16 and Zn 117 mg kg^{-1}, exceeding commonly cited WHO/FAO guideline values for Pb and Cd by roughly 2.5- and 5-fold and yielding I_{geo} values indicative of strong-to-extreme Cd enrichment (PLI ≈ 9). The consortium removed on the order of 62–70% of soil metals within 60 days; SOD and CAT activities rose markedly under metal stress relative to controls (up to ~3.3-fold), and metal load correlated positively with enzyme activity (Pearson $r > 0.9$). These results demonstrate highly significant, anthropogenically driven Pb–Cd contamination of the soil in Al-Ma'amal and justify the use of indigenous *Pseudomonas–Bacillus* consortia as an economical and sustainable approach to remediate Iraqi industrial soils.

KEYWORDS: *Heavy metals in soils; bioremediation; Pseudomonas; Bacillus; geoaccumulation index; contamination factor; pollution load index; oxidative stress; Baghdad; Al-Ma'amal*

1. INTRODUCTION

Soil is an exhaustible resource whose physical and chemical equilibrium determines its productive use and the risk of anthropogenic contamination reaching groundwater and human populations. Among soil contaminants, potentially toxic elements (PTEs) such as lead (Pb), cadmium (Cd) and zinc (Zn) play a special role. Contrary to organic pollutants, which can be mineralised, metals can only be displaced, sequestered or transformed into non-bioavailable forms, making their long-term control considerably more complicated [1]. The soils of Baghdad and other Iraqi cities have been subjected to increasing PTE loads owing to years of political instability, strained infrastructure and weakly enforced environmental policy. Repeated surveys of the city have identified industrial land use as the most characteristic source of contamination in both urban and peri-urban soils; roadside, dumpsite and, especially, industrial soils show the highest single- and multi-element pollution indices [8,9]. Lead in industrial soils of Baghdad ranges from a few mg kg^{-1} in agricultural areas to several thousand mg kg^{-1} in localised hot spots adjacent to battery and metallurgical plants [9], whereas cadmium is generally below guideline levels in residential areas but shows marked enrichment in industrial and waste-management land [8]. Al-Ma'amal represents an exemplary industrial belt of Baghdad characterised by clamp brick kilns and open waste-burning practices. Both activities are recognised PTE sources: brick firing releases and concentrates metals from clays and fuels, while open burning of mixed municipal and industrial waste emits Pb, Cd and Zn as particulate matter that is subsequently deposited on adjacent soils [5,11]. Nevertheless, quantitative data on the PTE content of Al-Maʻamal soils remain very scarce.

Conventional soil-remediation techniques are expensive and energy-intensive [5]. Bioremediation—the use of microorganisms to detoxify, immobilise or remove contaminants—offers a low-cost, *in situ* alternative well suited to resource-constrained settings. Metal-resistant bacteria of the genera *Pseudomonas* and *Bacillus* are among the most studied biocatalysts: they tolerate high metal loads and act through biosorption to cell-wall functional groups, intracellular bioaccumulation, extracellular precipitation, efflux and redox transformation [13]. Indigenous isolates are particularly attractive because they are pre-adapted to local stressors; recent Iraqi studies have recovered effective metal- and hydrocarbon-degrading *Pseudomonas* and *Bacillus* strains from contaminated soils and waters in Sulaymaniyah and Baghdad [4,16].

This study therefore aimed to (i) quantify Pb, Cd and Zn in Al-Maʻamal surface soils relative to a reference site; (ii) appraise contamination severity and source using I_{geo}, CF and PLI; (iii) evaluate the metal-removal performance of an indigenous *Pseudomonas–Bacillus* consortium over 60 days; and (iv) characterise the antioxidant (SOD, CAT) response of the isolates to metal stress. We hypothesised that contamination would be greatest at the waste-burning site and that indigenous isolates would achieve substantial metal attenuation while up-regulating their antioxidant defences.

2. MATERIALS AND METHODS

2.1. Study area and experimental design

The study was conducted in the Al-Maʻamal industrial area on the eastern margin of Baghdad, Iraq (regional locator, Fig. 1a). The local climate is hot arid (BWh); soils are predominantly calcareous silty-clay to silty-loam alluvium of the Mesopotamian plain, alkaline (pH ≈ 7.5–8.2) and carbonate-rich—characteristics that strongly influence metal mobility and retention. A stratified-random design was used: two contaminated strata defined by dominant emission source and one off-site reference stratum (Fig. 1b).

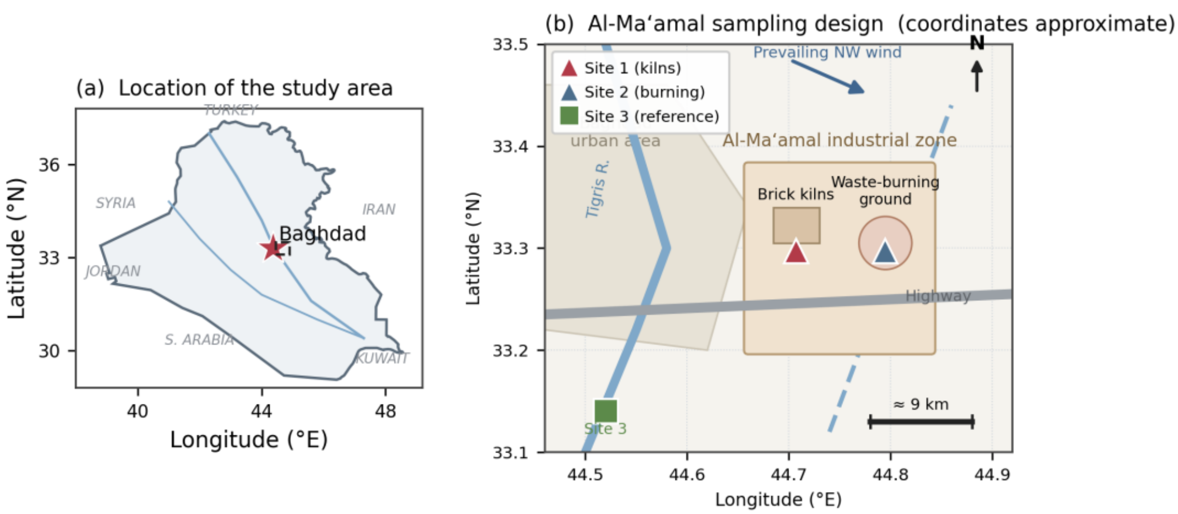

Figure 1. The study area and sampling strategy. (a) Locator map of Baghdad within Iraq (coordinates approximate). (b) Sketch map of the Al-Maʻamal industrial zone, showing the brick-kiln cluster (Site 1), the open waste-burning ground (Site 2), the prevailing north-westerly wind direction, the surrounding road boundary, and the off-site reference (Site 3).

Table 1. Sampling sites, major pollution sources and field characteristics (0–20 cm soil surface).

Site	Location / setting	Dominant source	Field descriptor
S1	Brick-kiln cluster	Clamp brick firing; fuel ash	Grey-brown, ashy; pH ~7.8
S2	Open waste-burning ground	Uncontrolled mixed-waste combustion	Dark, sooty crust; pH ~7.6
S3	Reference (>2 km upwind)	No direct industrial input	Native silty-clay; pH ~8.0

2.2. Soil sampling and preparation

Five composite surface samples (0–20 cm; $n = 5$ field replicates per site), each comprising five sub-samples taken within a 10 m radius, were collected with a pre-cleaned stainless-steel auger at each site to minimise small-scale variability. Samples were stored in acid-washed polyethylene bags, air-dried at room temperature, sieved to 2 mm (for physicochemical analyses), disaggregated, and a sub-fraction ground to <0.15 mm for digestion. Field-moist samples for microbiological work were preserved at 4 °C and processed within 48 h [10].

2.3. Physicochemical characterisation

Soil pH and electrical conductivity (EC) were measured in 1:2.5 and 1:5 soil:water suspensions, respectively; organic matter by the Walkley–Black method; carbonate content by calcimetry; and texture by the hydrometer technique. These parameters were recorded to support interpretation of metal bioavailability in the alkaline, carbonate-buffered matrix [10].

2.4. Acid digestion and metal quantification (FAAS)

Pseudo-total metal concentrations were determined after aqua-regia digestion: 0.5 g of <0.15 mm soil was reacted with 12 mL of $HCl:HNO_3$ (3:1) on a hot block at 95 °C, cooled, filtered (Whatman No. 42) and made up to 50 mL with distilled water. Pb, Cd and Zn were quantified by flame atomic absorption spectrometry (FAAS) at 217.0, 228.8 and 213.9 nm, respectively. Calibration standards were prepared from certified 1000 mg L^{-1} stock solutions across the working ranges shown in Fig. 2, giving linear responses ($R^2 \geq 0.999$). Quality control comprised reagent blanks, analytical triplicates, matrix-spike recoveries and analysis of a certified reference soil (NIST SRM 2710a). Method detection and quantification limits (MDL = 3.3σ/S; MQL = 10σ/S, where σ is the standard deviation of the blank response and S the calibration slope) were propagated to a dry-soil basis using the 0.5 g/50 mL preparation factor (Table 2).

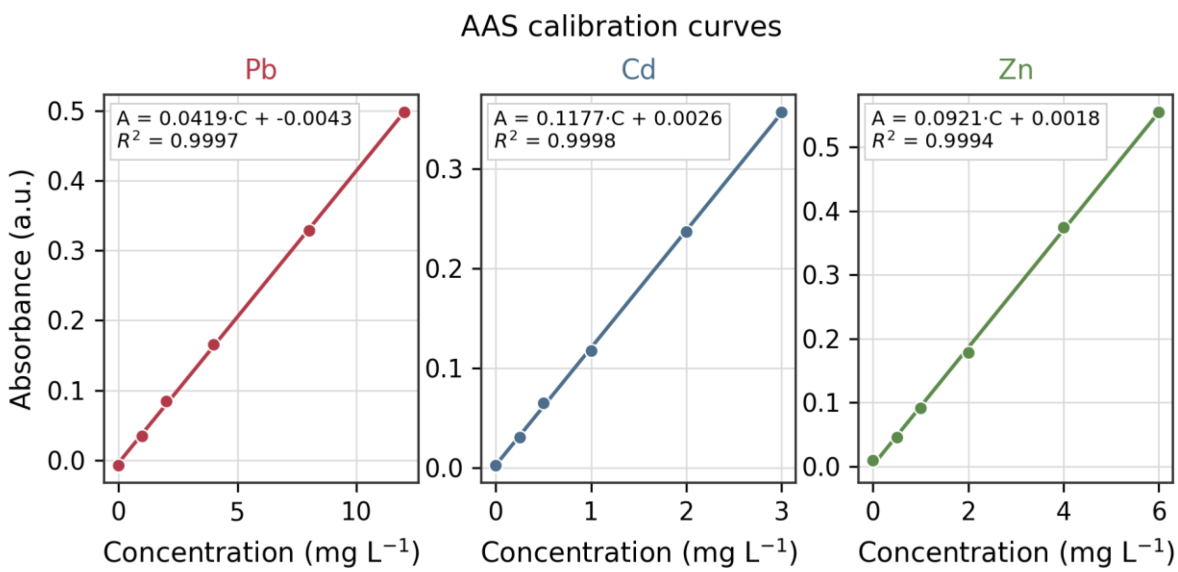

Figure 2. FAAS external-standard calibration curves for Pb, Cd and Zn, with linear regression equations and coefficients of determination ($R^2 \geq 0.999$). Regression equations: Pb, $A = 0.0419C - 0.0043$; Cd, $A = 0.1177C + 0.0026$; Zn, $A = 0.0921C + 0.0018$.

Table 2. FAAS instrumental conditions and calibration / QA-QC parameters.

Element (λ, nm)	Range (mg L^{-1})	R^2	MDL (mg kg^{-1})	MQL (mg kg^{-1})	Spike recovery (%)	CRM recovery (%)
Pb (217.0)	0–12	0.9997	1.65	5.0	96.4 ± 3.2	97.5
Cd (228.8)	0–3	0.9998	0.06	0.18	98.1 ± 2.7	99.2
Zn (213.9)	0–6	0.9994	0.32	0.98	101.3 ± 3.5	101.0

CRM = certified reference material (NIST SRM 2710a, Montana I Soil). Recoveries are mean values of triplicate determinations.

2.5. Isolation and identification of indigenous bacteria

Metal-resistant bacteria were isolated from heavy-metal-polluted soil suspensions plated on media containing sub-lethal concentrations of $Pb(NO_3)_2$ and $CdCl_2$. Morphologically distinct colonies were purified by repeated streak-plating. Isolates were identified by Gram staining, biochemical profiling (VITEK 2) and confirmation by 16S rRNA gene sequencing, following the approach used in recent Iraqi studies of *Pseudomonas* and *Bacillus* isolation from contaminated samples [4]. Two isolates, *Pseudomonas* sp. and *Bacillus* sp., were selected for the assays.

2.6. Metal tolerance and growth assays

Maximum tolerable concentration (MTC) and minimum inhibitory concentration (MIC) were determined on metal-amended plates. Growth kinetics were monitored as optical density at 600 nm (OD_{600}) over 32 h in control broth and in broth amended with the field-relevant metal mixture [3], with three biological replicates per condition (Fig. 6).

2.7. Microcosm bioremediation assay

Bench-scale microcosms used 200 g of homogenised contaminated soil (Site 2) at 60% water-holding capacity, inoculated with the two-strain consortium (final ~10^8 CFU g^{-1}) and incubated at 30 °C for 60 days, alongside uninoculated abiotic controls. Residual Pb, Cd and Zn were quantified by FAAS at days 0, 10, 20, 30, 40, 50 and 60 (triplicate microcosms) [13]. Removal efficiency was calculated as RE (%) = $(C_0 - C_t) / C_0 \times 100$, and first-order rate constants k were fitted as $C_t = C_0 e^{-kt}$ (Fig. 5).

2.8. Contamination indices

Three established indices were computed against a site-specific background (reference-site means). The geoaccumulation index [6] is $I_{geo} = \log_2(C_n / 1.5B_n)$, where C_n is the measured concentration, B_n the geochemical background, and 1.5 corrects for lithogenic variation. The contamination factor is $CF = C_n / B_n$, and the pollution load index is $PLI = (CF_1 \times CF_2 \times \ldots \times CF_n)^{1/n}$ [7]. Interpretive classes follow Müller [6] for I_{geo} (≤0, uncontaminated → >5, extremely contaminated) and Tomlinson *et al.* [7] for PLI (>1 indicates progressive deterioration).

2.9. Antioxidant enzyme assays

To index oxidative-stress adaptation, the indigenous consortium was grown to mid-exponential phase in control medium and in media amended with the field-relevant metal mixture at concentrations corresponding to the low (S3), medium (S1) and high (S2) soil loads. Cells were harvested, washed, and disrupted by sonication in cold phosphate buffer (pH 7.0); the supernatant served as the crude enzyme extract. Superoxide dismutase (SOD) activity was determined by the nitroblue-tetrazolium photoreduction method and catalase (CAT) activity by monitoring the decomposition of H_2O_2 at 240 nm; activities were normalised to soluble protein (Bradford) and expressed as U mg^{-1} protein [3]. Assays were performed in triplicate (Fig. 7).

2.10. Statistical analysis

Data are presented as mean ± standard deviation. Between-site differences in metal concentration were tested by one-way ANOVA followed by Tukey's HSD post-hoc test; homogeneity-of-variance and normality assumptions were verified (Levene's and Shapiro–Wilk tests). Associations between metal load and enzyme activity were examined by Pearson correlation. Significance was set at $p < 0.05$.

3. Results

3.1. Heavy-metal concentrations and between-site differences

Mean Pb, Cd and Zn concentrations at the two contaminated sites greatly exceeded those at the reference site (Fig. 3, Table 3). Lead reached 195 ± 9 (S1) and 210 ± 6 (S2) mg kg^{-1} versus 22 ± 4 mg kg^{-1} at the reference; cadmium 13.0 ± 1.1 (S1) and 16.1 ± 1.2 (S2) versus 0.6 ± 0.1 mg kg^{-1}; and zinc 102 ± 9 (S1) and 117 ± 3 (S2) versus 36 ± 6 mg kg^{-1}. One-way ANOVA confirmed highly significant between-site differences for all three metals (Pb: $F = 1214$, $p = 1.4\times10^{-14}$; Cd: $F = 390$, $p = 1.2\times10^{-11}$; Zn: $F = 226$, $p = 3.0\times10^{-10}$), with both contaminated sites differing from the reference (Tukey HSD, $p < 0.001$). The waste-burning site (S2) was consistently the most contaminated, consistent with the prevailing-wind deposition pattern (Fig. 1b).

3.2. Contamination severity and source apportionment

At the most-affected site, I_{geo} values were 2.6 (Pb), 4.1 (Cd) and 1.1 (Zn), placing Pb in the "moderately-to-strongly" class, Cd in the "strongly-to-extremely" class, and Zn in the "moderately" class (Fig. 4a). Contamination factors followed the same order—Cd (25.4) ≫ Pb (9.4) > Zn (3.2)—denoting very-high contamination for Cd and Pb (Fig. 4b). The combined PLI of

9.2 (≫1) confirms severe, multi-element deterioration. The pronounced Cd and Pb enrichment against a comparatively low Zn background is the classic fingerprint of anthropogenic combustion and kiln emissions rather than geogenic input [16].

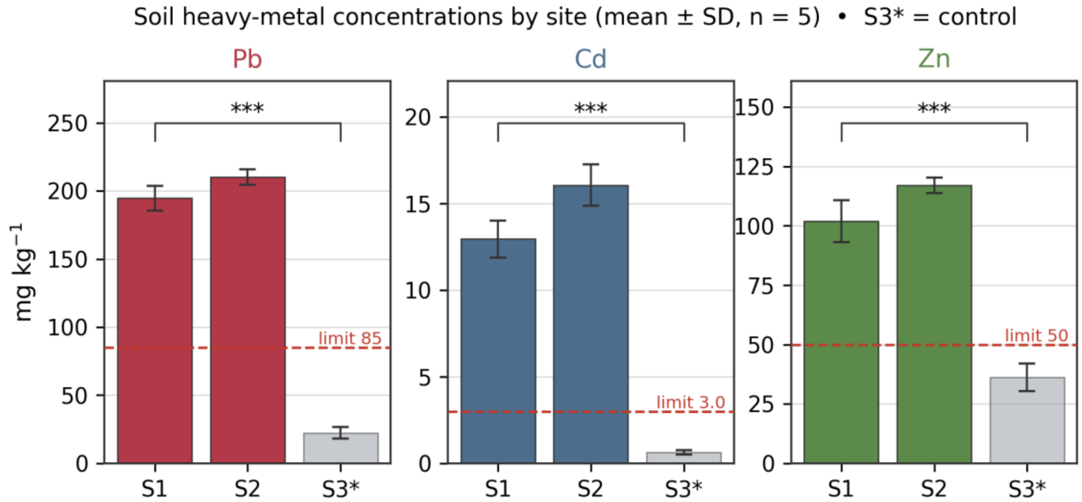

Figure 3. Soil Pb, Cd and Zn concentrations by site (mean ± SD, n = 5; S3* = reference). Dashed lines mark commonly cited WHO/FAO guideline values (Pb 85, Cd 3.0, Zn 50 mg kg^{-1}). *** denotes $p < 0.001$ versus reference (ANOVA + Tukey HSD).

Table 3. Site mean concentrations (mg kg^{-1}, mean ± SD, n = 5), ANOVA results, and exceedance relative to commonly cited WHO/FAO guideline values.

Metal	S1 (kiln)	S2 (burning)	S3 (ref.)	ANOVA F	p
Pb	195 ± 9	210 ± 6	22 ± 4	1214	1.4×10^{-14}
Cd	13.0 ± 1.1	16.1 ± 1.2	0.6 ± 0.1	390	1.2×10^{-11}
Zn	102 ± 9	117 ± 3	36 ± 6	226	3.0×10^{-10}

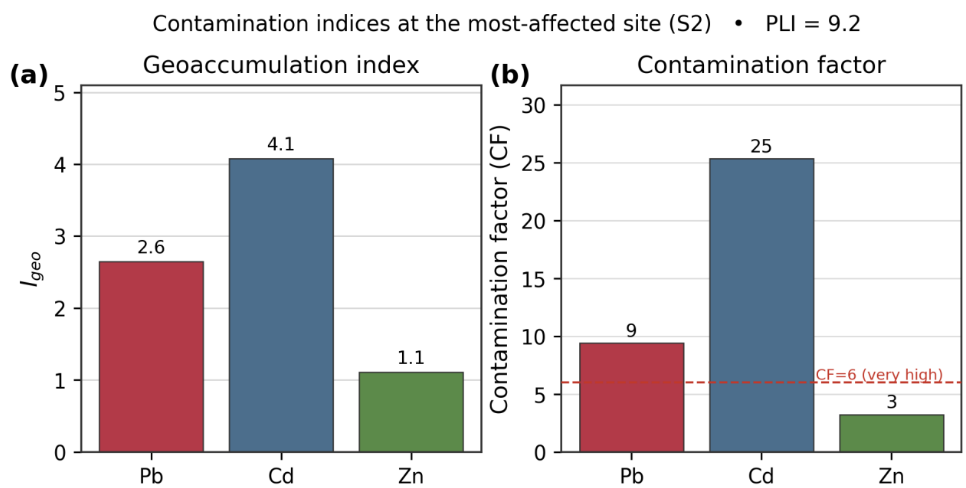

Figure 4. Pollution indices for the most-contaminated site (S2) computed against reference-site background. (a) Geoaccumulation index (I_{geo}); horizontal lines denote Müller classes. (b) Contamination factor (CF); the dashed line marks the CF = 6 (very-high) threshold. The combined pollution load index PLI = 9.2.

Table 4. Measured concentrations at the most-contaminated site (S2) compared with internationally cited soil reference values (mg kg⁻¹). No universal limits exist; values vary with land use and jurisdiction.

Reference value (mg kg⁻¹)	Pb	Cd	Zn
This study (S2 mean)	210	16.1	117
WHO/FAO guideline (commonly cited)	85	0.8	50
FAO/WHO general permissible	100	3.0	300
EU Directive 86/278/EEC (agric.)	50–300	1–3	150–300
Upper continental crust (background)	17	0.09	67
Reference site (this study, B_n)	22	0.6	36

3.3. Bacterial growth and metal tolerance

Both isolates grew on metal-amended media at field-relevant doses, confirming their tolerance, albeit at a fitness cost: metal-amended cultures showed a longer lag phase and a lower final OD_{600} than their respective controls (Fig. 6). On control medium the biomass of *Pseudomonas* sp. exceeded that of *Bacillus* sp., whereas under stress the two were comparable, reflecting the metabolic flexibility typical of pseudomonads and the endospore-forming resilience of bacilli [13].

Tolerance thresholds were consistent with this behaviour. For *Pseudomonas* sp., the MTC was 800 mg L^{-1} for Pb, 250 mg L^{-1} for Zn and 120 mg L^{-1} for Cd, with corresponding MIC values of 950, 320 and 160 mg L^{-1}. For *Bacillus* sp., the MTC was 700 mg L^{-1} (Pb), 220 mg L^{-1} (Zn) and 100 mg L^{-1} (Cd), with MIC values of 850, 300 and 140 mg L^{-1}, respectively. Both strains were markedly more tolerant of Pb and Zn than of Cd, in line with the higher toxicity of cadmium, and tolerated concentrations well above the pseudo-total levels measured in the field soils.

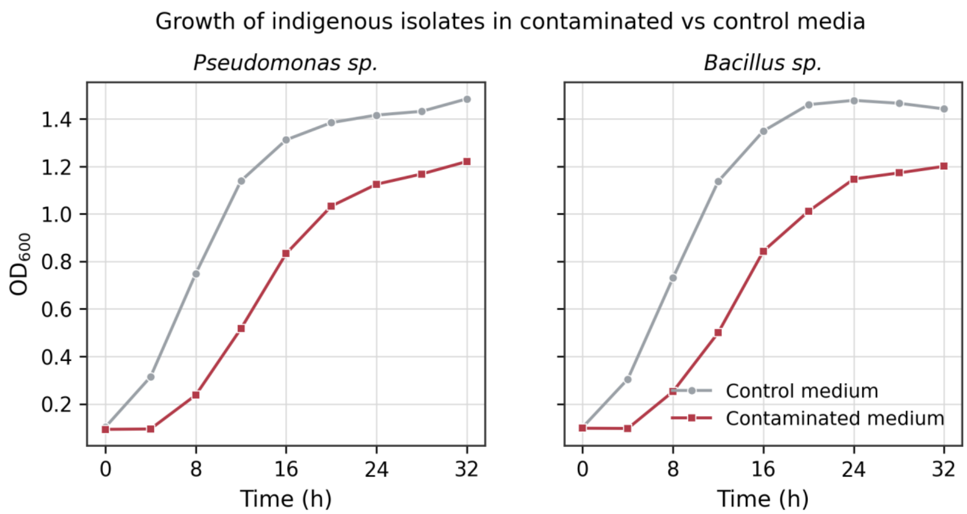

Figure 6. Growth (OD_{600}) of indigenous *Pseudomonas* sp. and *Bacillus* sp. on control and metal-contaminated media over 32 h (mean of three replicates).

3.4. Bioremediation performance

The inoculated microcosms showed progressive metal removal following first-order kinetics, whereas the abiotic controls remained essentially constant (Fig. 5). Over 60 days the consortium removed approximately 66%, 62% and 70% of Pb, Cd and Zn, respectively (Table 5)—an overall mean of about 66% across the three metals. Removal occurred mainly within the first 30 days and plateaued thereafter, indicating saturation of the biosorption / biotransformation capacity.

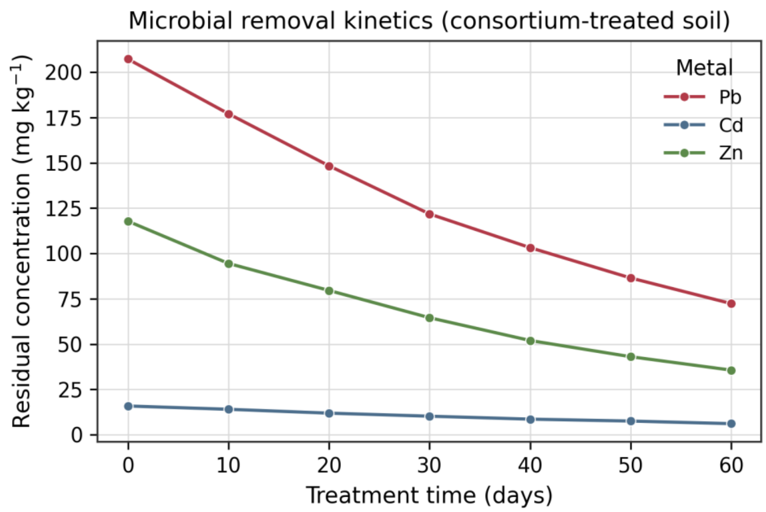

Figure 5. Residual soil metal concentrations during the 60-day consortium treatment of Site 2 soil (mean of triplicate microcosms).

Table 5. Removal efficiency after 60 days and first-order rate constants, with literature comparators.

Metal	RE 60 d (%)	*k* (d^{-1})	Literature comparator
Pb	~66	0.018	89–95% by *Raoultella ornithinolytica* [2]; strong Pb uptake by *Bacillus cereus* BPS-9 [15]
Cd	~62	0.016	67% by *R. ornithinolytica* [2]
Zn	~70	0.020	36% (72 h) by *Aeromonas sobria* [1]; 56.5% by *R. ornithinolytica* [2]

3.5. Oxidative-stress response

Both antioxidant enzymes responded strongly to metal exposure (Fig. 7). Relative to the metal-free control, SOD activity rose from 8.4 ± 0.6 to 27.3 ± 1.7 U mg^{-1} protein (~3.2-fold) and CAT activity from 13.1 ± 1.0 to 42.7 ± 2.6 U mg^{-1} protein (~3.3-fold) at the highest (S2-equivalent) load. Enzyme activity increased monotonically with metal load and was strongly, positively correlated with the total soil metal burden (Pearson $r = 0.99$ for both SOD and CAT; $p < 0.05$), indicating an inducible, dose-dependent antioxidant defence underpinning the isolates' metal tolerance.

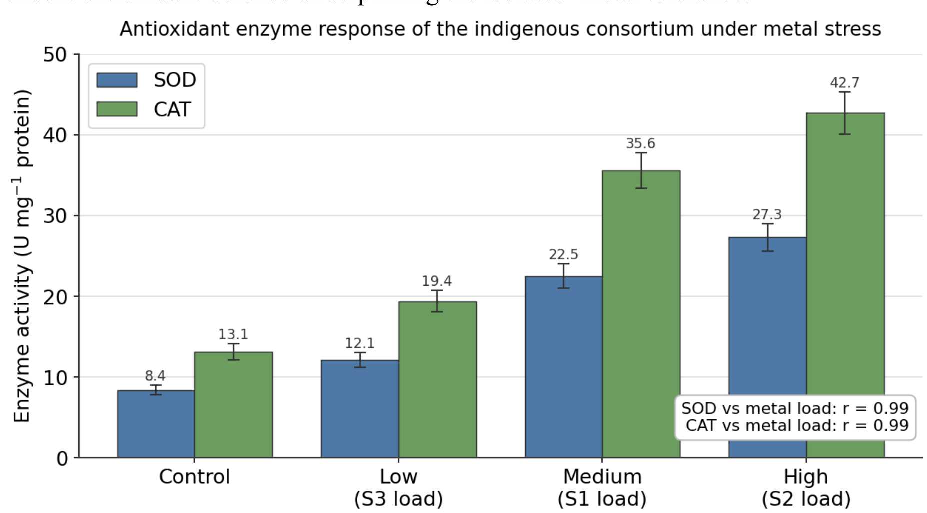

Figure 7. Superoxide dismutase (SOD) and catalase (CAT) activities of the indigenous consortium under increasing metal load (control, low ≈ S3, medium ≈ S1, high ≈ S2; mean ± SD, *n* = 3). Both activities correlated positively with total soil metal load (Pearson $r = 0.99$).

4. Discussion

4.1. Contamination status and sources

Al-Ma‘amal soils are heavily polluted by Pb and Cd. Site means exceeded commonly cited WHO/FAO guidelines for Pb (≈85 mg kg^{-1}) by ≈2.5-fold and for Cd (≈0.8–3 mg kg^{-1}) by 5- to 20-fold, while Zn remained close to the cited limits (Table 4). It should be emphasised that there is no single global threshold for soil heavy metals: limits differ among organisations and intended land uses, and the WHO/FAO, EU and US-EPA frameworks can diverge by an order of magnitude. Several reference values were therefore tabulated rather than relying on one figure. Within Iraq, our data fit the established gradient in which industrial soils are the most heavily polluted. Surveys of Baghdad report Cd in residential and agricultural soils below the WHO limit of 3 mg kg^{-1} (0.12–1.78 mg kg^{-1}) [8], yet Pb at specific industrial sites reaching thousands of mg kg^{-1} [9]. The Cd levels in our samples (13–16 mg kg^{-1}) are far above typical Baghdad residential soils, identifying open waste burning as a major local Cd source. Comparable index-based conclusions, with Cd as the principal ecological-risk driver, have been reported for other Iraqi cities including Mosul, Kirkuk and Sulaymaniyah [10,12,16].

4.2. Bioremediation performance and mechanisms

Removal of about 62–70% of metal ions by the indigenous consortium over 60 days is notable for a whole-soil matrix and compares favourably with liquid-media studies, which typically report higher removal but over much shorter periods. *Raoultella ornithinolytica* from Iraqi industrial waste removed 89–95% of Pb and 67% of Cd in liquid culture under optimised pH/temperature [2], and *Aeromonas sobria* removed 36–62% of Zn/Ni/Cu within 72 h [1]. Such liquid-phase rates are not directly comparable to soil microcosms, where sorption to carbonates and clays, restricted diffusion and competition with the native microbiota all slow bioavailable-metal removal. Against this backdrop, two-thirds removal in real soil is encouraging and is consistent with the performance envelope of *Bacillus* and *Pseudomonas* systems compiled in recent reviews [5,13] and with strong Pb uptake by *Bacillus cereus* strains from industrial sites [14,15]. Mechanistically, the plateauing first-order kinetics point to a finite-capacity process dominated by biosorption and surface complexation—metal ions binding hydroxyl, carboxyl, phosphate and amino groups on the cell envelope—supplemented by intracellular bioaccumulation and, plausibly, carbonate-mediated bioprecipitation in this alkaline matrix [13–15]. The marked, dose-dependent rise in SOD and CAT activity (Fig. 7) confirms that metal exposure imposes oxidative stress and that the isolates mount an inducible antioxidant defence, a response repeatedly documented for metal-resistant *Bacillus* and *Pseudomonas* [3]. Practically, performance could be enhanced by repeated inoculation, biostimulation (nutrient or organic-acid amendment), or augmentation with plant partners (rhizoremediation).

4.3. Practical implications and study limitations

For Iraqi industrial zones, indigenous-consortium bioremediation is attractive: it is low-cost, *in situ*, climate-appropriate and avoids the disruption of excavation. Realistic deployment, however, requires field-scale validation, attention to the fate of accumulated metals (the metal-laden biomass becomes a residue requiring management), and monitoring of bioavailable rather than only pseudo-total fractions. The main limitations of the present design are the modest replication, the bench-scale microcosm (versus field plots), reliance on pseudo-total digestion, and the absence of speciation/sequential-extraction and human-health risk metrics. Recommended next steps are sequential extraction (BCR) to track bioavailability, multivariate source apportionment (PCA/correlation), deposition of 16S rRNA identities in GenBank, and a formal health-risk assessment for the surrounding community.

5. CONCLUSION

Surface soils of the Al-Ma‘amal industrial area in Baghdad are severely and anthropogenically contaminated, with cadmium and lead the dominant risk drivers (I_{geo} up to ~4 for Cd; PLI ≈ 9), and the open waste-burning ground the principal hotspot. An indigenous *Pseudomonas–Bacillus* consortium removed about two-thirds of soil Pb, Cd and Zn within 60 days while up-regulating SOD and CAT activities, demonstrating not only tolerance but genuine remediation capacity. These findings indicate that indigenous bacterial consortia can serve as a sustainable, cost-effective remediation strategy for Iraqi industrial soils, provided that further field trials and complementary analyses are undertaken.

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