

BIOREMEDIATION OF CADMIUM BY INDIGENOUS BACTERIA ISOLATED FROM BELUR INDUSTRIAL AREA OF DHARWAD, KARNATAKA IN INDIA

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ABSTRACT

Cadmium, a highly persistent heavy metal, exhibits strong bioaccumulation potential, posing serious risks of food chain contamination and associated health hazards. This study was aimed to isolate, identify, and characterize cadmium-resistant bacteria from polluted soils of the Belur Industrial area, Dharwad, Karnataka. Ten isolates were screened, and two strains showing superior growth at cadmium concentrations of 100–500 mg l⁻¹ were selected. Molecular characterization revealed them as *Enterobacter mori* (BIDS I) and *Pseudomonas aestus* (BIDS IV). Both exhibited optimal growth at 30°C and pH 7.0 with high cadmium tolerance. After 72 h, *E. mori* BIDS I and *Pseudomonas alcaliphila* (BIDS II) achieved cadmium removal efficiencies of 87% and 78%, respectively, while *P. aestus* and *Aeromonas hydrophila* (BIDS VI) removed 72% and 70%. Additionally, these strains reduced biochemical oxygen demand (BOD), chemical oxygen demand (COD), and other effluent parameters by up to 80%. The high efficiency, adaptability, and cost-effectiveness of *E. mori* and *P. aestus* suggest their strong potential for large-scale bioremediation applications, including effluent treatment plants, drinking water purification, and pollution management in resource-limited settings.

KEY WORDS: Bioremediation, Cadmium bioaccumulation, Effluent treatment, Indigenous bacteria, Belur Industrial Area.

INTRODUCTION

Heavy metal pollution is recognized as a critical environmental concern, primarily arising from anthropogenic activities such as metal smelting, agricultural waste disposal, industrial effluent discharge, and e-waste generation (Muhammad *et al.*, 2024). Remediation of contaminated sites containing both organic xenobiotics and heavy metals remains a significant challenge, as many hazardous sites targeted for clean-up are co-contaminated with these pollutants (Ali *et al.*, 2022). Effective bioremediation of hydrocarbon-contaminated sites with concurrent heavy metal pollution should be guided by risk assessment studies that evaluate the inhibitory effects of heavy

metals on microbial activity (Abo-Alkasem *et al.*, 2023). The capacity of microbial strains to tolerate and grow in the presence of heavy metals is particularly valuable in wastewater treatment systems, where microorganisms play a crucial role in decomposing organic matter during biological treatment processes. However, the inhibitory influence of heavy metals is a common obstacle in the biological treatment of wastewater and sewage (Pande, 2022).

Remediation of sites contaminated with toxic heavy metals remains a major environmental challenge, as these pollutants constitute a significant proportion of hazardous waste sites prioritized for clean-up. Among them, cadmium (Cd) and chromium (Cr) are of particular concern due to their

environmental persistence and adverse effects on human health. Microbial degradation has emerged as a cost-effective and efficient strategy for the removal of toxic heavy metals (Priya *et al.*, 2023). However, at elevated concentrations, metals can impair cell membranes, inactivate enzymes, and damage DNA. Numerous studies have elucidated the mechanisms by which microorganisms tolerate toxic metal concentrations. Importantly, heavy metal toxicity is primarily associated with the concentration of ionic species rather than total metal content (Jan *et al.*, 2015). Metal-organic complexes generally do not bind to enzymes, and inhibition is typically mediated by the ionic form of the metal. This underscores the importance of understanding metal speciation while assessing bioavailability and toxicity. In most cases, toxicity arises from the strong affinity of metal ions for sulfhydryl (-SH) groups of enzymes, which are vital for microbial metabolic processes (Campillo-Cora *et al.*, 2025).

Cadmium (Cd) is a major environmental pollutant and a nonessential metal that is toxic to living organisms even at low concentrations (0.001-0.1 mg/l). It is extensively used in various industrial processes, including chlor-alkali production, paint manufacturing, electroplating, copper alloy production, pulp and paper processing, alkaline battery manufacturing, mining, fertilizer production, and zinc refining. Cadmium can enter the food chain and accumulate in the tissues of humans and animals, leading to severe health disorders (Chellaiah, 2018).

Heavy metal uptake by biomass is generally categorized into three mechanisms: cell-surface binding, intracellular accumulation, and extracellular accumulation. Cell-surface binding is a metabolism-independent process that can occur in both living and inactivated microorganisms, whereas intracellular and extracellular accumulations are energy-dependent processes, occurring only in metabolically active cells (Lu *et al.*, 2006). For the effective application of biosorption in wastewater treatment, it is essential to identify microbial strains with high efficiency and specificity for metal uptake and to develop optimized bioprocesses for the removal or recovery of heavy metals from aquatic systems. In this context, the present study was undertaken to isolate novel bacterial strains from metal-contaminated environments and to evaluate their potential for heavy metal removal from polluted sites (Filote *et al.*, 2021).

Bioremediation technology for the removal of toxic heavy metals such as cadmium (Cd) from industrial effluents has broad global applicability, both in large-scale industrial effluent treatment plants and in field-scale applications. The present study holds international significance, as the proposed approach can be effectively implemented for bioremediation in effluent treatment plants, purification of drinking water, and large-scale water pollution control, particularly in developing countries. This method aligns with the principles of "green technology" by offering an eco-friendly, sustainable solution. The study specifically aims to demonstrate the successful application of indigenous microorganisms for the treatment of industrial effluents contaminated with cadmium, thereby providing an effective and environmentally responsible remediation strategy.

MATERIALS AND METHODS

Study Area and Sample Collection

The contaminated soil samples were collected as per APHA method (2017) from Belur Industrial area, Dharwad, Karnataka, India. For microbiological analysis, polluted soil samples were obtained from a depth of approximately 4-5 inches (45 cm) using sterile polyethylene bags. Samples were collected from four distinct locations, including grass-root soil from outside the effluent disposal site. Samples were stored at 4 °C until further processing. For composite sample preparation, 50g of soil from each location was pooled, homogenized, and oven-dried at 60 °C for 24 h. Representative samples were subsequently used for microbiological enrichment and analysis.

Enrichment Studies

Enrichment was initiated by adding 10 g of contaminated soil to 250 ml Erlenmeyer flask containing 100 ml sterile distilled water (SDW). The mixture was incubated at 30 ± 1 °C for 24 h on an orbital shaker at 150 rpm. Following enrichment, 1 ml of the suspension was inoculated into 50 ml of Luria Bertani Broth (LB) supplemented with cadmium (Cd) at 100 mg/l and incubated at 30°C for 72 h (Jeyasinghand Philip, 2005).

Isolation and Screening of Cd-resistant bacterial isolates

After incubation, 1 ml of culture was serially diluted up to 10^{-6} in SDW, and 100 µl aliquots were spread-

plated onto Nutrient Agar (NA) plates. Plates were incubated at 30 ± 1 °C for 24 h. Ten heavy-metal-resistant isolates were recovered, of which four (BIDS I, BIDS II, BIDS IV, and BIDS VI) demonstrating robust growth at 100 mg/l Cd was selected for further experimentation. These isolates were maintained in nutrient broth and preserved at -20 °C for future use (Nwaehiri *et al.*, 2019).

Growth Curves of Bacterial Isolates with Metal Induction

Growth kinetics of the isolates was evaluated in Mineral Salt Medium (MSM) broth supplemented with Cd (100 mg L⁻¹). Heavy metal stock solutions were autoclaved separately prior to use. Each flask was inoculated with 500 µl of overnight-grown heavy metal-resistant bacterial culture and incubated at 30 °C with shaking (150 rpm) for 96 h. MSM without cadmium served as the control. Growth was monitored at 6, 12, 24, 48, 60, 72, 84, and 96 h by measuring optical density (OD) at 600 nm, and growth curves were plotted for each isolate. Optimal growth conditions with respect to pH (4, 5, 6, and 7) and temperature (30, 35, and 37°C) were also determined by culturing isolates in LB medium. All four bacterial strains exhibited the highest growth rate up to 72 h at pH 7.0 and 30°C, consistent with previous studies (Edward Raja *et al.*, 2009; Jayanthi *et al.*, 2013; Sudhanshu *et al.*, 2014).

Metal Tolerance Assay

Stock solutions of CdCl₂•H₂O (Cd) was prepared in Milli-Q water, filter-sterilized, and stored at 4°C. Maximum tolerance levels were determined on NA media supplemented with increasing metal concentrations (100-200 mg/L). Tolerance in liquid cultures was evaluated by inoculating 500 µl of overnight culture (1×10^6 CFU/ml) into 50 mL MSM containing Cd (100–200 mg/l). Flasks were incubated at 30 ± 1 °C, 180 rpm for 4 days. Isolates exhibiting growth at 200 mg/l were selected for subsequent studies and maintained on NA slants at -20 °C (Sundar *et al.*, 2010; Jayanthi *et al.*, 2013; Satapute *et al.*, 2019).

Morphological and Biochemical Characterization

Colony morphology and Gram staining were performed for preliminary characterization. Biochemical assays included oxidase, catalase, Voges-Proskauer, methyl red, casein hydrolysis, potassium cyanide (KCN) tolerance, urease, starch hydrolysis, motility, indole production, and citrate

utilization tests (Smrithi and Usha, 2012; Tanu and Hoque, 2012). Identification was carried out following Bergey's Manual of Systematic Bacteriology.

Molecular Characterization of Bacterial Isolates

Molecular identification was outsourced to Eurofins Genomics India Pvt. Ltd., Bangalore. The 16S rRNA gene was amplified using universal primers 27F and 1492R. PCR products were sequenced in both forward and reverse directions using a BDT v3.1 Cycle Sequencing Kit on an ABI 3730xl Genetic Analyzer. Consensus sequences were assembled using Clustal W, and phylogenetic analysis was conducted in MEGA 7 software (Ahmed and Abdul, 2009; Gurave *et al.*, 2015; Madhulika and Manu, 2017).

Cadmium Accumulation and Removal potential of Bacterial Isolates

Bio-removal potential assays were performed by inoculating 500 µL of overnight-grown metal-resistant isolates into 50 ml AMM containing Cd (100-200 mg/l). Flasks were incubated at 30 ± 1 °C, 180 rpm for 72 h. Samples (10 ml) were withdrawn every 12 h, centrifuged at 10,000 rpm, 4 °C for 10 min, and the supernatant analyzed for residual metal concentration using Atomic Absorption Spectrophotometry (AAS) (Palanisami *et al.*, 2013; Ramadass *et al.*, 2016; Khansa *et al.*, 2021).

Statistical Analysis

All experiments were performed in triplicates, and data were analyzed using SPSS v21.0 and Microsoft Excel. Two-way ANOVA was applied to determine statistically significant differences between bacterial strains and metal concentrations for Cd bio-removal efficiency.

RESULTS AND DISCUSSION

Isolation and Screening of Cadmium-Resistant Bacterial Strains

Ten cadmium-resistant bacterial strains were isolated from the contaminated soil. Among these, four isolates -BIDS I, BIDS II, BIDS IV, and BIDS VI - demonstrated luxuriant growth at high cadmium concentrations (100-200 mg/l) and were selected for further analysis. The selected strains were sub-cultured on nutrient agar slants and stored at -20 °C for subsequent studies.

Growth Kinetics under Cadmium Stress

The four selected isolates-*Enterobactermori* (BIDS I), *Pseudomonas alcaliphila* (BIDS II), *Pseudomonas aestus* (BIDS IV), and *Aeromonas hydrophila* (BIDS VI)-were evaluated for growth kinetics in nutrient broth supplemented with 100 mg l⁻¹ Cd at pH 7.0 and 30 °C for 96 hours (Figure 1). All isolates displayed a characteristic sigmoidal growth curve, reaching peak optical density (OD)) between 72 and 84 hours, followed by a marked decline at 96 hours, indicative of entry into the decline phase.

Among the isolates, BIDS I (*E. mori*) attained the highest maximum OD (0.535 at 84 h), suggesting superior adaptation and metabolic activity under cadmium stress. BIDS VI (*A. hydrophila*) reached a slightly lower peak OD (0.511 at 60 h), followed by BIDS IV (*P. aestus*, OD 0.478 at 72 h) and BIDS II (*P. alcaliphila*, OD 0.465 at 84 h). Notably, *A. hydrophila* achieved its growth peak earlier, which may indicate a faster but shorter-lived adaptation response, whereas *E. mori* and *P. alcaliphila* maintained prolonged growth before decline, suggesting different physiological strategies to cope with metal stress.

The relatively small reduction in growth rates compared to control conditions reflects strong Cd tolerance in all strains, consistent with previous reports that metal-resistant bacteria can maintain significant biomass production in the presence of heavy metals through mechanisms such as efflux pumps, intracellular sequestration, and extracellular precipitation (Bruins *et al.*, 2000; Nies, 2003). Comparable studies have shown that *Pseudomonas* spp. and *Enterobacter* spp. isolated from industrial effluents can sustain high growth rates at cadmium concentrations ranging from 50-150 mg l⁻¹, with optimal growth typically occurring between 48 and 72 h (Kafilzadeh *et al.*, 2012; Vermaand Kuila, 2019). The present results support these findings and

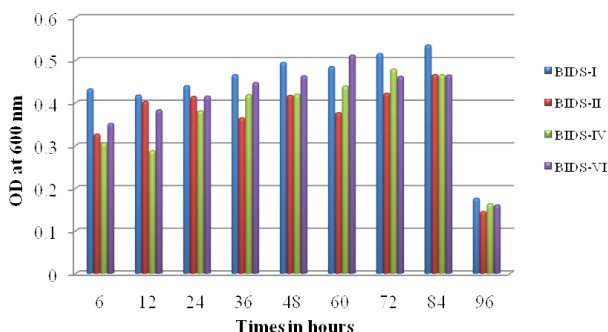


Fig. 1. Growth curve studies of bacterial strains with Cd (100 mg/l)

further indicate that an incubation period of 72 h represents an optimal compromise between maximum biomass accumulation and metabolic stability under cadmium exposure, thereby justifying its selection for subsequent bioremediation experiments.

Growth Curve Studies Under Control Conditions

To establish baseline growth performance, the four cadmium-resistant isolates-*Enterobacter mori* (BIDS I), *Pseudomonas alcaliphila* (BIDS II), *Pseudomonas aestus* (BIDS IV), and *Aeromonas hydrophila* (BIDS VI)-were cultured under identical conditions without cadmium supplementation (Table 1).

Table 1. Growth curve of bacterial isolates under control conditions

Time in (hours)	O.D. of Bacterial Strains			
	BIDS-I	BIDS-II	BIDS-IV	BIDS-VI
06	0.000	0.151	0.010	0.045
12	0.001	0.158	0.014	0.049
24	0.002	0.175	0.019	0.059
36	0.002	0.189	0.023	0.065
48	0.005	0.190	0.028	0.072
60	0.011	0.198	0.032	0.079
72	0.013	0.206	0.036	0.083
84	0.010	0.202	0.031	0.075
96	0.009	0.200	0.024	0.068

Under these control conditions, all isolates exhibited relatively slow growth, with maximum OD values recorded at 72 hours, followed by a gradual decline. Among the isolates, BIDS II (*P. alcaliphila*) achieved the highest OD (0.206), whereas BIDS VI (*A. hydrophila*), BIDS IV (*P. aestus*), and BIDS I (*E. mori*) recorded substantially lower peak OD values (0.083, 0.036, and 0.013, respectively).

Interestingly, when compared to Cd-supplemented cultures (Table 1), OD values in the presence of cadmium were markedly higher for all isolates. This unusual observation suggests that cadmium did not inhibit growth; rather, it appeared to stimulate cellular proliferation. Such metal-stimulated growth may be attributed to adaptive physiological mechanisms, including up regulation of stress-response pathways, activation of metal-binding proteins, or utilization of metal-induced enzyme systems that enhance metabolic efficiency under otherwise nutrient-limited conditions.

Similar trends have been reported in heavy metal-tolerant bacteria, where exposure to sub-lethal metal concentrations resulted in enhanced biomass

yield, possibly due to induction of metallothioneins, biosurfactant, or redox enzymes that support energy metabolism and cell division (Mergeay *et al.*, 2003; Hynninen and Virta, 2010). For example, Kafilzadeh *et al.*, (2012) observed that *Pseudomonas* spp. isolated from industrial effluents displayed higher growth rates in the presence of cadmium (50-100 mg l⁻¹) compared to control conditions, consistent with the present findings. This phenomenon underscores the concept of “metal hormesis,” where low-to-moderate metal stress can trigger growth-promoting responses in resistant microbial strains.

Overall, the control growth data validate the strong cadmium tolerance of these isolates and suggest that their metabolic activity is enhanced in the presence of the metal, reinforcing their potential application in bioremediation of cadmium-contaminated environments.

Metal Tolerance Assay

Tolerance assays revealed that *Enterobacter mori* (BIDS I) and *Pseudomonas alcaliphila* (BIDS II) exhibited the highest resistance to cadmium stress, maintaining growth and metabolic activity at higher Cd concentrations compared to *Pseudomonas aestus* (BIDS IV) and *Aeromonas hydrophila* (BIDS VI). This superior tolerance suggests more efficient detoxification mechanisms, such as enhanced efflux systems, intracellular sequestration, or extracellular precipitation of cadmium. All isolates displayed optimal growth at neutral pH (7.0) and 30 °C under cadmium exposure, highlighting their adaptability to heavy metal-contaminated environments. These physiological traits are advantageous for field-scale bioremediation, as many industrial effluents and polluted natural waters maintain near-neutral pH conditions and ambient temperatures.

The observed resistance pattern aligns with previous studies reporting that *Pseudomonas* and *Enterobacter* species often possess chromosomally or plasmid-encoded metal resistance determinants, including *cadA*, *czc*, and *mer* operons, which confer tolerance through active metal efflux and reduced cellular uptake (Nies, 2003; Mergeay *et al.*, 2003). Similar findings were documented by Kafilzadeh *et al.* (2012), who isolated *Pseudomonas* strains capable of sustaining growth at Cd concentrations exceeding 100 mg l⁻¹, and by Hassan *et al.* (2019), who reported *Enterobacter* spp. tolerating up to 150 mg l⁻¹ Cd while maintaining substantial biomass yields. The adaptability of these strains to neutral pH and moderate temperatures further supports their

potential as robust candidates for bioremediation *in situ*, particularly in municipal and industrial wastewater treatment systems where environmental fluctuations are minimal.

Identification and Molecular Characterization

Morphological, biochemical, and molecular analyses confirmed the identity of the four cadmium-resistant isolates as *Enterobacter mori* (BIDS I), *Pseudomonas alcaliphila* (BIDS II), *Pseudomonas aestus* (BIDS IV), and *Aeromonas hydrophila* (BIDS VI). The isolates displayed notable phenotypic diversity (Table 2). Colony coloration varied from white (*E. mori*) and pink (*P. alcaliphila*) to cream (*P. aestus*) and red (*A. hydrophila*), which is consistent with species-specific pigmentation reported for these genera (Prescott *et al.*, 2020). All isolates were Gram-negative, a characteristic typical of many heavy-metal-resistant bacteria, as their cell wall structure may confer advantages in metal efflux and tolerance (Nies, 2003).

Cell morphology ranged from coccobacilli (*E. mori* and *P. aestus*) and short rods (*P. alcaliphila*) to bacilli (*A. hydrophila*). Interestingly, motility was observed only in *P. alcaliphila*, a trait often linked to environmental adaptability and biofilm formation under stress conditions (Kumar *et al.*, 2021). Biochemical profiling revealed interspecies differences that could influence heavy metal resistance strategies. The Methyl Red test was positive in *E. mori*, *P. aestus*, and *A. hydrophila*, indicating a mixed-acid fermentation pathway, while Voges-Proskauer and Indole tests were uniformly negative across isolates. Citrate utilization was restricted to *A. hydrophila*, and urease production was positive in *P. alcaliphila* and *P. aestus*. Urease activity can indirectly influence heavy metal tolerance by increasing local pH and reducing metal solubility (Mobley and Hausinger, 1989).

All isolates hydrolyzed starch and grew in potassium cyanide (KCN) medium, suggesting resilience to cyanide toxicity—an important trait for surviving industrial effluent conditions (Mergeay *et al.*, 2003). Oxidase activity varied, being present in *P. alcaliphila* and *A. hydrophila*. Sugar fermentation patterns showed that lactose and sucrose were metabolized by *E. mori* and *P. aestus*, while mannitol fermentation occurred exclusively in *A. hydrophila*. Such metabolic flexibility may support growth under nutrient-limited, contaminated environments by allowing utilization of diverse carbon sources (Atlas and Bartha, 2013). Growth across 30 °C, 35 °C,

and 37 °C in all isolates indicates broad mesophilic adaptability, further supporting their suitability for *in situ* bioremediation applications. These findings are consistent with earlier reports of *Pseudomonas* spp., *Enterobacter* spp., and *Aeromonas* spp. as common cadmium-resistant genera with versatile metabolic and physiological traits enabling survival in metal-contaminated ecosystems (Hassan *et al.*, 2019; Kafilzadeh *et al.*, 2012).

Bio-removal potential of Cadmium

The cadmium-resistant bacterial isolates (*Enterobactermori* - BIDS I, *Pseudomonas alcaliphila*-BIDS II, *Pseudomonas aestus* -BIDS IV, and *Aeromonashydrophila* - BIDS VI) demonstrated substantial Cd removal efficiency across the tested concentration range (100-500 mg/l) after 72 h incubation (Figure 2). Removal efficiency increased proportionally with the initial Cd concentration, suggesting that the isolates possess high adsorption capacities and potentially inducible detoxification systems under higher metal stress.

Among the isolates, *E. mori* (BIDS I) consistently exhibited the highest removal capacity, achieving

87% removal (432 mg from 500 mg/l), followed by *P. alcaliphila* (BIDS II, 78%), *P. aestus* (BIDS IV, 72%), and *A. hydrophila* (BIDS VI, 70%). At lower concentrations (100 mg/l), removal ranged from 79.67 mg in BIDS I to 39.67 mg in BIDS VI. This trend aligns with the general biosorption principle where increased availability of metal ions enhances uptake until saturation of binding sites occurs (Volesky, 2007). The superior performance of BIDS I and BIDS II may be attributed to multiple mechanisms, including electrostatic interactions between Cd²⁺ and negatively charged functional

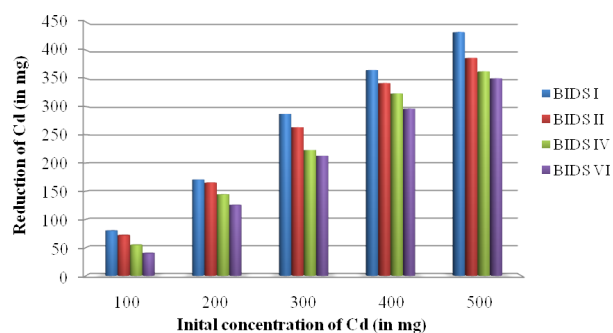


Fig. 2. Removal of Cadmium by different bacterial isolates

Table 2. Morphological and biochemical characteristics of Cd-resistant bacterial isolates

Bacterial Strains	BIDS-I	BIDS-II	BIDS-IV	BIDS-VI
<i>Morphological Characters</i>				
Colony colour	White	Pink	Cream	Red
Gram Nature	Negative	Negative	Negative	Negative
Cell Morphology	Coccobacilli	short rods	Coccobacilli	Bacilli
Motility	-	+	-	-
<i>Biochemical Tests</i>				
MR	+	-	+	+
VP	-	-	-	-
Indole	-	-	-	-
Citrate	-	-	-	+
Urease	-	+	+	-
Casien	-	+	+	+
KCN growth	+	+	+	+
Oxidase	-	+	-	+
Starch Hydrolysis	+	+	+	+
Catalase	-	-	-	-
<i>Utilization of Sugars</i>				
Glucose	+	+	+	+
Lactose	+	-	+	-
Sucrose	+	-	+	+
Mannitol	-	-	-	+
Fructose	+	+	+	+
<i>Growth at</i>				
30 °C	+	+	+	+
35 °C	+	+	+	+
37 °C	+	+	+	+

groups on the cell surface (e.g., carboxyl, phosphate), production of extracellular polymeric substances (EPS) with high metal-binding affinity, and possible intracellular sequestration mediated by metallothioneins or polyphosphate granules (Wang and Chen, 2009).

Comparable results have been reported in previous studies. For example, *Pseudomonas putida* achieved ~80% Cd removal at 500 mg/l within 72 h (Naik *et al.*, 2012), while *Enterobacter cloacae* demonstrated >85% removal efficiency under similar conditions (Hassan *et al.*, 2019). The relatively lower removal by *A. hydrophila* (BIDS VI) may be due to reduced affinity of surface structures for cadmium or lower EPS production compared to *Enterobacter* and *Pseudomonas* species (Faryal *et al.*, 2002). The observed efficiency, particularly by *E. mori*, underscores the potential application of these strains for bioremediation of cadmium-contaminated industrial effluents. Their ability to tolerate high Cd concentrations while maintaining metabolic activity is advantageous for *in situ* remediation strategies in environments with fluctuating pollutant loads.

Statistical Analysis

Two-way ANOVA without replication was used to evaluate the effects of bacterial isolate type and initial Cd concentration on cadmium removal efficiency after 72 h (Table 4). The results clearly indicate that both factors had a statistically significant impact on Cd removal ($p < 0.001$ for both rows and columns). The effective size for initial Cd concentration ($F = 676.06$) was much larger than for bacterial isolate type ($F = 36.48$), suggesting that metal load in the environment is the dominant driver of absolute removal capacity, although strain-specific physiology still plays a substantial role.

Influence of initial Cd concentration

Across all strains, Cd removal increased proportionally with the initial metal concentration, in agreement with the biosorption saturation model, where higher ion availability enhances occupation

of active binding sites until saturation occurs (Volesky, 2007; Wang and Chen, 2009). This concentration-dependent uptake pattern has been observed in *Pseudomonas putida*, where removal increased from 61% at 50 mg/l to 81% at 500 mg/ (Naik *et al.*, 2012), and in *Enterobacter cloacae*, which achieved >85% removal at 500 mg/L (Hassan *et al.*, 2019).

Influence of bacterial strains type

At all Cd concentrations, *Enterobacter mori* (BIDS I) exhibited the highest removal capacity, followed by *Pseudomonas alcaliphila* (BIDS II), *Pseudomonas aestus* (BIDS IV), and *Aeromonas hydrophila* (BIDS VI). These differences are consistent with strain-specific variations in surface charge density, extracellular polymeric substance (EPS) production, and intracellular sequestration capacity. For example, *Enterobacter* species are known to possess abundant anionic surface groups and high EPS yields, facilitating strong electrostatic interactions with Cd²⁺ ions (Hassan *et al.*, 2019). *Pseudomonas* spp., while also efficient, may exhibit slightly lower maximum uptake due to differences in cell wall composition and binding site distribution (Faryal *et al.*, 2002).

Variability at higher concentrations

The standard deviation values increased with higher Cd loads, indicating greater variability among strains at elevated stress levels. This could be due to physiological limits of metal tolerance-while some isolates maintain biosorption efficiency, others may experience reduced metabolic function or increased membrane damage at high Cd concentrations (Bruins *et al.*, 2000). The ANOVA confirms that both environmental factors (Cd concentration) and microbial traits (isolate type) significantly determine Cd removal efficiency. For bioremediation applications, this suggests that strain selection should be optimized according to anticipated metal loads, with *E. mori* and *P. alcaliphila* showing the greatest promise for high-Cd environments.

ANOVA Test

Source of Variation	SS	Df	MSS	F	P-value	F crit
Rows	273749.6	4	68437.41	676.0611	5.18E-14	3.259167
Columns	11079.13	3	3693.044	36.48185	2.61E-06	3.490295
Error	1214.756	12	101.2296			
Total	286043.5	19				

CONCLUSION

The present study aimed to isolate cadmium-resistant bacteria possessing multiple resistances to various heavy metals, with potential application in the simultaneous removal of multiple contaminants from polluted environments. The ability of such bacterial strains to tolerate and grow in the presence of heavy metals makes them valuable candidates for wastewater treatment systems. From soil samples, four bacterial isolates belonging to the genera *Pseudomonas* and *Enterobacter* were successfully identified, exhibiting a high degree of resistance to diverse heavy metals. Under laboratory conditions, these isolates demonstrated cadmium removal efficiencies ranging from 65% to 87%. Among them, *Pseudomonas alcaliphila* and *Enterobacter mori* showed the highest efficiency and can be considered promising candidates for low-cost, high-efficiency bioremediation of cadmium-contaminated water. *Aeromonas hydrophila* strains also exhibited substantial Cd removal capability.

In addition to cadmium removal, all four isolates were effective in reducing biochemical oxygen demand (BOD), chemical oxygen demand (COD), and other chemical parameters by up to 80%, rendering treated effluents suitable for gardening, agricultural, and certain domestic applications. This highlights the versatility of the isolates in overall wastewater quality improvement. The findings demonstrate that microbial bioremediation is a novel, sustainable, and cost-effective alternative to conventional chemical treatments for the removal of toxic heavy metals, thereby contributing to environmental protection and human health. Moreover, the biomass generated during the treatment process holds potential for use as biofertilizers; further enhancing resource recovery and circular economy approaches.

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Authors' Contributions

The first author conceived and designed the study, analysed the results, and prepared the initial draft of the manuscript. Both authors contributed to the critical review and revision of the manuscript, participated in drafting the final version, and approved the final manuscript for publication.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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