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Bioremediation Potentials of *Enterobacter cloacae* Exposed to Crude Oil-impacted Soil

By

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Abstract

This study investigated the potential of an autochthonous bacterium (Enterobacter cloacae) isolated from the Nekede dumpsite in remediating crude oil-contaminated soil. The bioremediation experiment involved treating soil contaminated with 5% crude oil concentration with Enterobacter cloacae and monitoring soil physicochemical parameters, heavy metal concentrations. Standard analytical methods were used for physicochemical analyses and the following mean concentrations were obtained for pH, moisture content, conductivity, organic matter, TOC, Ca, NO_3, PO_4, P and NH_3: 7.42, 2.34%, 110.0µS/cm, 3.27%, 4.30%, 196.14mg/L, 27.81mg/L, 6.82mg/kg, 1.84mg/kg and 1.329mg/kg in FUTO (control) soil; 6.50, 1.17%, 85.74µS/cm, 1.71%, 4.95%, 136.18mg/L, 38.18mg/L, 1.24mg/kg, 1.05mg/kg and 1.043mg/kg in FUTO soil + contaminant + treatment; 6.42, 1.78%, 107.48µS/cm, 3.24%, 4.81%, 134.5mg/L, 24.22mg/L, 4.78mg/kg, 1.83mg/kg and 2.067mg/kg in dumpsite soil; and 6.73, 1.55%, 100.5µS/cm, 1.14%, 5.08%, 134.14mg/L, 31.37mg/L, 2.44mg/kg, 1.12mg/kg and 0.196mg/kg in dumpsite soil + contaminant + treatment. The concentrations of heavy metals (Cr, Pb, Cu, Zn, Fe, Cd and Ni) in treated and untreated soil samples from FUTO and Nekede analyzed using Atomic Absorption Spectrophotometer obtained results showing the following mean concentrations: 50.45, 4.78, 18.13, 24.32, 10.2, 5.46 and 0.067 mg/kg in contaminated FUTO samples; 62.81, 8.11, 41.24, 23.85, 11.6, 6.86 and 0.054 mg/kg in uncontaminated Nekede samples and 74.11, 12.04, 49.07, 23.98, 14.8, 6.43 and 0.057 mg/kg in contaminated Nekede samples respectively. After bioremediation, the following mean concentrations were obtained: 35.21, 3.37, 9.52, 25.38, 7.2, 2.02 and 0.032 mg/kg in contaminated FUTO samples; 43.21, 5.11, 30.11, 24.79, 8.6, 4.23 and 0.027 mg/kg in uncontaminated Nekede samples and 51.83, 7.04, 32.07, 24.89, 9.8, 4.33 and 0.029 mg/kg in contaminated Nekede samples respectively. The findings from this study confirm that bioremediation with Enterobacter cloacae is effective in reducing heavy metal concentrations in crude oil-polluted soils.

Keywords: *Bioremediation, Enterobacter cloacae, Crude oil-contamination, Imo State.*

Introduction

The release of many types of contaminants is causing serious harm to all life-forms due to increasing global industrialization [1]. Pollutants such as oil hydrocarbons, heavy metals and pesticides are environmentally harmful, causing serious impacts on the health of ecosystems. Especially in humans, there is an incidence of carcinogenesis and mutagenesis as well as other toxic effects [2].

Oil contamination in water and soil is a worldwide environmental [3] posing a huge threat to human health and natural ecosystems [4]. Compared with physical and chemical remediation, bioremediation is regarded as the optimal method for remediation of oil-contaminated soil because it is inexpensive, efficient, and applies environmentally friendly processes [5]. The successful application of bioremediation techniques, such as bioaugmentation, bio-stimulation, and phytoremediation, for remediating oil spills has been reported in numerous studies [6]. Field-scale bioremediation works were also conducted in some oil-contaminated fields, and the obtained results were satisfactory. Most of them were ex-situ methods, such as bio-piles and prepared beds [7-8] which are always time-consuming and expensive [9] and therefore unsuitable for mass soil. However, few studies monitored microorganisms, so the status of degradation by microorganisms in the soil could not be determined [10]

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The success of bioremediation correlates with microorganisms' degradation ([11] which is potentially influenced by other microorganisms and nutrition enhancement. An understanding of the activities of biodegrading microorganisms and the relationships between microorganisms and environmental conditions is essential for the development of appropriate remediation procedures [12]. For this reason, this study focuses on the microbial community associated with crude oil-contaminated soil.

Materials and Methods

Study Area

The Nekede Dumpsite, located in Owerri West Local Government Area of Imo State, southeastern Nigeria, serves as a significant solid waste disposal site for the Owerri metropolis. Geographically, it is situated along the old Nekede road, adjacent to the Otamiri River, at approximately 5°25'59.99"N latitude and 7°01'60.00"E longitude [13]. It has an elevation of 194.4ft., is about two hectares in area and is surrounded by a stretch of residential buildings and farmlands [14].. It is about 3 km from Owerri town. Annual rainfall ranges from 2000-2500 mm, mean temperature ranges from 26-28°C and humidity ranges from 70-80% [15]. The dumpsite occupies an area that was previously an abandoned borrow pit, extending nearly 20 meters in depth. It has been operational for several years, receiving a heterogeneous mix of municipal solid wastes, including domestic, commercial, and industrial refuse. Notably, the site lacks engineered liners or leachate management systems, raising concerns about potential environmental impacts on surrounding soil and groundwater quality [16].

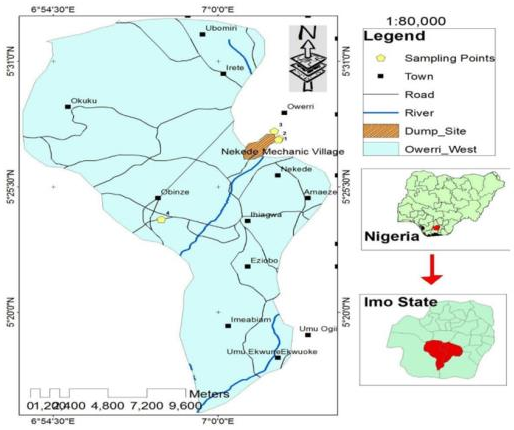

Fig. 1: Map of Owerri showing Nekede dumpsite and sampling stations

Sample Collection

Soil samples

Soil samples were collected from three random locations at the dumpsite, 10 m apart using a soil auger. Soil samples were also collected from the Federal University of Technology, Owerri (FUTO), which served as the control site. At each sample location, soil samples were collected at 15 cm and 30 cm depths. The soil samples were stored in sterilized plastic containers with lids.

More soil samples were subsequently collected from the control site (FUTO), which were used to plant *V. unguiculata*.

Crude oil sample

Exactly 75cl of crude oil was collected from Olumuru, Well 18, Ugheli South, Delta State

Sample Preparations

Pre-bioremediation preparations

Soil samples collected from the dumpsite and control site were split into two groups; one group was treated with crude oil while the second group was left untreated. The treated samples were covered and left undisturbed for two weeks (to enable the microorganisms acclimatize and proliferate). Each treatment was replicated three times.

Preparations for bioremediation

Soil samples

Soil samples were collected from the control site at FUTO for planting *V. unguiculata*. The soil samples collected were air-dried for 7 days and then sieved using a 2 mm sieve. Soil samples were sterilized using an oven at 90°C and allowed to cool before measuring and storing. 1500 g of soil was measured using a weighing balance and stored in each sterilized plastic bucket with lid.

Crude oil samples

The crude oil collected was subjected to 30% dilution with water. Then a 5% pollution concentration was calculated using the formula below:

$$\text{Percentage pollution} = \left(\frac{\text{Mass of Crude Oil Added}}{\text{Total Mass of Soil}}\right) \times 100$$

Equation 3.1

The 5% pollution concentration was then mixed vigorously with the soil stored in the plastic containers using a sterilized hand trowel.

Determination of physicochemical parameters in soil samples

Physicochemical parameters were assayed using the method of [17].

Determination of heavy metals content in soil samples

The heavy metals were determined using an atomic absorption spectrophotometer, in accordance with standard methods [18]. The samples were mixed gently and homogenized and sieved through 2mm mesh-sieve. The samples were first dried, and then placed in an electric oven at a temperature of 400°C approximately for 30 minutes. The resulting fine powder was kept at a room temperature for digestion. Flame atomic absorption spectrophometer apparatus (Buck scientific 210 spectrophometer) was used to measure the concentration of heavy metals in the specimens; after making the calibration graphs, three samples were taken for each element and the average concentration was recorded.

Statistical Analyses

The data collected during this study were analyzed using tables, charts and Analysis of Variance (ANOVA). Some of the analyses were determined at significant level of $P \leq 0.05$.

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RESULTS AND DISCUSSION

Physicochemical parameters of soil samples from Nekede dumpsite and FUTO, untreated and treated with 5% crude oil concentration and *Enterobacter cloacae*

Table 1 presents the physicochemical properties of soil samples from the FUTO and Nekede dumpsites before and after treatment with *Enterobacter cloacae* in the presence of crude oil contamination. The results indicate significant differences in key parameters, particularly pH, moisture content, conductivity, organic matter, nitrate, phosphate, and ammonia concentrations. pH in FUTO soil samples decreased from 7.42 to 6.50 while in the Nekede soil samples it increased from 6.42 to 6.73. Moisture content decreased from 1.17% to 2.34% in FUTO soil samples while it increased from 1.55% to 1.78% n Nekede soil samples. Electrical conductivity decreased from 110.0 µS/cm to 85.74 µS/cm and from 107.48 µS/cm to 78.07 µS/cm in FUTO and Nekede soil samples respectively. Organic matter content decreased from 3.27% to 1.71% and from 3.24% to 1.40% in FUTO and Nekede soil samples respectively, While nitrate (NO_3^-) levels increased from 27.81 mg/L to 38.18 mg/L and from 24.22 mg/L to 31.37 mg/L, phosphate levels decreased from 6.82 mg/L to 1.24 mg/L and from 4.78 mg/L to 2.43 mg/L in FUTO and Nekede soil samples respectively. Ammonia concentrations levels decreased from 1.329 mg/kg to 1.043 mg/kg and 2.067 mg/kg to 0.196 mg/kg in FUTO and Nekede soil samples respectively.

Table 1: Physicochemical parameters of soil samples from Nekede dumpsite and FUTO, untreated and treated with 5% crude oil concentration and *Enterobacter cloacae*

Parameters	FMEnv STD	FUTO (Control)	FUTO + 5% COC + 30 ml of *E. cloacae*	Nekede dump site + 30 ml of *E. cloacae*	Nekede dump site + 5% COC + 30 ml of *E. cloacae*
pH	6.0-7.5	7.42 ± 0.54^{a}	6.50 ± 0.29^{b}	6.42 ± 0.27^{b}	6.73 ± 0.42^{b}
Moisture content %	-	2.34 ± 0.37^{a}	1.17 ± 0.22^{c}	1.78 ± 0.28^{b}	1.55 ± 0.30^{b}
Conductivity (µS/cm)	100	110.0 ± 28.45^{a}	85.74 ± 18.27^{c}	107.48 ± 25.42^{b}	100.5 ± 26.08^{b}
Organic Matter %	-	3.27 ± 0.25^{a}	1.71 ± 0.09^{b}	3.24 ± 0.21^{a}	1.14 ± 0.07^{b}
TOC %	5.0	4.30 ± 0.03^{c}	4.95 ± 0.04^{b}	4.81 ± 0.03^{b}	5.08 ± 0.03^{a}
Calcium (mg/L)	25.0	196.14 ± 21.17^{a}	136.18 ± 27.22^{b}	134.50 ± 25.18^{b}	134.14 ± 15.14^{b}
Nitrate (mg/L)	50.00	27.81 ± 7.05^{c}	38.18 ± 7.05^{a}	24.22 ± 7.05^{d}	31.37 ± 7.05^{b}
Phosphate (mg/kg)	20-60	6.821 ± 0.32^{a}	1.241 ± 0.09^{d}	4.781 ± 0.25^{b}	2.438 ± 0.19^{c}
Phosphorus (mg/kg)	10-50	1.84 ± 0.03^{a}	1.05 ± 0.02^{c}	1.83 ± 0.04^{a}	1.12 ± 0.02^{b}
Ammonia (mg/kg)	-	1.329 ± 0.02^{b}	1.043 ± 0.01^{c}	2.067 ± 0.02^{a}	0.196 ± 0.05^{c}

Legend: TOC = Total Organic Carbon, FMENV STD = Federal Ministry of Environmental Standard, mg/kg = Milligram Per Kilogram, µS/cm = micro-Siemens per centimeter, COC = Crude oil concentration, *E. cloacae* = *Enterobacter cloacae*, mean along the row having different superscript of alphabets differ significantly at $P \leq 0.05$ level.

Heavy metals content of soils polluted with crude oil before bioremediation

Table 2 shows the impact of crude oil contamination on heavy metal concentrations in FUTO and Nekede soils. Across all metals, crude oil pollution led to a significant increase in concentrations, with higher accumulation observed in the Nekede soil. Chromium (Cr) was absent in control soil but increased to 50.45 mg/kg in crude oil-contaminated FUTO soil and 74.11 mg/kg in Nekede soil. Lead (Pb) followed a similar trend, rising from 0.00 mg/kg to 4.78 mg/kg in FUTO and 12.04 mg/kg in Nekede. Copper (Cu) increased significantly, from 0.00 mg/kg in control soil to 18.13 mg/kg in crude oil-treated FUTO soil and 49.07 mg/kg in Nekede. Zinc (Zn) levels fluctuated, decreasing in FUTO soil (43.9 to 24.32 mg/kg) but slightly increasing in Nekede (23.85 to 23.98 mg/kg). Iron (Fe) rose from 0.7 mg/kg to 10.2 mg/kg in FUTO soil and from 11.6 mg/kg to 14.8 mg/kg in Nekede. Cadmium (Cd) was undetectable in control soil but reached 5.46 mg/kg in crude oil-treated FUTO and 6.43 mg/kg in Nekede. Nickel (Ni) appeared at low levels post-contamination, with slightly higher accumulation in FUTO soil (0.067 mg/kg) than Nekede (0.057 mg/kg).

Table 2: Heavy metal contents of soils polluted with crude oil before bioremediation

Heavy metals (mg/kg)	WHO	FUTO soil (Control)	FUTO soil with 5% COC	Nekede dump site soil	Nekede dump site soil with 5% COC
Chromium	64	0.00 ± 0.00^{d}	50.45 ± 7.22^{c}	62.81 ± 4.35^{b}	74.11 ± 5.81^{a}
Lead	85	0.00 ± 0.00^{d}	4.78 ± 0.19^{c}	8.11 ± 0.21^{b}	12.04 ± 0.39^{a}
Copper	36	0.00 ± 0.00^{b}	18.13 ± 2.33^{c}	41.24 ± 5.59^{b}	49.07 ± 5.79^{a}

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Zinc	50	43.9 ± 6.52^{a}	24.321 ± 4.04^{b}	23.854 ± 3.7^{b}	23.984 ± 3.97^{b}
Iron	0.5-10	0.7 ± 0.01^{c}	10.2 ± 1.00^{b}	11.6 ± 1.01^{b}	14.8 ± 1.02^{a}
Cadmium	5.3	0.00 ± 0.00^{c}	5.462 ± 1.00^{b}	6.358 ± 1.01^{a}	6.432 ± 1.03^{a}
Nickel	-	0.00 ± 0.00^{c}	0.067 ± 0.08^{a}	0.054 ± 0.04^{b}	0.057 ± 0.05^{b}

COC = Crude oil concentration, mean along the row having different superscript of alphabets differ significantly at P = 0.05 level.

Heavy metals content of soils polluted with crude oil after bioremediation with *Enterobacter cloacae*

Table 3 shows the heavy metal concentrations in crude oil-contaminated FUTO and Nekede soils after bioremediation with *E. cloacae*. A notable reduction in all heavy metals was observed compared to pre-bioremediation levels. Chromium (Cr) decreased from 74.11 mg/kg to 51.83 mg/kg in Nekede soil and from 50.45 mg/kg to 35.21 mg/kg in FUTO soil. Lead (Pb) reduced significantly, dropping to 7.04 mg/kg in Nekede and 3.37 mg/kg in FUTO. Copper (Cu) declined from 49.07 mg/kg to 13.71 mg/kg in Nekede and from 18.13 mg/kg to 9.52 mg/kg in FUTO. Zinc (Zn) showed slight reductions, with Nekede decreasing to 24.89 mg/kg and FUTO to 25.82 mg/kg. Iron (Fe) levels dropped to 9.4 mg/kg in Nekede and 7.2 mg/kg in FUTO. Cadmium (Cd) reduced to 4.33 mg/kg in Nekede and 2.03 mg/kg in FUTO. Nickel (Ni) levels remained low, decreasing slightly in both soils.

Table 3: Heavy metal contents of soils polluted with crude oil after bioremediation with *Enterobacter cloacae*

Heavy metals	WHO	FUTO soil (Control)	FUTO soil with 5% COC + 30 ml of *E. cloacae*	Nekede dump site soil + 30 ml of *E. cloacae*	Nekede dump site soil with 5% COC + 30 ml of *E. cloacae*
Chromium	64	0.00 ± 0.00^{d}	35.21 ± 2.38^{c}	43.21 ± 3.21^{b}	51.83 ± 4.54^{a}
Lead	85	0.00 ± 0.00^{d}	3. 37± 0.09^{c}	5.11 ± 0.05^{b}	7.04 ± 0.13^{a}
Copper	36	0.00 ± 0.00^{c}	9.52 ± 1.73^{b}	30.11 ± 3.04^{a}	32.07 ± 3.91^{a}
Zinc	50	43.9 ± 6.52^{a}	25.382 ± 4.11^{b}	24.789 ± 3.04^{b}	24.893 ± 3.12^{b}
Iron	0.5-10	0.7 ± 0.01^{c}	7.2 ± 0.07^{b}	8.6 ± 0.10^{a}	9.8 ± 0.12^{a}
Cadmium	5.3	0.00 ± 0.00^{c}	2.023 ± 0.54^{b}	4.231 ± 0.91^{a}	4.332 ± 0.04^{a}
Nickel	-	0.00 ± 0.00^{b}	0.032 ± 0.05^{a}	0.027 ± 0.02^{a}	0.029 ± 0.03^{a}

COC = Crude oil concentration, mean along the row having different superscript of alphabets differ significantly at P = 0.05 level.

Discussion

Soil contamination with crude oil significantly alters its physicochemical properties, impacting nutrient availability, microbial diversity, and overall soil health [19].

Soil pH: The mean concentration of pH of the control soil (7.42 ± 0.54) was slightly alkaline, but crude oil contamination led to a decrease (6.50 ± 0.29 and 6.42 ± 0.27) in polluted soils. However, bioremediation with *Enterobacter cloacae* resulted in a slight increase (6.73 ± 0.42). This is consistent with the study by [1] where crude oil contamination induced soil acidification due to hydrocarbon oxidation, but microbial degradation neutralized pH over time.

Moisture Content (%): Crude oil pollution significantly reduced soil moisture content (1.17 ± 0.22 in polluted FUTO soil), compared to the control (2.34 ± 0.37). However, bioremediation increased moisture content (1.55 ± 0.30). This aligns with findings by [20] who reported that microbial activity enhances water retention by improving soil porosity and organic matter content.

Electrical Conductivity (µS/cm): Electrical conductivity was highest in the control soil (110.0 ± 28.45) but decreased after crude oil contamination (85.74 ± 12.77). Bioremediation led to a partial recovery (107.48 ± 25.42), indicating microbial degradation of hydrocarbons and restoration of ionic balance [21]. The decline in electrical conductivity due to crude oil contamination is well-documented, as hydrocarbons displace ionic species in soil [22].

Organic Matter and Total Organic Carbon (TOC) (%): Crude oil contamination significantly elevated total organic carbon (TOC)—from 4.30 ± 1.03 in control soil to 4.95 ± 0.04 in polluted sites—reflecting the input of hydrocarbon-derived organic matter. Subsequent microbial bioremediation reduced TOC to 3.08 ± 0.03, as heterotrophic bacteria consumed hydrocarbons as a carbon source. A similar pattern—initial TOC increase followed by notable microbial-driven reduction—has been observed in oil-polluted soils treated with compost-amended or consortium-based bioremediation approaches [23].

Calcium (Ca) (mg/kg): Calcium levels were highest in control soil (196 ± 21 mg/kg) but declined after crude oil contamination (136 ± 27 mg/kg). Bioremediation restored Ca levels partially to approximately 134 ± 15 mg/kg, supporting observations that hydrocarbon degradation can liberate or mobilize bound nutrients, thereby aiding soil recovery [24].

Nitrate (NO_3^-) and Phosphate (PO_4^{3-}) (mg/L): Crude oil contamination significantly reduced nitrate and phosphate availability, likely because hydrocarbon toxicity suppresses

nitrifiers and immobilizes nutrient pools. Bioremediation restored nitrate to 31.37 ± 7.05 mg·kg⁻¹ as nitrogen-cycling and diazotrophic activity recovered under stimulated microbial communities. This recovery of N cycling after hydrocarbon removal has been documented in recent bioremediation syntheses and field/laboratory studies. Phosphate likewise increased from 1.241 ± 0.09 mg·kg⁻¹ (polluted) to 2.438 ± 0.19 mg·kg⁻¹ after treatment; such increases are consistent with inoculation or enrichment by phosphate-solubilizing bacteria — for example, *Enterobacter cloacae* strains that release organic acids and solubilize mineral P, increasing plant-available phosphate [25].

Ammonia Concentration (NH_3) (mg/kg): Ammonia concentration declined during post-bioremediation (0.196 ± 0.05), indicating microbial nitrogen cycling [4]. Similar observations were reported by [12] where nitrogen transformation was enhanced in bioremediated oil-contaminated soils.

The physicochemical results demonstrated that crude oil pollution disrupts soil physicochemical properties, but bioremediation with *Enterobacter cloacae* significantly improved soil health. pH, moisture content, conductivity, and nutrient availability improved post-treatment, supporting microbial-driven restoration. These findings are consistent with previous studies on bioremediation effectiveness in crude oil-polluted environments.

Crude oil pollution is known to introduce heavy metals into soil, significantly impacting its quality and biological activity [1] The data presented in Tables 4.2 and 4.3 highlight the changes in heavy metal concentrations before and after bioremediation using *Enterobacter cloacae*. Bioremediation relies on microbial metabolic processes to immobilize, transform, or bioaccumulate heavy metals, making them less bioavailable and toxic [11].

Chromium (Cr) (mg/kg): Before bioremediation, chromium levels were highest in the polluted soil at 74.11 ± 5.81, significantly above the WHO permissible limit of 64. After treatment with *Enterobacter cloacae*, Cr levels dropped to 51.83 ± 4.54, showing a 30% reduction. This aligns with the findings of González Henao and Ghneim-Herrera (2021), who reported a 28% reduction in chromium levels in hydrocarbon-contaminated soil treated with hydrocarbon-degrading bacteria. The observed decrease in chromium (Cr) concentration can be attributed to microbial mechanisms including bioaccumulation and enzymatic reduction (e.g., Cr (VI) to the less toxic Cr (III)). For instance, *Acinetobacter junii* strain b2w demonstrated remarkable bioremediation capability by bioaccumulating Cr and reducing up to 98.2% of Cr (VI) to Cr (III) under lab conditions, via enzymatic reduction coupled with bioaccumulation and efflux systems [26]. Likewise, *Bacillus cereus* WHX-1 immobilized on biochar transformed 94.2% of Cr (VI) to Cr (III) in contaminated soil, demonstrating soil-based enzymatic reduction and immobilization of chromium [27]

Lead (Pb) (mg/kg): Lead, which was undetectable in the control soil, reached 12.04 ± 0.39 in crude oil-polluted soil before treatment. Post-bioremediation, Pb levels reduced to 7.04 ± 0.13, indicating microbial-mediated lead immobilization. Studies by [3] found a 40-50% reduction in Pb levels in contaminated soil using *Pseudomonas aeruginosa* and *Bacillus subtilis*. The reduction in lead (Pb) concentration can be attributed to microbial mechanisms such as biosorption—where functional groups on the cell surface bind Pb (II)—and precipitation of Pb as less bioavailable forms like PbS and $Pb_3(PO_4)_2$ [20]. For example, *Paraclostridium bifermentans* and *Klebsiella pneumoniae* isolated from an industrial consortium removed up to 100% of Pb (II) in solution, forming precipitates such as lead sulfide, while a *Bacillus cereus* strain immobilized Pb through biosorption linked to cell wall functional groups [10].

Copper (Cu) (mg/kg): Copper concentrations decreased significantly—from 49.07 ± 5.79 to 30.27 ± 3.91 mg/kg—after treatment. Similar outcomes have been documented in oil-polluted or contaminated soils, where bacteria like *Enterobacter cloacae* and other strains achieved notable copper removal via biosorption and intracellular [11]. In particular, *Enterobacter cloacae* strains isolated from river environments demonstrated effective Cu (II) removal, while *Penicillium* species from kefir grains showed strong copper biosorption, confirming both cell-surface binding and internalization processes as key removal mechanisms.

Zinc (Zn) (mg/kg): Zinc levels decreased slightly from 23.98 ± 3.97 to 24.89 ± 3.12 post-bioremediation, showing limited reduction. Zinc (Zn) levels often remain relatively stable after bioremediation because Zn is an essential micronutrient for microorganisms, functioning as a cofactor in numerous enzymes and regulatory proteins. Consequently, microbes actively maintain Zn homeostasis, limiting its removal compared to non-essential toxic metals [15]. Similar trends were observed in research by [18], who noted that Zn remained relatively stable in petroleum-contaminated soils treated with biosurfactant-producing bacteria.

Iron (Fe) (mg/kg): Iron levels, before bioremediation were 14.8 ± 1.02, which decreased to 9.8 ± 1.02 after microbial treatment. The slight reduction is consistent with microbial Fe (III)/Fe (II) redox cycling during hydrocarbon degradation—processes that transform Fe rather than permanently remove it from the soil matrix [19]. Field syntheses also note that, unlike hydrocarbons, bulk metals such as Fe typically show limited change after bioremediation, reflecting transformation and redistribution instead of extraction [15]. Similar observations have been reported in oil-impacted wetlands where stimulating dissimilatory iron reduction altered Fe speciation without wholesale removal [1].

Cadmium (Cd) (mg/kg): Cadmium concentrations in contaminated soil were initially 6.432 ± 1.03 mg/kg, but after bioremediation, levels fell to 4.332 ± 0.04 mg/kg—representing approximately a 33 % reduction. This aligns with reports indicating that microbial and plant-assisted remediation typically yields 20–40 % decreases in bioavailable Cd, driven by immobilization mechanisms like precipitation and biosorption rather than complete metal

removal [22]. The observed reduction is likely attributable to microbial-induced precipitation—the formation of cadmium carbonate or other low-solubility compounds bound to microbial metabolites, reducing Cd mobility and bioavailability [28].

Nickel (Ni) (mg/kg Nickel levels decreased modestly from 0.057 ± 0.05 mg/kg to 0.029 ± 0.03 mg/kg, suggesting only minimal microbial interaction with Ni. This persistence is well-documented—even after remediation efforts, Ni often remains in soils more tenaciously than other heavy metals, partly due to its strong binding with soil organic matter and its tendency to form stable complexes [29]. These strong soil–organic associations reduce Ni mobility and limit microbial-mediated removal processes.

Conclusion

The findings from this study confirm that bioremediation with *Enterobacter cloacae* is effective in reducing heavy metal concentrations in crude oil-polluted soils. The most significant reductions were observed for chromium, lead, copper, and cadmium, while zinc, iron, and nickel showed more moderate changes. These results align with previous studies on microbial-assisted heavy metal remediation, confirming the role of bacteria in detoxifying crude oil-polluted environments.

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