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Bioremediation of Heavy Metals in Crude Oil-Polluted Water: A Case Study of the Erhioke River, Niger Delta

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Abstract

The Niger Delta region of Nigeria is highly vulnerable to crude oil pollution, affecting riverine water quality and aquatic biodivhz+ersity. The present study evaluated the seasonal physicochemical properties and Water Quality Index (WQI) of Erhioke River, Kokori, Delta State, and assessed, under controlled laboratory microcosm conditions, the comparative heavy metal bioremediation potential of four indigenous bacterial isolates: *Pseudomonas putida* AY001, *Winkia neuii* B12, *Alcaligenes aquatilis* AYB61, and *Enterococcus casseliflavus* AAOO-1, all previously recovered from the same crude oil-polluted river. Physicochemical analysis revealed generally poor water quality in both seasons, with BOD_5, COD, ammonia, TSS, and sulphate levels consistently exceeding permissible limits set by WHO (2022). The river was found unsuitable for drinking water, with WQI values of 83.20 ("Very Poor") and 111.64 ("Unsuitable for Drinking") during the rainy and dry seasons, respectively. In laboratory-spiked crude oil water microcosms under non-optimised conditions, the highest removal efficiencies were recorded after 28 days, with 49.4% cadmium removal by *E. casseliflavus* AAOO-1 and 49.1% nickel removal by *P. putida* AY001. Two-way ANOVA confirmed that removal performance was significantly influenced by bacterial isolate identity (ηp^2= 0.976–0.999). Principal Component Analysis and Spearman rank correlation identified distinct operational groupings of metal removal, suggesting that active metabolic mechanisms, including proposed enzymatic reduction and efflux systems, predominantly drove cadmium, vanadium, and chromium removal, while passive biosorption primarily governed nickel and lead removal. Overall, these findings demonstrate the bench-scale feasibility and bioremediation potential of indigenous Niger Delta bacterial isolates for the simultaneous heavy metal removal from crude oil-polluted water.

Keywords: Bioremediation; biosorption; crude oil; heavy metal; bacteria; microcosm; river

Introduction

Crude oil pollution represents one of the most persistent environmental threats of the twenty-first century, with millions of metric tonnes of petroleum hydrocarbons entering aquatic ecosystems annually through spillages, pipeline vandalism, illegal bunkering, and refinery discharges (Ossai *et al*., 2020; Onyena and Sam, 2020). The Niger Delta region of Nigeria has recorded thousands of documented oil spill incidents releasing millions of barrels of crude oil into surrounding aquatic and terrestrial environments, making it one of the most petroleum-impacted regions globally (Onyena and Sam, 2020; Sam *et al*., 2017). These recurrent spillages have caused severe deterioration of surface water quality, loss of aquatic biodiversity, and elevated risks of chronic human exposure to toxic petroleum constituents including polycyclic aromatic hydrocarbons (PAHs) and heavy metals (Ordinioha and Brisibe, 2013; Onyena and Sam, 2020). Beyond the organic hydrocarbon fraction, crude oil contains substantial quantities of heavy metals — notably nickel, vanadium, chromium, cadmium, and lead — which persist in receiving water bodies long after volatile organic fractions have degraded (Balali-Mood *et al*., 2021; Mitra *et al*., 2022). In petroleum-impacted aquatic systems, heavy metals and hydrocarbon fractions exist as a complex co-contaminated matrix. Crude oil inherently contains trace metals (particularly nickel and vanadium, bound within porphyrin complexes) alongside metals introduced through industrial oilfield infrastructure, drilling fluids, and anthropogenic run-off (such as lead, chromium, and cadmium). Consequently, indigenous microorganisms inhabiting these ecosystems are subjected to dual chemical stress: metabolizing recalcitrant petroleum hydrocarbons while simultaneously tolerating or sequestering non-biodegradable toxic metals. Unlike organic contaminants, these metals are non-biodegradable, bioaccumulate through aquatic food chains, and exert carcinogenic, nephrotoxic, and neurotoxic effects even at trace concentrations (Zaynab *et al*., 2022; Mitra *et al*.,

2022). The World Health Organization has therefore established stringent permissible limits for these metals in drinking water (WHO, 2022).

Integrated assessment of water quality in oil-impacted rivers increasingly relies on the Water Quality Index (WQI), which translates multiple physicochemical parameters into a single interpretable score reflecting overall water suitability (Uddin *et al*., 2021; Chidiac *et al*., 2023). Recent studies applying WQI to Nigerian surface waters have consistently reported "Poor" to "Unsuitable for Drinking" classifications, driven primarily by elevated BOD, ammonia, TSS, and heavy metal loads (Ighalo and Adeniyi, 2020; Adesakin *et al*., 2020). Erhioke River in Delta State exemplifies this trend, having experienced repeated crude oil spillages between 2014 and 2021 (NOSDRA, 2021). Conventional remediation strategies for heavy metal-contaminated water — including chemical precipitation, ion exchange, and membrane filtration — are often expensive, energy-intensive, and generate secondary pollutants (Qasem *et al*., 2021). Bioremediation, which exploits the metabolic versatility of microorganisms to detoxify or immobilise pollutants, has emerged as an eco-friendly, cost-effective, and sustainable alternative (Bala *et al*., 2022; Sharma *et al*., 2022). Bacterial removal of heavy metals proceeds through biosorption, intracellular bioaccumulation, enzymatic biotransformation, extracellular precipitation, and efflux-mediated resistance (Priyadarshanee and Das, 2021; Pande *et al*., 2022; Igiri *et al*., 2018). Among bacterial genera with demonstrated multi-metal remediation potential, *Pseudomonas* species — particularly *P. putida* — are notable for their metabolic flexibility, hydrocarbon tolerance, and well-characterised metal resistance operons (Roy *et al*., 2014; Ayangbenro and Babalola, 2017). *Enterococcus* species have exhibited biosorption capacity for cadmium and chromium in polluted matrices (Igiri *et al*., 2018), while *Alcaligenes* species possess documented capacity for hydrocarbon degradation and heavy metal tolerance under saline conditions (Nzila *et al*., 2016). *Winkia neuii*, recently reclassified from the genus *Actinomyces* (Nouioui *et al*., 2018), remains poorly characterised for bioremediation applications. Despite growing interest in bacterial bioremediation, most published studies have evaluated isolates individually against single metals in synthetic growth media, with limited comparative assessments of multiple indigenous isolates against a suite of co-occurring heavy metals under complex crude oil-polluted water matrices (Sharma *et al*., 2022; Ojuederie and Babalola, 2017). Indigenous bacterial isolates — those recovered directly from contaminated sites — are widely recognised as superior candidates for site-specific bioremediation owing to their pre-adaptation to local physicochemical conditions and prevailing pollutants (Ojuederie and Babalola, 2017; Ayangbenro and Babalola, 2017). However, evaluating the practical baseline performance of such isolates requires controlled bench-scale microcosm simulations using environmental water matrices before field-scale deployment can be designed. Comprehensive comparative evaluations of indigenous Niger Delta isolates against multiple co-occurring heavy metals remain scarce, and no published study has systematically assessed the multi-metal remediation performance of *Pseudomonas putida* AY001, *Winkia neuii* B12, *Alcaligenes aquatilis* AYB61, and *Enterococcus casseliflavus* AAOO-1 — four isolates recently recovered from Erhioke River — using integrated multivariate statistical analyses.

The present study was therefore designed to: (i) characterise the seasonal physicochemical properties and compute the Water Quality Index of Erhioke River water; (ii) evaluate in a laboratory microcosm model the comparative efficiencies of the four indigenous bacterial isolates in removing nickel, vanadium, chromium, cadmium, and lead from crude oil-contaminated water over a four-week incubation period; and (iii) elucidate the multivariate relationships among heavy metal removal patterns using Principal Component Analysis and Spearman's rank correlation. The findings provide baseline feasibility data on the performance of native Niger Delta isolates, offering a foundation for developing tailored bacterial consortia for ecological restoration in petroleum-impacted aquatic systems.

2. Materials and Methods

2.1 Study Area and Sample Collection

Erhioke River is located in the Kokori community, Ethiope East Local Government Area, Delta State, Nigeria (5°40′0″N; 6°4′0″E; 13 m above mean sea level). The community is surrounded by Okuenebele (4.1 km north-west), Isiokolo (5.2 km south-west), and Orokpor (9.3 km south-west) (Google Earth, 2020). The river has a documented history of recurrent crude oil spill incidents, with reported occurrences in 2014, March 2018, November 2019, and 2021 (NOSDRA, 2021). Water samples were collected from three designated sampling stations (upper, middle, and lower reaches) along the river during two distinct seasons: the rainy season (June–August) and the dry season (November–January). At each sampling station, water samples were collected from a depth of approximately 20–30 cm below the surface using pre-cleaned, acid-washed 2-litre sterile amber glass bottles with Teflon-lined lids under aseptic conditions. Samples were stored in ice-packed insulated containers and transported immediately to the Microbiology Laboratory of the Federal University of Technology, Akure, Ondo State, Nigeria. All sampling was conducted in triplicate (n = 3) at each station per season.

2.2 Physicochemical Analysis of River Water

Ten physicochemical parameters were determined following the standard methods of the American Public Health Association (APHA, 2017). Water temperature was measured in situ using a calibrated digital thermometer (Brannan Precision Thermometer, Model 12/108/2, UK; ±0.1°C precision). pH was measured in situ using a portable pH meter (Hanna Instruments HI 9813-6, USA), calibrated with standard buffer solutions of pH 4.0, 7.0, and 10.0 before each use. Electrical conductivity was determined using a portable conductivity meter (Jenway Model 4510, Bibby Scientific, UK), calibrated with a standard potassium chloride (KCl) solution. Total dissolved solids (TDS) were measured using a calibrated portable TDS meter (Hanna Instruments HI 98301 DiST1, USA). Total suspended solids (TSS) were determined gravimetrically by filtering a measured volume of water sample through a pre-weighed Whatman GF/C glass fibre filter paper (pore size 1.2 µm; Whatman International Ltd., UK), drying at 105°C for 2 hours in a laboratory oven (Memmert Model UN55, Germany), and calculating the weight difference before and after filtration using an analytical balance (Mettler Toledo ML204, Switzerland; ±0.0001 g precision). Total hardness was determined by the EDTA titrimetric method using standardised 0.01 M EDTA solution with Eriochrome Black T as the indicator. Sulphate was determined spectrophotometrically by the barium sulphate turbidimetric method at 420 nm using a UV-Visible spectrophotometer (Shimadzu UV-1800, Japan). Ammonia nitrogen was determined by the indophenol blue colorimetric method at 640 nm using the same Shimadzu UV-1800 spectrophotometer. Biochemical oxygen demand (BOD_5) was determined by the standard 5-day incubation method at 20°C in the dark using a BOD incubator (Lovibond ET 650, Germany) and dissolved oxygen was measured using a portable dissolved oxygen meter (YSI Pro20, YSI Inc., USA). Chemical oxygen demand (COD) was determined by the open reflux dichromate method using a COD digestion reactor (HACH DRB200, USA) and absorbance read at 620 nm on the Shimadzu UV-1800 spectrophotometer. All measurements were performed in triplicate and results expressed as mean ± SE.

2.3 Water Quality Index Computation

The WQI was computed using the Weighted Arithmetic Index method of Brown *et al*. (1972), as described by Ramakrishnaiah *et al*. (2009), using WHO (2022) permissible limits as reference standards for parameter weighting.

2.4 Bacterial Isolates

The four bacterial isolates used in this study — Pseudomonas putida AY001, Winkia neuii B12, Alcaligenes aquatilis AYB61, and Enterococcus casseliflavus AAOO-1 were previously isolated from crude oil-polluted water of Erhioke River, Kokori, Delta State, Nigeria, and characterised by morphological, biochemical, and 16S rRNA gene sequencing methods as described in Abe *et al*. (2024). Their GenBank accession numbers are OR882120, PP436817, PP527761, and OR882868, respectively. The isolates were selected for this study based on their previously demonstrated capacity to utilise crude oil hydrocarbons as a sole carbon source, as confirmed (Abe *et al*., 2024). Prior to the bioremediation experiment, isolates were retrieved from glycerol stock cultures maintained at −70°C, subcultured on nutrient agar (Oxoid, UK) at 30°C for 24 hours, and confirmed for viability before use.

2.5 Preparation of Crude Oil-Polluted Experimental Water

Crude oil used in this study was obtained from a petroleum industry operative in the Niger Delta region of Nigeria and stored at 4°C until use. Experimental crude oil-polluted water was prepared by adding 1% (w/v) crude oil to Erhioke River water and mixing thoroughly on a magnetic stirrer at 200 rpm for 30 min to obtain a homogeneous mixture. Residual concentrations of nickel, vanadium, chromium, cadmium, and lead determined during the incubation period therefore reflected the metal content of the crude oil-polluted water matrix used in the experiment.

2.6 Bioremediation Experiment

2.6.1 Inoculum Preparation

Each bacterial isolate was cultured overnight in nutrient broth (Oxoid, UK) at 30°C on a rotary shaker at 150 rpm. The optical density of each culture was adjusted to 0.5 at 600 nm (OD_{600} = 0.5; approximately 1.5×10^8 CFU/mL) using sterile normal saline (0.85% NaCl), equivalent to McFarland Standard 1, to ensure uniform inoculum density across all treatments.

2.6.2 Experimental Setup

The bioremediation experiment was conducted in 250 mL Erlenmeyer flasks, each containing 100 mL of 1% (w/v) crude oil-polluted water. Each flask was inoculated with 1 mL of the standardised bacterial suspension to achieve a final inoculum concentration of approximately 1.5×10^6 CFU/mL. A non-inoculated control flask containing 100 mL of the crude oil polluted water without bacterial inoculation was included for each metal to account for abiotic metal reduction. All flasks were sealed with sterile cotton wool plugs covered with aluminium foil to allow gaseous exchange while preventing contamination. The flasks were incubated at 30°C on a rotary shaker at 150

rpm for four weeks. Each treatment and control were prepared in triplicate (n = 3). Sampling was conducted at weekly intervals over the four-week incubation period.

2.6.3 Measurement of Residual Heavy Metal Concentrations

At weekly intervals (weeks 1, 2, 3, and 4), 5 mL aliquots were withdrawn aseptically from each flask. Samples were centrifuged at 10,000 rpm for 10 minutes to remove bacterial biomass and particulate matter. The resulting supernatant was acidified with 0.1% (v/v) concentrated nitric acid (HNO_3; Merck, Germany) and stored at 4°C until analysis. Residual concentrations of nickel, vanadium, chromium, cadmium, and lead in the supernatants were determined by Atomic Absorption Spectrophotometry (AAS) following APHA (2017) standard methods. Calibration curves were prepared using certified standard solutions (Merck Certipur, Germany) for each metal. All measurements were performed in triplicate and expressed as mean ± SE (mg/L). Metal removal efficiency (%) was calculated as the percentage decrease in residual metal concentration at week 4 compared with the corresponding initial concentration measured at week 1.

2.7 Statistical Analysis

All data are expressed as mean ± standard error (SE) of triplicate measurements (n = 3). The effects of bacterial isolate and incubation week on residual heavy metal concentrations were evaluated using two-way analysis of variance (ANOVA), with bacterial isolate and incubation week as fixed between-subjects factors. Where significant main effects or interactions were detected ($p < 0.05$), Tukey's Honestly Significant Difference (HSD) post hoc test was applied to partition treatment means into homogeneous subsets. Effect sizes were reported as partial eta squared (η^2_p). Homogeneity of variances was assessed using Levene's test. Seasonal differences in physicochemical parameters were assessed by independent samples t-test. All parametric analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA).

Multivariate relationships among heavy metal removal efficiencies were examined using Principal Component Analysis (PCA) with Varimax rotation and Kaiser Normalization. The suitability of the correlation matrix for PCA was verified using the Kaiser–Meyer–Olkin (KMO) measure of sampling adequacy and Bartlett's test of sphericity. Spearman's rank correlation analysis was performed to quantify pairwise associations among metal removal efficiencies across all isolates and sampling time points (N = 16). The Water Quality Index was computed using Microsoft Excel 2019 (Microsoft Corp., Redmond, WA, USA). Figures were generated using Microsoft Excel 2019.

3. Results

3.1 Physicochemical Properties of Erhioke River Water

Table 1 and Fig. 1 present the physicochemical properties of the water from the Erhioke River during the rainy and dry seasons. All the parameters showed significant difference between the seasons except pH ($t = 1.59$; $p = 0.188$). The pH value was 6.41 ± 0.11 during rainy season and 6.63 ± 0.09 during dry season, which is slightly lower than the permissible range (6.5–8.5). The temperature was significantly higher during the dry season (32.90 ± 0.19°C) than during the rainy season (28.59 ± 0.08°C) and was above the recommended range (25–30°C). The conductivity was within the permissible limit (1000 µS/cm) in both seasons, but higher in rainy season (518.42 ± 11.73 µS/cm) than in dry season (417.88 ± 5.05 µS/cm). BOD and COD were significantly higher in the rainy season (30.98 ± 0.42 and 982.18 ± 6.53 mg/L, respectively) than in the dry season (18.86 ± 0.26 and 550.67 ± 10.94 mg/L), and both exceeded permissible limits in both seasons. The total hardness was found to be higher during dry season (391.15 ± 1.27 mg/L) than in rainy season (248.48 ± 2.26 mg/L) but within the permissible limit. Sulphate and ammonia were significantly higher during the dry season, with sulphate only being above the limit during the dry season and ammonia being above the limit in both seasons. The TSS was significantly higher during the rainy season, and the TDS was higher during the dry season, with both seasons having TSS levels above the permissible limit and TDS levels within the permissible limit.

Table 1 Physicochemical properties of water from Erhioke River, Kokori, Delta State, during the rainy and dry seasons

Parameter	Rainy season	Dry season	Permissible limit	t (df = 4)	p
pH	6.41 ± 0.11[a]	6.63 ± 0.09[a]	6.5–8.5	1.59	0.188
Temperature (°C)	28.59 ± 0.08[a]	32.90 ± 0.19[b]	25–30	20.54	< 0.001
Conductivity (µS/cm)	518.42 ± 11.73[a]	417.88 ± 5.05[b]	1000	7.88	0.001
BOD_5 (mg/L)	30.98 ± 0.42[a]	18.86 ± 0.26[b]	3–6	24.62	< 0.001
COD (mg/L)	982.18 ± 6.53[a]	550.67 ± 10.94[b]	250	33.85	< 0.001
Total hardness (mg/L)	248.48 ± 2.26[a]	391.15 ± 1.27[b]	500	54.93	< 0.001
Sulphate (mg/L)	242.25 ± 4.15[a]	382.63 ± 3.78[b]	250	25.02	< 0.001

Parameter	Rainy season	Dry season	Permissible limit	*t* (df = 4)	*p*
Ammonia (mg/L)	2.78 ± 0.40[a]	5.36 ± 0.12[b]	1.5	6.11	0.004
TSS (mg/L)	122.37 ± 3.07[a]	66.59 ± 0.42[b]	25–30	17.99	< 0.001
TDS (mg/L)	680.81 ± 3.27[a]	853.59 ± 4.32[b]	1000	31.88	< 0.001

Values are mean ± SE of three sampling stations (n = 3), each analysed in triplicate. Means within the same row bearing different superscript letters differ significantly between seasons (independent samples t-test, $p < 0.05$). BOD_5 biochemical oxygen demand, COD chemical oxygen demand, TSS total suspended solids, TDS total dissolved solids. Permissible limits are based on WHO (2022) guideline and acceptability values, supplemented with national standards for parameters lacking WHO guideline values.

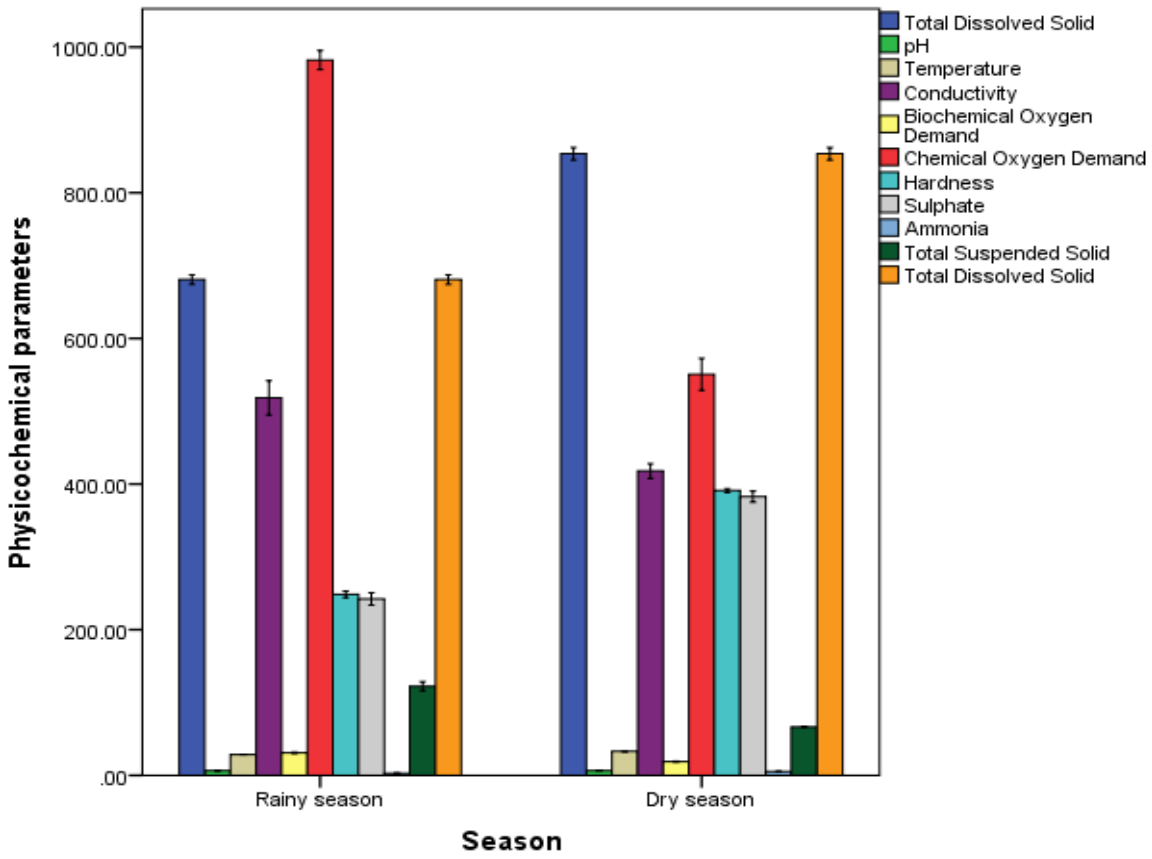

Fig.1 Seasonal variation in physicochemical parameters of water from Erhioke River, Kokori, Delta State. Values are mean ± SE (n = 3).

3.2 Water Quality Index

The Water Quality Index (WQI) values computed using the Weighted Arithmetic Index method (Brown *et al.,* 1972) are presented in Table 2. Relative weights (Rw) ranged from 0.0004 for conductivity to 0.2435 for ammonia. The rainy season WQI was 83.20 ("Very Poor"), driven primarily by ammonia (sub-index = 45.13), BOD (31.44), and TSS (4.97). The dry season WQI increased to 111.64, shifting the classification to "Unsuitable for Drinking," with ammonia (86.96), BOD (19.14), and TSS (2.70) as the dominant contributors. The pH sub-index was 0.00 in both seasons.

Table 2: Relative contribution of physicochemical parameters to the Water Quality Index (WQI) of Erhioke River during rainy and dry seasons.

Parameter	Relative Weight (Rw)	SI (Rainy)	SI (Dry)
pH	0.0522	0.00	0.00
Temperature	0.0122	0.79	2.10
Conductivity	0.0004	0.02	0.02
BOD	0.0609	31.44	19.14

Parameter	Relative Weight (Rw)	SI (Rainy)	SI (Dry)
COD	0.0015	0.58	0.32
Total Hardness	0.0007	0.03	0.05
Sulphate	0.0015	0.14	0.23
Ammonia	0.2435	45.13	86.96
TSS	0.0122	4.97	2.70
TDS	0.0007	0.10	0.12
Overall WQI	—	83.20	111.64

3.3 Bioremediation of Heavy Metals in Crude Oil-Polluted Water

Residual concentrations of nickel, vanadium, chromium, cadmium, and lead declined progressively across the four-week incubation period in all bacterially inoculated treatments, while non-inoculated controls showed comparatively negligible reductions (Tables 3a–7a; Fig. 2A–E). Two-way ANOVA revealed highly significant effects of bacterial isolate, incubation week, and their interaction on residual concentrations of all five metals (all $p < 0.001$), with model R^2 values of 0.981–0.999, confirming that the experimental factors accounted for 98.1–99.9% of total variance (Tables 3b–7b). Significant differences among isolates were identified by Tukey HSD post hoc analysis in all cases ($p < 0.05$).

3.3.1 Nickel

Residual nickel concentration declined progressively in all bacterially inoculated treatments, while the control remained nearly unchanged (Table 3a; Fig. 2A). *Pseudomonas putida* AY001 recorded the lowest mean residual concentration (0.01035 ± 0.00079 mg/L), followed by *Enterococcus casseliflavus* AAOO-1 (0.01048 ± 0.00055 mg/L), *Winkia neuii* B12 (0.01060 ± 0.00053 mg/L), and *Alcaligenes aquatilis* AYB61 (0.01174 ± 0.00044 mg/L), compared to the control (0.02497 ± 0.00003 mg/L). Nickel removal efficiency ranged from 21.6% (*A. aquatilis* AYB61) to 49.1% (*P. putida* AY001; Fig. 3). All main effects and their interaction were highly significant (Table 3b; $R^2 = 0.996$).

Table 3a. Weekly changes in residual nickel concentration (mg/L) during bacterial bioremediation over a four-week incubation period.

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Degradation
Control	0.02497 ± 0.00003	0.02500 ± 0.00000	0.02490 ± 0.00010	0.02500 ± 0.00000	0.02497 ± 0.00003[c]	—
Pseudomonas putida AY001 (A)	0.01407 ± 0.00007	0.01130 ± 0.00015	0.00887 ± 0.00026	0.00717 ± 0.00009	0.01035 ± 0.00079[a]	49.1
Alcaligenes aquatilis AYB61 (B)	0.01293 ± 0.00020	0.01297 ± 0.00102	0.01093 ± 0.00012	0.01013 ± 0.00007	0.01174 ± 0.00044[b]	21.6
Winkia neuii B12 (C)	0.01300 ± 0.00006	0.01133 ± 0.00018	0.00967 ± 0.00015	0.00840 ± 0.00015	0.01060 ± 0.00053[a]	35.4
Enterococcus casseliflavus AAOO-1 (D)	0.01287 ± 0.00009	0.01133 ± 0.00037	0.00971 ± 0.00010	0.00800 ± 0.00006	0.01048 ± 0.00055[a]	37.8

KEY: Values are mean ± standard error (SE), n = 3 per isolate per week. Means in the Mean ± SE column with different superscript letters are significantly different according to Tukey HSD post hoc test ($p < 0.05$).
% Degradation = [(Week 1 concentration − Week 4 concentration) / Week 1 concentration] × 100. Lower residual nickel concentration indicates greater nickel removal.
Two-Way ANOVA: Isolate ($F_{4,40} = 2237.75$, $p < 0.001$), Week ($F_{3,40} = 197.07$, $p < 0.001$), Isolate × Week ($F_{12,40} = 17.96$, $p < 0.001$); $R^2 = 0.996$.

Table 3b. Two-way ANOVA summary for the effects of bacterial isolate and incubation week on residual nickel concentration.

Source of Variation	Sum of Squares	df	Mean Square	*F*	*p*-value	Partial η^2
Corrected model	2.119×10^{-3}	19	1.115×10^{-4}	513.57	< 0.001	0.996
Bacterial isolate	1.943×10^{-3}	4	4.859×10^{-4}	2237.75	< 0.001	0.996
Incubation week	1.284×10^{-4}	3	4.279×10^{-5}	197.07	< 0.001	0.937
Isolate × Week	4.680×10^{-5}	12	3.900×10^{-6}	17.96	< 0.001	0.843

Source of Variation	Sum of Squares	df	Mean Square	*F*	*p*-value	Partial η^2
Error	8.685×10^{-6}	40	2.171×10^{-7}	—	—	—

$R^2 = 0.996$; Adjusted $R^2 = 0.994$.
df = degrees of freedom; η^2 = partial eta squared.

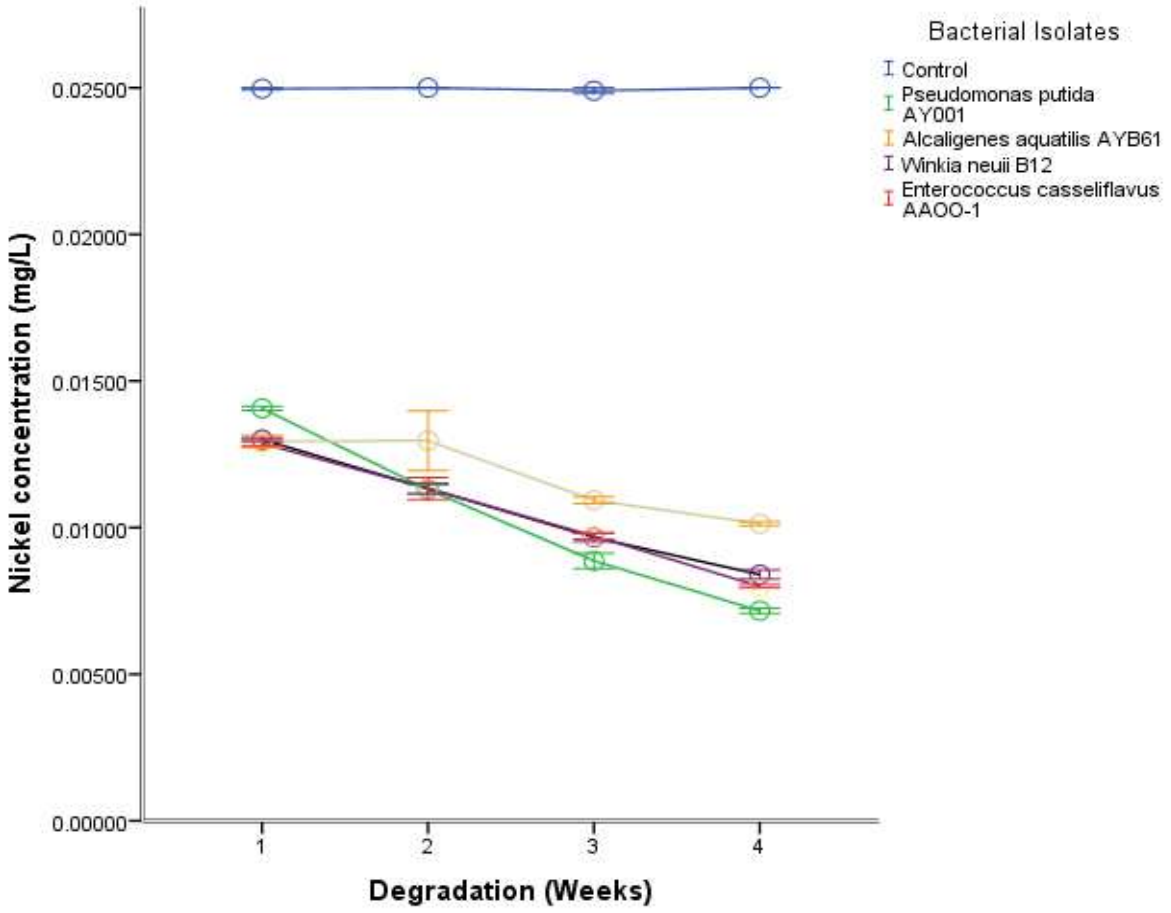

Fig. 2A Bioremediation of Nickel in 1% (w/v) crude oil-polluted water by four bacterial isolates over four weeks of incubation

3.3.2 Vanadium

Residual vanadium concentration decreased across all treatments including the control, which recorded a 12.8% reduction (Table 4a; Fig. 2B). Among the inoculated treatments, *E. casseliflavus* AAOO-1 had the lowest mean residual concentration (0.02171 ± 0.00116 mg/L), followed by *P. putida* AY001 (0.02241 ± 0.00134 mg/L), *A. aquatilis* AYB61 (0.02248 ± 0.00089 mg/L), and *W. neuii* B12 (0.02282 ± 0.00090 mg/L); all four isolates were significantly lower than the control (0.03740 ± 0.00065 mg/L) but did not differ significantly from one another (Tukey HSD, $p < 0.05$). Vanadium removal efficiency ranged from 27.7% (*A. aquatilis* AYB61) to 39.6% (*P. putida* AY001; Fig. 3). All main effects and their interaction were highly significant (Table 4b; $R^2 = 0.981$).

Table 4a. Weekly changes in residual vanadium concentration (mg/L) during bacterial bioremediation over a four-week incubation period.

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Degradation
Control	0.03980 ± 0.00015	± 0.03897 0.00009	± 0.03613 0.00070	± 0.03470 0.00036	± 0.03740 0.00065[b]	± 12.8
Pseudomonas putida strain AY001 (A)	0.02827 ± 0.00064	± 0.02347 0.00043	± 0.02083 0.00142	± 0.01707 0.00187	± 0.02241 0.00134[a]	± 39.6

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Degradation
Alcaligenes aquatilis AYB61 (B)	0.02650 ± 0.00029	0.02370 ± 0.00012	0.02053 ± 0.00026	0.01917 ± 0.00107	0.02248 ± 0.00089[a]	27.7
Winkia neuii B12 (C)	0.02643 ± 0.00022	0.02420 ± 0.00025	0.02217 ± 0.00012	0.01847 ± 0.00084	0.02282 ± 0.00090[a]	30.1
Enterococcus casseliflavus AAOO-1 (D)	0.02645 ± 0.00008	0.02410 ± 0.00015	0.01980 ± 0.00006	0.01650 ± 0.00006	0.02171 ± 0.00116[a]	37.6

KEY: Values are means ± standard error (n = 3). Means in the "Mean ± SE" column followed by different lowercase superscript letters ($^{a-b}$) are significantly different (Tukey HSD, $p < 0.05$). % Degradation = [(Week 1 − Week 4) / Week 1] × 100.

Two-Way ANOVA: Isolate ($F_{4,40} = 407.49$, $p < 0.001$), Week ($F_{3,40} = 145.76$, $p < 0.001$), Isolate × Week ($F_{12,40} = 2.66$, $p = 0.010$); $R^2 = 0.981$

Table 4b. Two-way ANOVA summary for the effects of bacterial isolate and incubation week on residual vanadium concentration.

Source of Variation	SS	df	MS	*F*	*p*-value	η^2_p
Isolate	2.180×10^{-3}	4	5.451×10^{-4}	407.49	< 0.001	0.976
Week	5.849×10^{-4}	3	1.950×10^{-4}	145.76	< 0.001	0.916
Isolate × Week	4.270×10^{-5}	12	3.559×10^{-6}	2.66	0.010	0.444
Error	5.353×10^{-5}	40	1.338×10^{-6}	—	—	—

SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; η^2_p = partial eta squared. $R^2 = 0.981$; Adjusted $R^2 = 0.972$.

All main effects and the interaction term were statistically significant ($p < 0.05$).

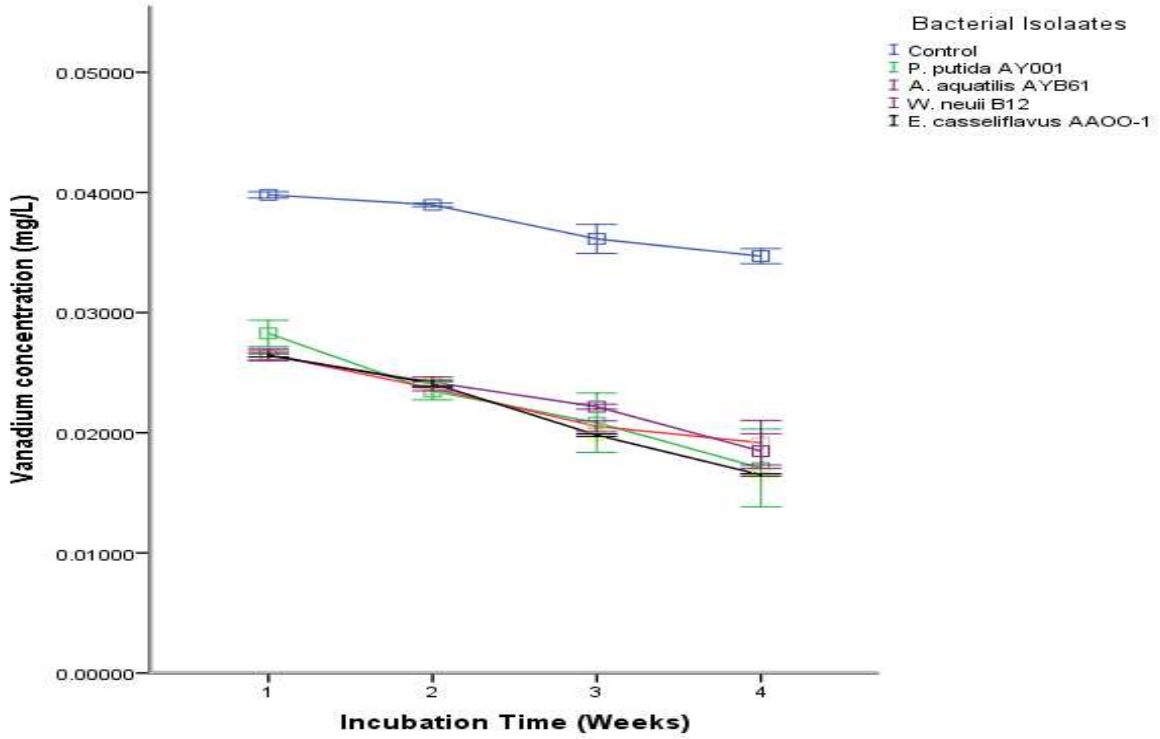

Fig. 2B Bioremediation of Vanadium in 1% (w/v) crude oil-polluted water by four bacterial isolates over four weeks of incubation

3.3.3 Chromium

Residual chromium concentration decreased in all inoculated treatments, while the control showed only a marginal reduction (Table 5a; Fig. 2C). *P. putida* AY001 recorded the lowest mean residual concentration (0.007450 ± 0.000423 mg/L), followed by *W. neuii* B12 (0.007711 ± 0.000406 mg/L), *E. casseliflavus* AAOO-1 (0.007723 ± 0.000459 mg/L), and *A. aquatilis* AYB61 (0.007976 ± 0.000370 mg/L), all significantly below the control (0.014847 ± 0.000034 mg/L). Chromium removal efficiency ranged from 33.5% (*A. aquatilis* AYB61) to 40.9% (*E. casseliflavus* AAOO-1; Fig. 3). All main effects and their interaction were highly significant (Table 5b; R^2 = 0.998).

Table 5a. Weekly changes in residual chromium concentration (mg/L) during bacterial bioremediation over a four-week incubation period.

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Removal
Control	0.015000 ± 0.000000	0.014897 ± 0.000003	0.014797 ± 0.000003	0.014693 ± 0.000007	0.014847 ± 0.000034[d]	—
Pseudomonas putida AY001	0.009237 ± 0.000121	0.008167 ± 0.000088	0.006883 ± 0.000017	0.005513 ± 0.000144	0.007450 ± 0.000423[a]	40.3
Alcaligenes aquatilis AYB61	0.009550 ± 0.000050	0.008657 ± 0.000072	0.007347 ± 0.000087	0.006350 ± 0.000087	0.007976 ± 0.000370[c]	33.5
Winkia neuii B12	0.009480 ± 0.000042	0.008453 ± 0.000179	0.006857 ± 0.000043	0.006053 ± 0.000074	0.007711 ± 0.000406[b]	36.1
Enterococcus casseliflavus AAOO-1	0.009600 ± 0.000153	0.008630 ± 0.000119	0.006990 ± 0.000067	0.005673 ± 0.000228	0.007723 ± 0.000459[b]	40.9

Values are mean ± SE (n = 3). Means in the "Mean ± SE" column followed by different superscript letters differ significantly (Tukey HSD, $p < 0.05$). % Removal = [(Week 1 − Week 4) / Week 1] × 100. Two-way ANOVA: Isolate ($F_{4,40} = 4048.06$, $p < 0.001$); Week ($F_{3,40} = 819.58$, $p < 0.001$); Isolate × Week ($F_{12,40} = 43.42$, $p < 0.001$); $R^2 = 0.998$.

Table 5b. Two-way ANOVA summary for the effects of bacterial isolate and incubation week on residual chromium concentration.

Source of Variation	SS	df	MS	F	p-value	η^2_p
Bacterial isolate	4.900×10^{-4}	4	1.225×10^{-4}	4048.06	< 0.001	0.998
Incubation week	7.439×10^{-5}	3	2.480×10^{-5}	819.58	< 0.001	0.984
Isolate × Week	1.576×10^{-5}	12	1.314×10^{-6}	43.42	< 0.001	0.929
Error	1.210×10^{-6}	40	3.026×10^{-8}	—	—	—

SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; η^2_p = partial eta squared. $R^2 = 0.998$; Adjusted $R^2 = 0.997$. Levene's test: $F(19, 40) = 3.48$, $p < 0.001$, indicating heterogeneity of variances; however, ANOVA is robust to this violation given the balanced design (n = 3 per cell).

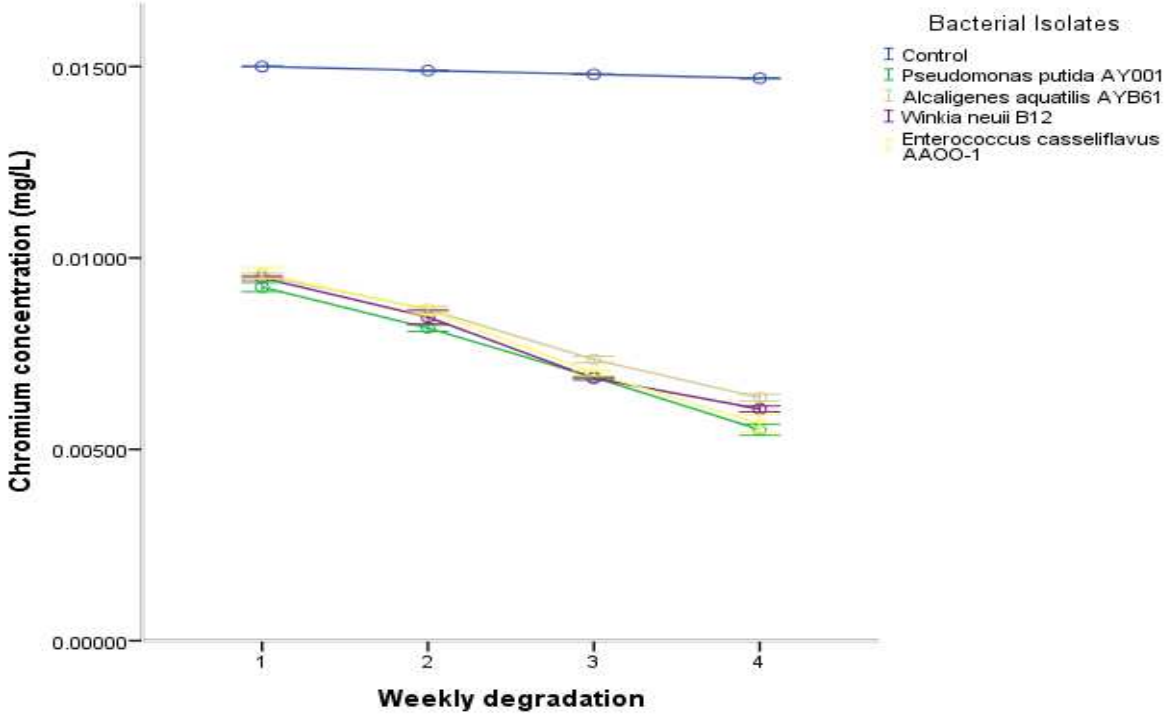

Fig. 2C Bioremediation of Chromium in 1% (w/v) crude oil-polluted water by four bacterial isolates over four weeks of incubation

3.3.4 Cadmium

Residual cadmium concentration declined across all treatments, with the control showing only a 2.0% reduction (Table 6a; Fig. 2D). *P. putida* AY001 and *E. casseliflavus* AAOO-1 recorded the lowest mean residual concentrations (0.00229 ± 0.00015 and 0.00242 ± 0.00018 mg/L, respectively; Tukey subset a), followed by *A. aquatilis* AYB61 (0.00284 ± 0.00010 mg/L; subset b), the control (0.00500 ± 0.00002 mg/L; subset c), and *W. neuii* B12 (0.01036 ± 0.00034 mg/L; subset d). Cadmium removal efficiency ranged from 19.4% (*A. aquatilis* AYB61) to 49.4% (*E. casseliflavus* AAOO-1; Fig. 3). All main effects and their interaction were highly significant (Table 6b; $R^2 = 0.999$).

Table 6a. Weekly changes in residual cadmium concentration (mg/L) during bacterial bioremediation over a four-week incubation period.

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Degradation
Control	0.00506 ± 0.00004	± 0.00502 0.00003	± 0.00497 0.00003	± 0.00496 0.00001	± 0.00500 0.00002[c]	± 2.0
Pseudomonas putida AY001	0.00302 ± 0.00004	± 0.00232 0.00006	± 0.00213 0.00007	± 0.00167 0.00009	± 0.00229 0.00015[a]	± 44.7
Alcaligenes aquatilis AYB61	0.00320 ± 0.00006	± 0.00307 0.00012	± 0.00253 0.00018	± 0.00258 0.00004	± 0.00284 0.00010[b]	± 19.4

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Degradation
Winkia neuii B12	0.01223 ± 0.00015	± 0.01023 0.00009	± 0.00976 0.00004	± 0.00923 0.00009	± 0.01036 0.00034[d]	± 24.5
Enterococcus casseliflavus AAOO-1	0.00330 ± 0.00006	± 0.00235 0.00003	± 0.00237 0.00004	± 0.00167 0.00009	± 0.00242 0.00018[a]	± 49.4

Values are means ± standard error (n = 3). Means in the "Mean ± SE" column followed by different lowercase superscript letters ([a–d]) are significantly different (Tukey HSD, $p < 0.05$). % Degradation = [(Week 1 − Week 4) / Week 1] × 100.
Two-Way ANOVA: Isolate ($F_{4,40} = 7544.17$, $p < 0.001$), Week ($F_{3,40} = 263.95$, $p < 0.001$), Isolate × Week ($F_{12,40} = 39.31$, $p < 0.001$); $R^2 = 0.999$

Table 6b: Two-Way ANOVA Summary for the Effects of Bacterial Isolate, Incubation Week, and Their Interaction on Residual Cadmium Concentration

Source of Variation	SS	df	MS	*F*	*p*-value	η^2_p
Isolate	1.117×10^{-3}	4	2.793×10^{-4}	7544.17	< 0.001	0.999
Week	1.467×10^{-5}	3	4.888×10^{-6}	263.95	< 0.001	0.952
Isolate × Week	8.736×10^{-6}	12	7.280×10^{-7}	39.31	< 0.001	0.922
Error	7.408×10^{-7}	40	1.852×10^{-8}	—	—	—

SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; η^2_p = partial eta squared. $R^2 = 0.999$; Adjusted $R^2 = 0.998$. All main effects and the interaction term were highly significant ($p < 0.001$).

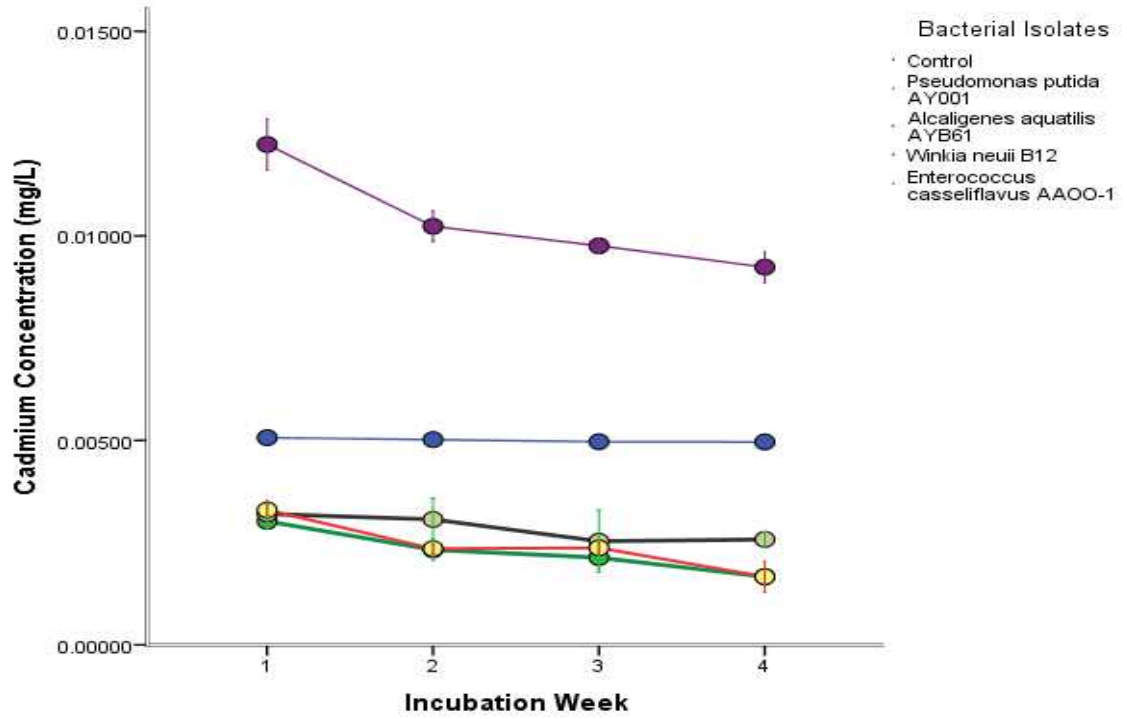

Fig. 2D Bioremediation of Cadmium in 1% (w/v) crude oil-polluted water by four bacterial isolates over four weeks of incubation

3.3.5 Lead

Residual lead concentration decreased progressively in all inoculated treatments, while the control showed only a marginal decline (Table 7a; Fig. 2E). *P. putida* AY001 recorded the lowest mean residual concentration (0.00947 ± 0.00052 mg/L), followed by *E. casseliflavus* AAOO-1 (0.00971 ± 0.00042 mg/L), *W. neuii* B12 (0.01023 ± 0.00034 mg/L), and *A. aquatilis* AYB61 (0.01082 ± 0.00029 mg/L), all significantly below the control (0.01979 ± 0.00005 mg/L). Lead removal efficiency ranged from 21.5% (*A. aquatilis* AYB61) to 37.8% (*P. putida* AY001; Fig. 3). All main effects and their interaction were highly significant (Table 7b; $R^2 = 0.998$).

Table 7a. Weekly changes in residual lead concentration (mg/L) during bacterial bioremediation over a four-week incubation period.

Bacterial Isolate	Week 1	Week 2	Week 3	Week 4	Mean ± SE	% Removal
Control	0.01997 ± 0.00003	± 0.01986 0.00001	± 0.01976 0.00001	± 0.01957 0.00003	± 0.01979 0.00005[e]	—
Pseudomonas putida AY001	0.01190 ± 0.00006	± 0.01010 0.00006	± 0.00847 0.00019	± 0.00740 0.00017	± 0.00947 0.00052[a]	37.8
Alcaligenes aquatilis AYB61	0.01210 ± 0.00006	± 0.01110 0.00006	± 0.01060 0.00017	± 0.00950 0.00020	± 0.01082 0.00029[d]	21.5
Winkia neuii B12	0.01210 ± 0.00006	± 0.01010 0.00006	± 0.00957 0.00014	± 0.00913 0.00007	± 0.01023 0.00034[c]	24.5
Enterococcus casseliflavus AAOO-1	0.01150 ± 0.00029	± 0.01055 0.00013	± 0.00867 0.00012	± 0.00813 0.00009	± 0.00971 0.00042[b]	29.3

Values are mean ± SE (n = 3). Means in the "Mean ± SE" column followed by different superscript letters differ significantly (Tukey HSD, $p < 0.05$). % Removal = [(Week 1 − Week 4) / Week 1] × 100. Two-way ANOVA: Isolate ($F_{4,40} = 5076.96$, $p < 0.001$); Week ($F_{3,40} = 476.23$, $p < 0.001$); Isolate × Week ($F_{12,40} = 34.16$, $p < 0.001$); $R^2 = 0.998$.

Table 7b. Two-way ANOVA summary for the effects of bacterial isolate and incubation week on residual lead concentration.

Source of Variation	SS	df	MS	F	p-value	η^2_p
Bacterial isolate	9.22×10^{-4}	4	2.30×10^{-4}	5076.96	< 0.001	0.998
Incubation week	6.49×10^{-5}	3	2.16×10^{-5}	476.23	< 0.001	0.973
Isolate × Week	1.86×10^{-5}	12	1.55×10^{-6}	34.16	< 0.001	0.911
Error	1.82×10^{-6}	40	4.54×10^{-8}	—	—	—

SS = Sum of Squares; df = degrees of freedom; MS = Mean Square; η^2_p = partial eta squared. $R^2 = 0.998$; Adjusted $R^2 = 0.997$. Levene's test: $F(19, 40) = 2.143$, $p = 0.021$, indicating heterogeneity of variances; however, ANOVA is robust to this violation given the balanced design (n = 3 per cell).

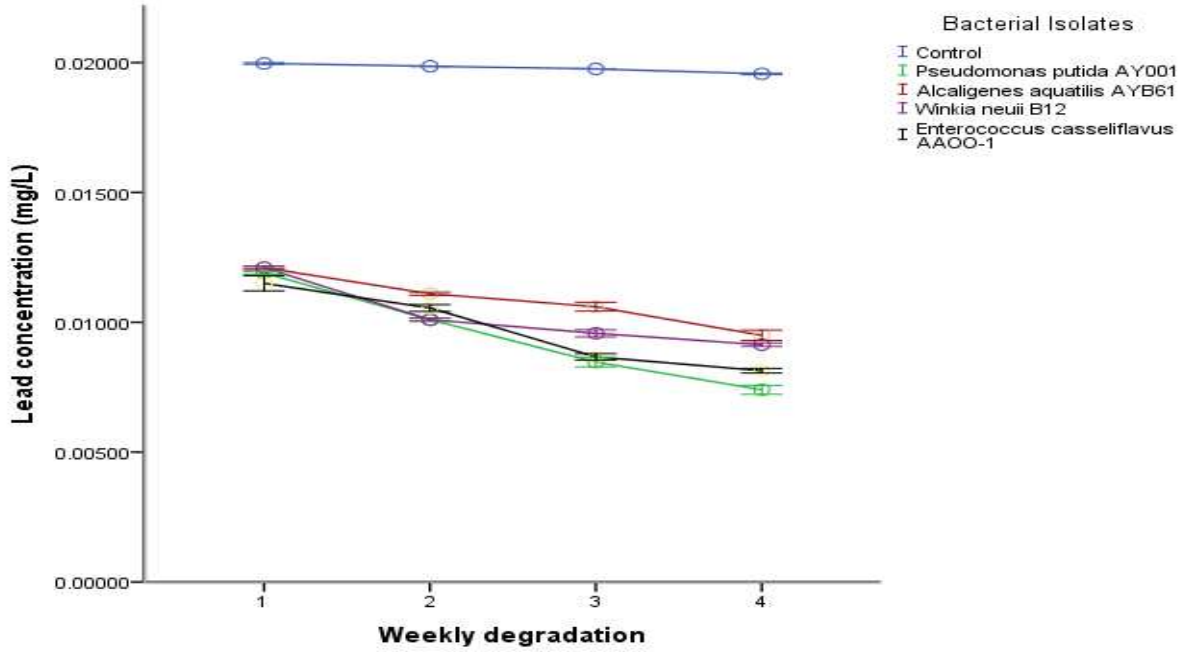

Fig. 2E Bioremediation of Lead in 1% (w/v) crude oil-polluted water by four bacterial isolates over four weeks of incubation

3.3.6 Overall Pattern

Across all five metals, all four bacterial isolates consistently reduced residual metal concentrations below control levels by week 4 (Tables 3a–7a; Figs. 2 and 3). The highest removal efficiencies recorded were 49.4% for

cadmium (*E. casseliflavus* AAOO-1), 49.1% for nickel (*P. putida* AY001), 40.9% for chromium (*E. casseliflavus* AAOO-1), 39.6% for vanadium (*P. putida* AY001), and 37.8% for lead (*P. putida* AY001; Fig. 3). Effect sizes for bacterial isolate were uniformly large across all metals (η^2_p = 0.976–0.999), confirming isolate identity as the dominant source of variance in metal removal performance. Significant isolate × week interactions across all metals ($p \leq 0.010$) confirmed that temporal removal trajectories were isolate-dependent.

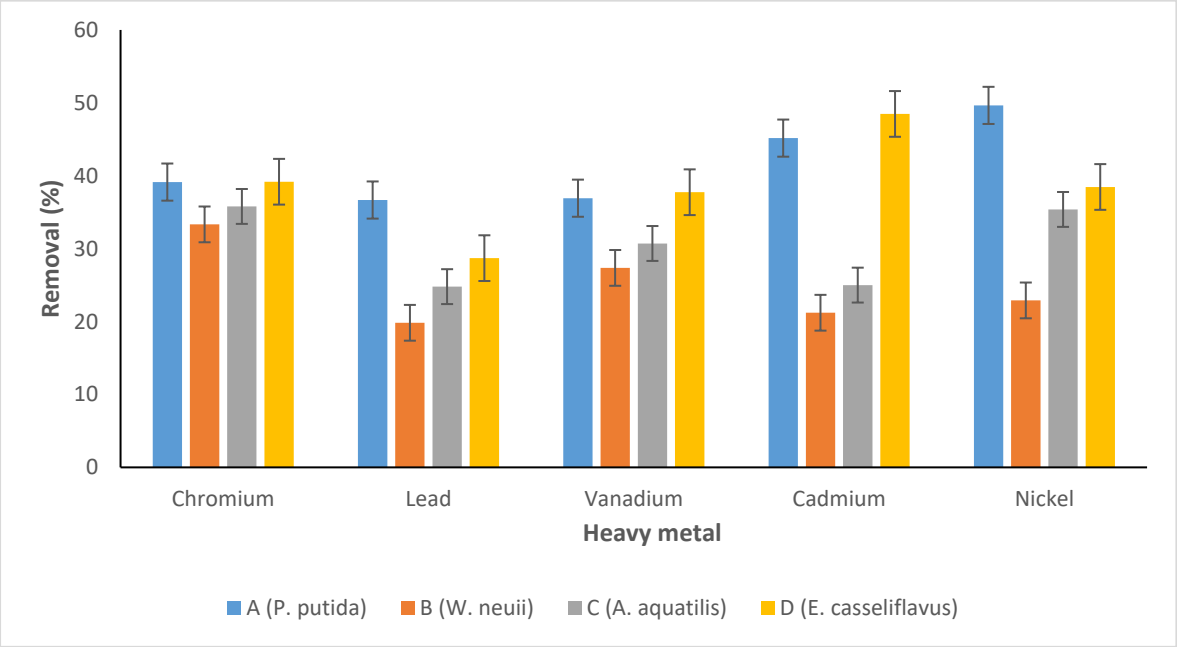

Fig. 3 Removal efficiency (%) of vanadium, chromium, cadmium, nickel, and lead by four bacterial isolates from 1% (w/v) crude oil-polluted water after four weeks of incubation. A = *Pseudomonas putida* AY001; B = *Winkia neuii* B12; C = *Alcaligenes aquatilis* AYB61; D = *Enterococcus casseliflavus* AAOO-1.

3.4 Multivariate Analysis of Heavy Metal Removal

Principal Component Analysis (PCA) and Spearman's rank correlation were performed to examine the multivariate relationships among heavy metal removal efficiencies of the four bacterial isolates (Figs. 3 and 4). The Kaiser–Meyer–Olkin measure of sampling adequacy was 0.561 and Bartlett's test of sphericity was highly significant ($\chi^2 = 114.58$, df = 10, $p < 0.001$), confirming the factorability of the correlation matrix. Two principal components were extracted, together accounting for 99.15% of the total variance (PC1 = 53.35%; PC2 = 45.80%; Table 8). Rotated component loadings revealed that PC1 was strongly associated with cadmium (0.901), vanadium (0.861), and chromium (0.808) removal, reflecting the group of metals for which isolates showed the widest divergence in removal efficiency (19.4–49.4%; Fig. 3; Table 8). PC2 was dominated by nickel (0.879) and lead (0.865) removal, metals for which removal efficiencies ranged from 21.6–49.1% and 21.5–37.8%, respectively (Fig. 3; Table 8).

Table 8. Principal Component Analysis of heavy metal removal efficiencies by bacterial isolates.

Variable	PC1 (53.35%)	PC2 (45.80%)
Cadmium % removal	0.901	0.426
Vanadium % removal	0.861	0.504
Chromium % removal	0.808	0.577
Nickel % removal	0.465	0.879
Lead % removal	0.494	0.865
Eigenvalue (Rotated)	2.67	2.29
Cumulative variance (%)	53.35	99.15

KMO = 0.561; Bartlett's test: $\chi^2 = 114.58$, df = 10, $p < 0.001$. Extraction Method: Principal Component Analysis; Rotation: Varimax with Kaiser Normalization. Bold values indicate dominant loadings (>0.7).

The PCA ordination plot (Fig. 4) demonstrated clear separation of the four isolates across the principal component space, with duplicate samples clustering tightly within each isolate group (Fig. 4), confirming the reproducibility of the removal patterns. *E. casseliflavus* AAOO-1 (Isolate D), which recorded the highest cadmium removal (49.4%; Fig. 3), grouped in the upper-left quadrant (positive PC1, negative PC2; Fig. 4). *P. putida* AY001 (Isolate A), which recorded the highest nickel (49.1%) and vanadium (39.6%) removal (Fig. 3), positioned in the right region (positive PC2; Fig. 4). *A. aquatilis* AYB61 (Isolate C), which consistently recorded the lowest removal efficiencies across all five metals (19.4–33.5%; Fig. 3), occupied the lower-central area (negative PC1; Fig. 4). *W. neuii* B12 (Isolate B) grouped in the lower-left quadrant (Fig. 4), consistent with its intermediate removal performance (24.5–36.1%).

Spearman's rank correlation analysis (Table 9) revealed strong positive associations among chromium, lead, vanadium, and nickel removal efficiencies (ρ = 0.921–0.940; $p < 0.01$), indicating closely coordinated temporal removal patterns across these four metals (Fig. 3). Cadmium removal showed moderate positive correlations with chromium (ρ = 0.501), lead (ρ = 0.574), and vanadium (ρ = 0.540) at $p < 0.05$, but a weak and non-significant correlation with nickel (ρ = 0.457; Table 9), consistent with the distinct loading pattern of cadmium on PC1 relative to nickel in the PCA (Table 8; Fig. 4).

Table 9. Spearman's rank correlation matrix for heavy metal removal efficiencies (N = 16).

Variable	Chromium (Cr)	Lead (Pb)	Vanadium (V)	Cadmium (Cd)	Nickel (Ni)
Chromium (Cr)	1.000				
Lead (Pb)	0.928**	1.000			
Vanadium (V)	0.939**	0.932**	1.000		
Cadmium (Cd)	0.501*	0.574*	0.540*	1.000	
Nickel (Ni)	0.921**	0.931**	0.940**	0.457	1.000

Note. All values represent Spearman's correlation coefficient **(ρ)**.
* Correlation is significant at the 0.05 level (2-tailed).
** Correlation is significant at the 0.01 level (2-tailed).

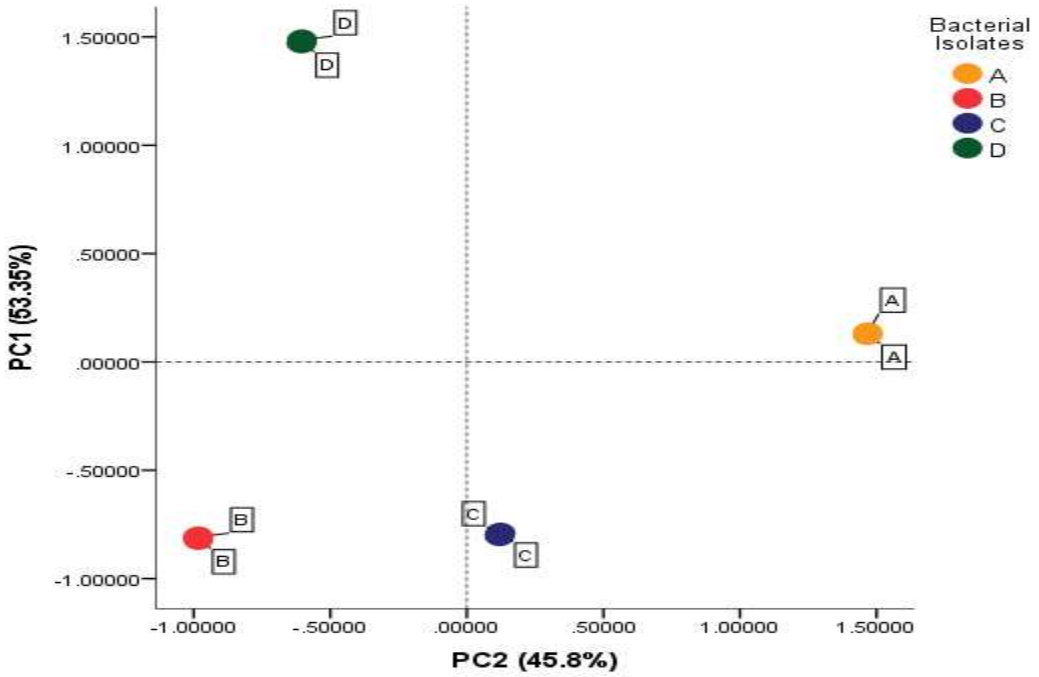

Fig. 4 Principal Component Analysis (PCA) ordination plot of heavy metal removal efficiencies by four bacterial isolates from 1% (w/v) crude oil-polluted water. PC1 and PC2 together explained 99.15% of the total variance.

Duplicate samples clustered tightly within each isolate group. A = *Pseudomonas putida* AY001; B = *Winkia neuii* B12; C = *Alcaligenes aquatilis* AYB61; D = *Enterococcus casseliflavus* AAOO-1.

DISCUSSION

4.1 Physicochemical Properties of Erhioke River Water

The physicochemical characterization of the Erhioke River showed that the water quality was generally poor in both sampling seasons, with most of the parameters measured in the river exceeding the permissible limits set by WHO (2022), indicating that the river has been subjected to cumulative ecological damage due to the frequent crude oil spill incidents reported at this location. The results are in line with the many reports of physicochemical degradation of crude oil polluted riverine systems in the Niger Delta (Anyanwu *et al*., 2023; Ukhurebor *et al*., 2021; Ewim *et al*., 2023). The significantly higher temperature of the dry season (32.90 ± 0.19°C) compared to the rainy season (28.59 ± 0.08°C) both exceeded the recommended threshold by WHO (2022) is indicative of the thermal amplification characteristic of tropical riverine environment during harmattan period. The increased water temperature promotes the volatilisation of dissolved oxygen, enhances the metabolic activity of microorganisms, and increases the toxicity of dissolved contaminants (Akinpelumi *et al*., 2025; Akpodjee *et al*., 2026). The lack of significant seasonal variation in pH ($p = 0.188$) and the values within the acceptable range of 6.5–8.5 as recommended by WHO (2022) may be attributed to the high total hardness values recorded at this site (248.48–391.15 mg/L) as observed by Iwegbue *et al*. (2023) in waterways of the Niger Delta impacted by oil spills and by Arojojoye *et al*. (2021) in similar freshwater systems. The higher conductivity in the rainy season (518.42 ± 11.73 µS/cm) compared to the dry season (417.88 ± 5.05 µS/cm) is contrary to the traditional assumption that conductivity decreases during high flow periods due to dilution. This is probably because of the mobilization of ionic species like dissolved petroleum constituents, remnants of formation water, and remobilised sediment-associated ions observed in petroleum-impacted Niger Delta freshwater systems by Ebong and John (2021) and Onwugbuta and Egbunefu (2026).

The BOD values for both seasons (30.98 ± 0.42 and 18.86 ± 0.26 mg/L) were higher than the WHO (2022) guideline of 3–6 mg/L, by 5.2 and 3.1 times respectively, which is a sign of severe organic enrichment, which is associated with flushing of petroleum residues into the water column during high discharge periods (Ogbonna *et al*., 2021; Onuigbo *et al*., 2025). The COD values were especially high, reaching 982.18 ± 6.53 mg/L during the rainy season, which is almost four times the WHO (2022) guideline of 250 mg/L, indicating the high oxidant demand of the polycyclic aromatic hydrocarbons, alkanes and BTEX compounds typical of water bodies contaminated by petroleum (Ighalo and Adeniyi, 2020; Ite *et al*., 2018). The high concentration of the sulphate ion (382.63 ± 3.78 mg/L) in the dry season is probably due to the concentration of petroleum-derived sulphur compounds during low flow conditions, as reported by Popoola *et al*. (2023) for oilfield-produced water from onshore facilities in the Niger Delta region. The ammonia nitrogen exceeded the WHO (2022) limit by 1.9 and 3.6 times in the rainy and dry seasons, respectively, which is consistent with the decomposition of nitrogenous organic matter under reducing conditions typical of oil-contaminated sediments (Raimi *et al*., 2022; Amesi *et al*., 2025). The significantly higher TSS (122.37 ± 3.07 mg/L) recorded during the rainy season compared to the dry season (66.59 ± 0.42 mg/L) both of which are far higher than the WHO (2022) standard, is indicative of the mobilisation of suspended particulate matter and oil-coated sediment particles during high discharge events, as similarly reported by Nkeeh *et al*. (2021) and Amaewhule *et al*. (2025) in similar crude oil-impacted Niger Delta systems.

4.2 Water Quality Index

The computed WQI values of 83.20 ("Very Poor") for rainy season and 111.64 ("Unsuitable for Drinking") for dry season indicate that Erhioke River is not potable in both seasons. These classifications are similar to the WQI classification of oil-polluted rivers in the Niger Delta. Azuamah *et al*. (2023) calculated WQI values ranging from Very Poor to Unsuitable for Drinking for surface and groundwater in the vicinity of illegal crude oil refining sites in a developing economy, which is similar to the present results. In the same vein, Sam *et al*. (2023) also reported WQI values that indicated water unsuitability for drinking in some communities in the Niger Delta region contaminated by petroleum products, and Akharame and Biose (2025) reported similar WQI deterioration in the rivers of Delta State after oil spill events, which is the same geographical area as the present study. The findings of the present study are also consistent with the regional evidence base, as reported by Onyena (2022) who reported WQI classifications of Very Poor to Unsuitable for Drinking from highly oil-contaminated sites in the Niger Delta. The high relative weight (Rw = 0.2435) of ammonia and the significant measured exceedances are

typical of the weighted arithmetic method of Brown *et al*. (1972) in impacted tropical water bodies (Ajadi *et al*., 2025; Lukhabi *et al*., 2023). The shift in the dry season from "Very poor" to "Unsuitable for Drinking" is attributed to the increase in the ammonia sub-index, which reflects the high sensitivity of this water body during low flow conditions when dilution capacity is low and evaporative concentration is high (Aigberua *et al*., 2020; Egbueri *et al*., 2020).

4.3 Bioremediation of Heavy Metals in Crude Oil-Polluted Water

4.3.1 The removal of metals from the environment

The progressive decline in residual concentrations of all five metals in inoculated treatments versus non-inoculated controls confirms the active role of bacteria in metal removal. When contextualized with published literature on bacterial bioremediation in hydrocarbon-contaminated environments, the unoptimised removal efficiencies achieved in this study (21.5%–49.4% over 28 days) compare favorably with established wild-type reference strains. For instance, wild-type *Pseudomonas* and *Bacillus* strains evaluated by Roy *et al*. (2014) and Razzak *et al*. (2022) demonstrated single-metal removal efficiencies ranging between 30% and 60% in synthetic growth media. In comparison, *P. putida* AY001 and *E. casseliflavus* AAOO-1 in this study achieved up to 49.1% Ni removal and 49.4% Cd removal, respectively, within a complex co-contaminated matrix containing 1% crude oil without external nutrient supplementation or pH optimization. This highlights the practical remediation potential of these indigenous isolates.

4.3.2 Nickel Removal

The highest nickel removal efficiency (49.1%) was achieved by *P. putida* AY001. *Pseudomonas* species are known to possess membrane-bound metal-binding proteins and exopolysaccharide matrices that facilitate extracellular biosorption (Razzak *et al*., 2022). The lower efficiency of *A. aquatilis* AYB61 (21.6%) may reflect energetic prioritization of hydrocarbon metabolism over metal biosorption under multi-contaminant stress (Adeniran *et al*., 2023; Alao *et al*., 2026).

4.3.3 Vanadium Removal

Isolates achieved vanadium removal efficiencies of 27.7%–39.6%. Vanadium is a signature trace metal in Niger Delta crude oils, occurring within organometallic porphyrin complexes (Anyanwu *et al*., 2023). Based on literature precedents, proposed bacterial mechanisms for vanadium removal include biosorption onto cell wall functional groups, enzymatic reduction of soluble vanadate [V(V)] to less soluble vanadyl [V(IV)], or co-precipitation with biogenic iron oxides (Razzak *et al*., 2022). Non-significant differences among isolates (Tukey HSD, $p>0.05p>0.05$) suggest that vanadium bioavailability was constrained by organometallic complexation in the crude oil matrix (Ola *et al*., 2024).

4.3.4 Chromium Removal

Chromium removal ranged from 33.5% to 40.9%. In similar bacterial systems, chromium removal is typically attributed to biosorption on cell wall carboxyl and hydroxyl groups or enzymatic reduction of hexavalent Cr(VI) to Cr(III) via chromate reductases (e.g., encoded by *chrR* operons; Razzak *et al*., 2022). While genetic expression was not directly measured in this study, the observed removal trends in *P. putida* AY001 and *E. casseliflavus* AAOO-1 align with established models of bacterial chromate reduction.

4.3.5 Cadmium Removal

E. casseliflavus AAOO-1 achieved the highest cadmium removal (49.4%), followed by *P. putida* AY001. *Enterococcus* species often utilize cell wall teichoic acids and peptidoglycan carboxyl groups for passive biosorption alongside active P-type ATPase efflux pumps (Razzak *et al*., 2022). In contrast, *W. neuii* B12 exhibited higher residual cadmium levels in early weeks, which could be hypothesized as biosurfactant-mediated metal mobilization from the crude oil matrix or localized cell lysis under cadmium toxicity (Rehman *et al*., 2021).

4.3.6 Lead Removal

Lead removal efficiencies ranged from 21.5% to 37.8%. Reported mechanisms for bacterial lead remediation include cell-surface complexation with lipopolysaccharides, intracellular metallothionein binding, or extracellular biomineralization as lead phosphate/carbonate precipitates (Priyadarshanee and Das, 2021).

4.4 Multivariate Analysis and Mechanistic Insights

PCA accounted for 99.15% of total variance across two principal components. The statistical grouping of cadmium, vanadium, and chromium on PC1 versus nickel and lead on PC2 reflects operational differences in removal patterns. While specific molecular pathways were not experimentally assayed in this work, this statistical separation is consistent with literature models distinguishing active metabolic biotransformation/efflux (commonly associated with Cr, V, and Cd handling) from passive cell-wall biosorption (typically dominant for Ni and Pb; Razzak *et al*., 2022). It should be emphasized that these mechanistic interpretations represent literature-derived hypotheses supported by multivariate clustering rather than directly verified biochemical pathways. Overall, the four indigenous isolates demonstrated substantial heavy metal co-remediation potential within crude oil-contaminated water matrices under non-optimised laboratory conditions. Future studies should build upon these bench-scale microcosm findings by evaluating multi-strain consortium formulations, conducting pilot-scale mesocosm trials under dynamic field conditions, and utilizing omics tools (transcriptomics and proteomics) to definitively confirm the underlying enzymatic pathways.

4.4 Multivariate Analysis of Heavy Metal Removal

The PCA results, which accounted for 99.15% of the total variance across two principal components, offered a solid multivariate basis for understanding the differential metal removal performance. The partitioning of cadmium, vanadium and chromium onto PC1 (which is often mediated by enzymatic reduction and active efflux) and nickel and lead onto PC2 (where passive biosorption is likely to be the dominant mechanism) is biochemically interpretable and fits well with the mechanistic diversity of bacterial heavy metal resistance as discussed by Razzak *et al*. (2022). The multi-metal removal capacity reported here is also applicable to the actual environmental contamination profiles of the Niger Delta region, as all the five metals were reported to co-occur in the crude oil-contaminated water bodies in the region (Anyanwu *et al*., 2023; Isueken *et al*., 2025; Amaewhule *et al*., 2025). The high positive Spearman correlation between the removal efficiencies of chromium, lead, vanadium and nickel ($\rho = 0.921–0.940$; $p < 0.01$) suggests that the simultaneous multi-metal removal is due to common general metal resistance mechanisms: efflux pump overexpression, production of extracellular polymeric substance and broad-spectrum biosorption. The remaining four metals, cadmium, were less strongly correlated with each other ($\rho = 0.457–0.574$), suggesting that the cadmium resistance is more specialised and isolate-specific, as opposed to being uniformly distributed across all isolates, and is biochemically different from the loading of nickel and lead onto PC2. The four isolates are well separated in PCA space, and the samples within each isolate are tightly clustered, further supporting the robustness of the differential removal profiles and experimental reproducibility.

Overall, the four bacterial isolates demonstrated genuine and substantial heavy metal bioremediation potential in crude oil-polluted water, with efficiencies reaching 49.4% for cadmium and 49.1% for nickel within four weeks under non-optimised conditions without external amendments. These efficiencies are ecologically significant given the well-documented severity of simultaneous heavy metal and petroleum hydrocarbon contamination across Niger Delta water bodies (Ukhurebor *et al*., 2021; Ola *et al*., 2024; Gbadamosi and Aldstadt, 2025), where co-occurring stressors impose combined toxic burdens on aquatic ecosystems and riparian communities (Alao *et al*., 2026; Ewim *et al*., 2023). Future investigations should evaluate consortium-based inoculation strategies exploiting synergistic inter-species interactions under optimised physicochemical conditions, and apply metagenomics and functional proteomics approaches to elucidate the molecular mechanisms underpinning multi-metal removal performance, providing a rational basis for the design of enhanced bioremediation strategies tailored to the complex co-contaminated environments of the Niger Delta (Rehman *et al*., 2021).

5 Conclusion

Physicochemical assessment revealed that Erhioke River is severely polluted, with BOD_5, COD, ammonia, and TSS exceeding WHO (2022) limits, yielding WQI scores of 83.20 ("Very Poor", rainy) and 111.64 ("Unsuitable for Drinking", dry). In laboratory microcosm tests using spiked river water, the indigenous isolates *Pseudomonas putida* AY001 and *Enterococcus casseliflavus* AAOO-1 demonstrated promising multi-metal removal performance, achieving up to 49.1% Ni removal and 49.4% Cd removal within 28 days under unoptimised conditions. This bench-scale study establishes the practical feasibility of using native Niger Delta bacterial isolates for heavy metal bioremediation in petroleum-impacted waters, providing a solid foundation for future optimized consortium design and field-scale validation.

Declarations

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Competing Interests
The authors declare no competing interests.

Data Availability
The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

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