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Heavy Metal Tolerance and Hydrocarbon-Degrading Potential of Indigenous Soil Bacteria Isolated from Oil Spill Sites in Bere Community, Rivers State, Nigeria

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Abstract

Contamination from oil spills in Nigeria's Niger Delta has led to widespread co-contamination of soils with petroleum hydrocarbons and heavy metals, creating significant environmental and ecological problems. This research aimed to test the hydrocarbon-degrading capacity and heavy metal tolerance of indigenous soil heterotrophic bacteria isolated from oil-polluted sites in the Bere community in the Gokana Local Government Area of Rivers State, and to assess their potential for bioremediation. The results of the physical and chemical analysis of the soils showed a pH range between 6.51 and 6.79 in the polluted soils as compared to 5.60 in the control soil; the organic carbon range was 2.9–3.18% in the polluted soils and 2.50% in the control soil. The mean concentration of copper and Iron in the polluted soil was 199.5 and 143.6 mg/kg, respectively. The total heterotrophic bacterial (THB) counts ranged from 2.8×10^5 to 3.4×10^5 CFU/g, and the hydrocarbon-utilizing bacterial (HUB) counts ranged from 6.7×10^5 to 8.8×10^5 CFU/g. Potential for degradation of hydrocarbons was determined by the 2,6- dichlorophenolindophenol (DCPIP) reduction assay in Bushnell–Haas broth containing 1% crude oil after 144 hours. Among the sixteen isolates characterized, *Salmonella spp* (DJ15) and *Proteus spp* (DJ16) showed exceptional capability for hydrocarbon degradation. Heavy metal tolerance assays with Fe^{3+} and Cu^{2+} revealed different resistance profiles, with isolates JD14 and JD16 being very high copper-tolerant. The results suggest that indigenous bacteria from oil-polluted soils may possess hydrocarbon-degrading and heavy-metal tolerance traits that may be useful for future bioremediation applications.

Keywords: Bioremediation, Heavy metal tolerance, Hydrocarbon, Indigenous microorganisms, Oil-contaminated soils.

Introduction

Crude oil-contaminated soil ecosystems are among the most complex and persistent environmental contamination systems, in which hydrocarbons from the oil and heavy metals coexist. Petroleum hydrocarbons (PHCs), which are extensively used in industrial and energy applications, are among the most ubiquitous environmental pollutants (Mekonnen *et al*., 2024) and are being released into soils, posing an ecological concern globally. In oil spills, they are frequently bound with toxic heavy metals like cadmium (Cd), lead (Pb), nickel (Ni), and chromium (Cr), etc. (Muhammad *et al.,* 2024). This co-contamination leads to a complex stress environment, which strongly affects the physical, chemical and microbial properties of the native soil. The toxic effects

of heavy metals on microbial cells may disrupt enzymatic activity, damage cell structure, and cause oxidative stress. Heavy metal toxicity has been reported to have a negative effect on the growth and metabolic activity of hydrocarbon degrading bacteria and to change the microbial community structure and diversity (Odion *et al.,* 2025). However, some microbes develop tolerance and adaptive strategies to withstand and even grow in heavy metal-contaminated habitats. The metabolic transformation of a petroleum hydrocarbon to simpler compounds like carbon dioxide, water and biomass by microorganisms is called hydrocarbon biodegradation. This process is the major mechanism of natural attenuation in oil-contaminated environments and is a key component of the restoration of polluted ecosystems. Hydrocarbon-degrading microorganisms can degrade petroleum components, decreasing the concentration of contaminants in soil and water, as well as using the hydrocarbons as carbon and energy sources (Atlas, 1981; Leahy & Colwell, 1990). The aliphatic hydrocarbons are more easily biochemically degraded than the aromatic hydrocarbons because they have less complex structures. Some of the more complex compounds, like polycyclic aromatic hydrocarbons (PAHs), may need a longer time to degrade and specific microbial enzymes (Atlas & Bartha, 1992). Hence, the ability of native hydrocarbon-degrading bacteria to be identified is an important approach in the development of sustainable remediation technology in the Niger Delta area.

Heavy metals are not biodegradable and can be retained in soil for a long time, causing ecological damage in the long run. The elevated levels of metals, like copper, iron, cadmium, lead, zinc and chromium, cause selective pressure on the microorganisms and can induce resistance mechanisms (Nies, 1999). Oil spills result in dramatic shifts in microbial ecology with enrichment of microorganisms that are able to degrade hydrocarbons and loss of sensitive microorganisms (Atlas, 1984). These changes are important for the natural recovery rates and proportions. Microbial adaptation to oil contamination can be either physiological or ecological. Some bacteria carry genes for the catabolism of hydrocarbons and some bacteria have indirect roles in the processes of nutrient cycling, biosurfactant production, and cooperation in metabolism. The biosurfactants also have the ability to lower the surface tension and emulsify hydrophobic hydrocarbons, thus aiding microbial degradation (Ron & Rosenberg, 2002).

Despite the number of studies to date that have reported hydrocarbon contamination and microbial diversity in the Niger Delta, limited information is available on the ability of the indigenous bacterial isolates from Bere community in Gokana LGA, Rivers State, not only to tolerate the heavy metals but to degrade hydrocarbons. Moreover, very little research has examined the ability of indigenous bacteria to survive high levels of heavy metals (e.g., copper and iron) and also possess hydrocarbon degradation ability. This is because there are several stress conditions, such as petroleum hydrocarbons and associated heavy metal(s) present in crude oil-contaminated soils in the Niger Delta, which affect the survival or remediation efficiency of the microbes. Despite its past of frequent oil spills and environmental degradation, the Bere community is still relatively understudied.. The community of Bere was selected for this study based on the fact that the community has been plagued with crude oil contamination resulting from petroleum exploration, production and transportation activities in Ogoni land for a long time. The community is situated along the Trans-Niger Pipeline (TNP) corridor and has experienced several oil spill incidents, which have negatively impacted the soil, vegetation, water resources, livelihoods and socio-economic status of the community. A general contamination of hydrocarbons across Ogoni

land, including communities in the Gokana Local Government Area (LGA), was reported by the United Nations Environment Programme (UNEP) (UNEP, 2011). In addition, research has been conducted in Gokana, which has revealed the susceptibility of the local population to the effects of crude oil exploitation and the continued environmental pollution (Nwilo & Badejo, 2023; Sam *et al*., 2024). The increasing recurrence of crude oil spills in Bere is becoming alarming, which further highlights the potential for continued exposure to petroleum contamination; Bere is an ideal location to investigate microbial adaptation and bioremediation potential in oil-impacted soils. There is a lack of knowledge about the impact of heavy metal stress on the ability of microbial communities to degrade hydrocarbons and the distribution of these dual functions in different environmental contexts. These gaps need to be addressed to maximize bioremediation strategies and to provide better forecasts of ecosystem recovery after contaminating events. Based on the need to investigate the dual ability of soil microbes in an oil-polluted environment and the tolerance of the soil microbes to the presence of heavy metals and their ability to degrade hydrocarbons, the present study is warranted.

"Therefore, the objective of this study is to isolate indigenous bacteria from oil-polluted soils, evaluate their hydrocarbon-degrading capacity, and assess their tolerance to selected heavy metals (Fe and Cu)."

Materials and methods

Study site description.

Bere is a community in the oil-rich Local Government Area of Gokana in Rivers State, Nigeria. Humid tropical climate with mean annual rainfall of about 2000–2500 mm mostly in March to November (Peter and Gbaraneh, 2021). The average annual temperatures vary between 26–32 °C and are comparatively low, with not much difference between seasons because of its proximity to the Atlantic coast. The dominant soils are the coastal plain sands and the hydromorphic soils, mostly of the Ultisol and Inceptisol classes, which are naturally low in fertility, sandy loam to loamy sand and tend to be acidic.

Bere has a long history of crude oil exploration and transportation because it is home to oil facilities and the Trans-Niger pipeline network runs through Ogoniland. Multiple incidents of crude oil contamination from pipelines, operations and oil-related activities have occurred in the community. These activities have altered the soil's physical, chemical, and microbial properties and the livelihoods of the locals. The inhabitants of the study area primarily engage in farming and fishing, activities that are directly affected by environmental pollution.

Soil sample collection

Soil samples were collected from visibly oil-polluted sites located in the Niger Delta region of Nigeria, specifically within the Bera community, Gokana Local Government Area, Ogoni, Rivers State. Soil samples were collected from both contaminated and uncontaminated locations. All soil sampling points were within the Bera community to represent the oil-contaminated sites. The first sampling point (BD1) was located at latitude 4.673176° N and longitude 7.276586° E, while the second sampling point (BD2) was located at latitude 4.673123° N and longitude 7.276492° E. The third sampling point (BD3) was also collected. The control sample (CT1) was located at latitude 4.66068032° N and longitude 7.25583569° E. Soil samples were collected using a sterile stainless-steel auger at a depth of 0–15 cm with an auger. Four soil sub-samples were randomly collected per location and thoroughly mixed to obtain a composite sample. Each composite sample was replicated three times in the laboratory. The collected samples were transferred into sterile, airtight polyethylene bags, properly labelled, and stored at approximately 4°C. Samples for Soil

physical and chemical analysis were air-dried, sieved and moved to the laboratory.

Laboratory analysis

Physical and chemical analysis of soil samples

Physical and chemical analyses were performed to determine soil parameters influencing microbial activity and degradation potential. The pH was measured using a digital meter in a 1:2.5 soil-water suspension. Moisture content was determined gravimetrically by oven drying. Organic matter was estimated by dichromate oxidation. Total nitrogen was determined using the Kjeldahl method. Total concentrations of heavy metals were determined using the Aqua Regia digestion method, employing a 3:1 mixture of hydrochloric acid (HCl) and nitric acid (HNO_3), as outlined by Ehi-Eromosele *et al*. (2012). The heavy metal concentration was measured using Atomic Absorption Spectrophotometry (PerkinElmer PinAAcle 900 Series). Total petroleum hydrocarbons were quantified using Gas Chromatography with Flame Ionization Detection (GC-FID). Copper and iron were chosen for the tolerance assay (TA) because they are environmentally relevant metals commonly found in petroleum-contaminated soils and are micronutrients that play important roles in microbial metabolic processes. Copper and iron allow for evaluation of microbial adaptive responses under increasing stress with a high degree of ecological relevance to contaminated environments, unlike the non-essential and highly toxic metals of lead and cadmium.

Isolation of Microorganisms from Soil

Isolation of microorganisms was carried out using standard serial dilution and plating methods as described by Tudararo-Aherobo & Osaide, 2025.

Biochemical Tests, Characterization, and Identification

A series of standard biochemical tests was conducted to characterize and tentatively identify the bacterial isolates obtained from the crude oil–impacted soil samples. These tests included the Triple Sugar Iron (TSI) agar test, catalase, indole, glucose fermentation, citrate utilization, lactose fermentation, and methyl red Voges-Proskauer tests. All tests were carried out following standard microbiological procedures as described by Cheesbrough (2000). Pure bacterial cultures obtained from nutrient agar were used for the tests, and all media were prepared according to the manufacturer's instructions and sterilized before use.

Enrichment and Isolation of Bacteria.

Enrichment of hydrocarbon-degrading bacteria was achieved using Bushnell-Haas broth supplemented with 1% (v/v) crude oil as the sole carbon source, as described by Valencia-Luna *et al*. (2025).

Heavy metal tolerance assay

Pure cultures of the test isolates were inoculated onto Nutrient Agar supplemented with predetermined concentrations of copper sulfate ($CuSO_4 \cdot 5H_2O$) or ferric chloride ($FeCl_3.6H_2O$). Different concentrations of the metal solutions (e.g., 0.1, 0.2, 0.3, and 0.4 mg/mL were incorporated into the sterilized medium before plating. After inoculation, the plates were incubated at 28 ± 2°C for 24–72 h. Growth was monitored by measuring the colony diameter of bacterial isolates. Isolates that exhibited visible growth at a particular metal concentration were considered tolerant, whereas those showing no growth were considered sensitive. Tolerance was assessed based on the growth response of each isolate relative to the control medium without added metal. In this study, the term "Resistant" is used, which indicates that the isolate maintained normal growth with no

observable inhibition at the tested metal concentration.

Statistical analysis

All data obtained from soil physical and chemical analyses were subjected to Analysis of variance, while others were presented using their mean values in charts and tables.

Results and discussion

The results of the selected soil physical and chemical parameters obtained in this study are presented in Table 1. Substantial variations were observed in the physical and chemical properties of the soils sampled from the control site (CNTRL) and the three contaminated sites (BD1, BD2 and BD3) due to the petroleum contamination and associated anthropogenic activities. The soil textural class indicated that the control soil and BD1 and BD3 sites were sandy loams, and the BD2 site was a loamy sand. The moisture content of the soil also showed a wide range from 7.2 % in BD3 to 23.5 % in BD2. The highest moisture content in BD2 may be a result of the higher amount of organic matter build-up and lower drainage conditions caused by hydrocarbon contamination. In contrast, the lower moisture content observed for BD3 could result from increased soil degradation or reduced water-holding capacity due to prolonged exposure to crude oil contaminants.

The level of organic carbon (OC) ranges from 3.2 % (control site) to 4.3 % (BD2), with a significant difference between locations. The organic carbon contents observed in contaminated sites are probably due to the build-up of petroleum hydrocarbons, which add further carbon compounds to the soil matrix. Other studies also recorded a rise in soil organic matter (SOC) after crude oil contamination in the soil of the Niger Delta region; for instance, Chikere *et al*. (2019) observed that crude oil residues usually lead to an increase in total carbon but decrease nutrient cycling processes.

The observed pH values (5.60 – 6.79) indicate that the soils were generally slightly acidic to near-neutral, a characteristic commonly reported for soils within the Niger Delta region due to the humid tropical climate, high rainfall, and the nature of coastal plain sand parent materials (Kamalu *et al*., 2022). The polluted soils showed an increase in pH, moving towards near neutral (6.51–6.79), which meant there was a moderate increase in alkalinity. This observation is in agreement with recent research indicating that crude oil contamination can modify soil pH by adding alkaline hydrocarbon fractions and related base cations to the soil. Agbogidi *et al*. (2025) reported higher pH in crude oil-polluted soils in Delta State and the higher pH was said to be due to chemical changes brought about by hydrocarbons in the soil. The pH values close to neutral, as observed in this study, are conducive to the proliferation of microorganisms that break down hydrocarbons, since many microorganisms have optimum activity at a near-neutral pH (Das & Chandran, 2011).

The total nitrogen (TN) content had a decreasing trend from the control site (0.04%) to polluted sites (0.01 – 0.02%) and significant differences were found. The decrease is likely due to the inhibitory effects of the petroleum chemicals on nitrogen-fixing microorganisms and nutrient cycles. Analysis of heavy metals showed significant increases in the concentration of lead (Pb), cadmium (Cd), iron (Fe), and copper (Cu) in the contaminated soils when compared with the control soils. The treatment BD3 had a higher lead concentration of 143.8 mg/kg, which is considered highly contaminated. Similarly, the cadmium concentration was higher, ranging from 12.6 mg/kg in control soil to 68.9-94.2 mg/kg in BD3. The copper content increased from 12.4 mg/kg in the control to 265.4 mg/kg in BD2. The concentrations of Fe and Cu in the polluted soils are much higher than those in the

control soils, implying long-term exposure to petroleum-related contaminants and associated industrial inputs. Heavy metals can remain in soils for a long time and can cause toxicity to soil microorganisms, plants and ecosystem functions by interfering with enzymatic activities and nutrient cycles (Ayangbenro & Babalola, 2023). These elevated levels of heavy metals (Pb, Cd, Fe, and Cu) could lead to changes in the composition of microorganisms, biodiversity loss, and changes in soil ecosystem function.

Total Petroleum Hydrocarbon content in the soil samples

The chromatograms of the total petroleum hydrocarbon analysis of the different soil samples collected are depicted in Figures 2, 3, 4 and 5. The results for the Total Petroleum Hydrocarbon (TPH) analysis in the control (CNTRL) soil samples are shown in Figure 2, revealing different levels of TPH values. The chromatogram (Figure 2) shows several sharp peaks, representing different hydrocarbons, with molecular weights spanning from C8 to C40, suggesting the presence of both low and high-molecular-weight components. The predominance of medium-chain n-alkanes (C15 to C22) indicates their high concentration in the sample, a characteristic frequently found in crude oil and the diesel fractions (Wang *et al.,* 2023). The relatively higher concentrations of the heavier hydrocarbons (C23-C40) indicate the presence of less volatile and more resistant to biodegradation hydrocarbons. Based on the information in this profile, it is suggested that the area may have experienced some biodegradation, but a high level of residual hydrocarbons remains in the area.

The chromatogram of BD1 is shown in Figure 3, which shows a large peak at the beginning, followed by several smaller peaks representing hydrocarbons with estimated carbon numbers from about C8 to C37, suggesting the presence of a complex petroleum mixture. The sharp initial peak corresponds to very low molecular weight hydrocarbons that are very volatile and elute early because they have lower boiling points and less affinity to the stationary phase in the chromatographic column (Wang *et al.,* 2023). The lower intensity of the later peaks could indicate partial biodegradation, as microorganisms are more likely to break down the shorter-chain hydrocarbons first before the high molecular weight compounds (HMWCs), which are more difficult to degrade (Varjani and Upasani, 2024). Biodegradation experiments resulting in a reduction in peak abundance and complexity with time had similar chromatographic profiles (Mekonnen *et al.,* 2024). In addition, a decrease in maximum peak intensity indicates that the hydrocarbons are continuously degraded to intermediate metabolites during microbial degradation processes (Atlas & Hazen, 2011

The chromatogram of BD2 as shown in Fig. 4, consists of a large early peak with smaller peaks between about C8 and C33, which are typical of a weathered mixture of petroleum hydrocarbons. The early signal is very strong, which is composed of volatile and low molecular weight hydrocarbons that elute quickly because of their low boiling points and less interaction with the stationary phase (Wang *et al.,* 2023). Medium- and long-chain n-alkanes (C20–C26) are known to be compounds that are difficult for microbes to break down rapidly due to their structural complexity and hydrophobicity, as indicated by peaks (Varjani *et al.,* 2025). The reduction in peak intensity indicates that biodegradation is continuing, with microorganisms breaking down lighter hydrocarbons first and leaving the heavier hydrocarbons to be degraded. Microbial transformation and natural attenuation of contaminated soils have been previously reported with similar chromatographic profiles (Prince *et al.,* 2022). In addition, a large number of partially degraded crude oil products and weathered crude oil residues are usually identified near the late retention time of the unresolved complex mixtures

(Liu *et al.,* 2024). The microbial degradation processes have been proven to modify the content of hydrocarbons by decreasing the number of biodegradable n-alkanes and increasing the amount of recalcitrant aromatic hydrocarbons through the use of the GC–MS method (Al-Hawash *et al.,* 2023). Thus, the chromatographic pattern reveals partial degradation and some persistence of resistant fractions of hydrocarbons in the sample.

The chromatogram of BD3 is shown in Fig. 5, which displays a dominant early peak, followed by a nearly flat baseline with minimal later peaks, indicating that the sample contains predominantly low-molecular-weight volatile hydrocarbons and very few heavy hydrocarbon fractions. The rapid decline in signal intensity suggests extensive biodegradation or weathering, where microorganisms preferentially degrade lighter and more bioavailable hydrocarbons first (Mekonnen *et al.,* 2024). The absence of significant peaks at longer retention times indicates depletion of medium- and long-chain n-alkanes, which commonly occurs during advanced microbial degradation processes (Varjani *et al.,* 2025). Similar chromatographic profiles have been associated with highly degraded petroleum residues and reduced hydrocarbon complexity after bioremediation treatment (Liu *et al.,* 2024).

Heterotrophic and hydrocarbon-Utilizing bacteria count.

The total heterotrophic bacterial (THB) and hydrocarbon-utilizing bacterial (HUB) counts in the present study reflect a strong microbial reaction against the presence of petroleum hydrocarbons in the soil samples taken from the Bera area of Gokana LGA. Polluted samples (BD1, BD2 and BD3) showed significantly higher counts of THB ($2.8 \times 10^5 \pm 0.20 – 3.4 \times 10^5 \pm 0.15$ CFU/g) than that of the control sample ($1.9 \times 10^4 \pm 0.20$ CFU/g). Likewise, there was a significant difference in the number of HUB between the contaminated soils ($6.7 \times 10^5 \pm 0.17$ to $8.8 \times 10^5 \pm 0.19$ CFU/g) and control ($9.0 \times 10^4 \pm 0.35$ CFU/g). The results indicate that crude oil contamination favoured the proliferation and survival of the microorganisms that are able to live and multiply in such environments. The higher THB numbers in contaminated soils could be due to the greater availability of the organic carbon that has been added from the petroleum hydrocarbons. Crude oil is composed of various carbon compounds that could provide nutrients for microbial growth and thus promote the growth of heterotrophic bacteria (Mekonnen *et al.,* 2024). In oil-polluted soils of the Niger Delta, the abundance of THB (hydrocarbon degrading bacteria) was reported to have increased as a result of the favourable conditions for adaptation and metabolic activities by microorganisms when exposed to hydrocarbon pollution (Chikere *et al.,* 2019). The bacterial load was higher in the sample from BD3 than in the other samples, which could be due to the intensity of the contamination, the availability of nutrients, and/or the environment (e.g., soil aeration and moisture content). In all the polluted samples the HUB counts have been higher than the THB counts, which indicates a good selective enrichment of the hydrocarbon-degrading microorganisms. The result indicates that exposure to petroleum hydrocarbons for a long time promoted the development of specialized bacterial communities that could use hydrocarbons as carbon and energy sources. Hydrocarbon-utilizing bacteria have catabolic enzymes like oxygenases and dehydrogenases, which help in the degradation of aliphatic and aromatic hydrocarbons into simpler metabolites (Varjani *et al.,* 2025). The high HUBs recorded in BD1 (8.8×10 5 CFU/g) indicate the high level of adaptation of the microorganisms in the contaminated soil, as well as the high biodegradation potential of the soil. The relatively lower level of THB in the control soil is also supportive of the effects of hydrocarbon contamination on the population dynamics of microorganisms. Hydrocarbon degraders are less dominant in

the microbial communities in uncontaminated soils because hydrocarbons are not the primary carbon source (Atlas & Hazen, 2011). In polluted environments, on the other hand, metabolically diverse bacteria that can tolerate toxic hydrocarbons as well as the associated heavy metals are selected. Polluted soils are ecologically important since HUB is prevalent in these soils, and natural attenuation processes are also important in them. Bacterial strains of indigenous origin play a key role in bioremediation as they can break down petroleum compounds into less toxic products like carbon dioxide, water and biomass (Das & Chandran, 2011). A recent study (Varjani *et al.,* 2025) has shown that microbial communities in soils can be more effective in biodegrading hydrocarbons than in uncontaminated soil due to their adaptation to the hydrocarbons over a period of time. Therefore, in this study, the high counts of HUB obtained indicate that the polluted soils have high intrinsic bioremediation potential.

Hydrocarbon Degradation Efficiency

The bacterial isolates from the oil-polluted soils are found to be different in terms of their potential to degrade the hydrocarbons, as evident from the result obtained from the 2,6-dichlorophenolindophenol assay (DCPIP). DCPIP is an artificial electron acceptor that can be employed to reliably track microbial oxidation of hydrocarbons, being reduced from blue to colourless (Hanson *et al.,* 1993). The gradual appearance of positive reactions (+) over time suggests that the isolates utilized hydrocarbons in their metabolic process. The 14 isolates showed a positive reaction after 24 hours and remained positive for the rest of the incubation period, with J.D15 and J.D16 achieving a positive reaction after 48 hours and remaining positive throughout the incubation period. Proposes a fast metabolism of adaptation and efficient enzymatic oxidation of petroleum hydrocarbons from early reduction of DCPIP. Generally, fast degraders are associated with an oxygenase and highly active degradation enzymes in bacteria that can start the degradation process early in the degradation of hydrocarbons (Varjani *et al.,* 2025). The isolates might thus have high catabolic abilities and high bioremediation potential. Isolates JD4 demonstrated positive activity at the 96-hour mark and JD3, JD6, JD7, JD8, JD11, JD12 and JD13 demonstrated positive activity at 120 hours. These isolates may be regarded as moderate degraders as a longer adaptation period before active hydrocarbon metabolism was noticed. However, the reduction of DCPIP can be delayed due to the nature of the hydrocarbon substrates, induction time of the enzyme and/or tolerance of the enzymes to toxic compounds found in crude oil (Mekonnen *et al.,* 2024). Several isolates such as JD1, JD2, JD5 and JD9 were only positive after 144 h, suggesting relatively slow degradation rates. Slow degraders may have fewer enzymes to metabolize hydrocarbons or may have developed a preference for the use of simpler fractions of hydrocarbons after an extended period of acclimatization. One notable exception was the isolate JD10 that gave a positive response at 120 h and then a negative response at 144 h. This could be due to depletion of easily available carbon fractions leading to a reduction in metabolism over time, or when toxic metabolic waste products build up to stop further metabolism. This behaviour indicated that JD10 had a lower physiological resilience and reduced long-term substrate utilization efficiency compared to the other isolates (e.g. JD15 and JD16) that exhibited positive response throughout the incubation period. It was interesting that JD14 was negative over the entire 144-hour incubation period, suggesting that the hydrocarbons in the assay medium were not metabolized by JD14. It indicates either that the isolate does not have the enzymes for oxidizing hydrocarbons or that they may not use the substrate utilized in

the experimental conditions. JD10 on the other hand, showed a positive reaction at 120 hours and turned negative at 144 hours. This unusual response could be a sign of temporary metabolic activity due to reduced available substrates. This predominance of positive reactions is a result of the adaptation of the microorganisms to the contaminated environment, which was exposed to hydrocarbons for a long time. The ability of the rapid degraders of this study, especially JD15 and JD16, shows great potential for future bioremediation efforts in ameliorating oil-contaminated soils.

Heavy metal tolerance and implications

Iron (Fe) tolerance assay results show different degrees of resistance of the bacterial isolates retrieved from the oil-contaminated soils. The growth diameters ranged from 1.0 mm to 2.0 mm across Fe concentrations (0.1–0.4 mM) which suggests that most isolates were capable of surviving and sustaining metabolic activity during iron stress conditions. Some isolates (JD10, JD15) showed consistently high growth diameters in all Fe concentrations, suggesting high tolerance to Fe exposure. The growth was consistent, indicating efficient metal resistance mechanisms like extracellular polymeric substance production that neutralizes metal toxicity, intracellular sequestration and metal efflux system (Igiri *et al.,* 2018). These bacteria were able to grow at higher Fe concentrations suggesting significant physiological adaptation to contaminated environments where they may be useful in bioremediation processes with co-contamination of hydrocarbons and heavy metals. The other isolates, JD4, JD6 and JD7, were also relatively tolerant, growing 2.0 mm at several concentrations. This rise in tolerance could be due to environmental exposure to hydrocarbons containing metals that have selected for microorganisms resistant to oxidative stress and metal toxicity. Interestingly, some isolates showed stable growth at all concentrations (JD1, JD9 and JD14) with minor variations in the inhibition zones. This stability implies moderate resistance and effective regulation of intracellular iron. This tolerance of iron among the observed iron isolates has ecological and biotechnological significance. The ability of microorganisms to withstand high concentrations of heavy metals and to degrade hydrocarbons is especially desirable for the treatment of multi-metal and multi-hydrocarbon contaminated sites. Heavy metals can have inhibitory effects on microbial growth and enzyme activity in petroleum-polluted soils, thus affecting biodegradation. The copper tolerance assay showed significant differences in tolerance among the bacterial isolates from the oil-polluted soils, which indicated the adaptation of the microbes to the stress of the heavy metals in polluted soils. The resistance of the isolates to the increasing level of Cu concentration indicates the presence of efficient resistance mechanisms developed in the presence of contaminated environments for a longer time duration. The copper-tolerant isolates were the most tolerant, with JD13, JD14 and JD16 being the most tolerant. JD14 and JD16 were resistant up to 0.3 mM Cu and were still growing at 0.4 mM, which was an outstanding tolerance capacity. The resistance may be due to adaptive mechanisms that minimize copper toxicity in the cell. Studies have now demonstrated the ability of hydrocarbon-degrading bacteria isolated from hydrocarbon-polluted environments, such as aquatic and soil samples, to be tolerant of the same metals, as a result of long-term selection in co-contaminated environments (Ashade *et al.,* 2026). Isolates JD1, JD6, JD8, and JD10 also exhibited good resistance at lower copper levels and measurable resistance at higher levels. The progressive toxic effects of increasing Cu concentration are reflected in the increasing inhibition zones observed. Ironically, however, the fact that these isolates continue to survive means that there is considerable physiological adaptation and stress tolerance. Most of the isolates (JD2, JD3, JD4, JD5, JD7, JD9, JD11, JD12, JD15) had moderate tolerance with inhibition zones

ranging from 1.0 mm to 1.5 mm. Recently, moderate resistance was also observed in hydrocarbon-utilizing bacteria recovered from petroleum-contaminated sites, where long-term exposure to hydrocarbons and heavy metals fosters microbial adaptations and boosts stress-response mechanisms (Al Hoqani *et al.,* 2025). These microorganisms can frequently break down hydrocarbons and are tolerant of toxic metals and could be used in bioremediation applications. A high proportion of resistant and moderately tolerant isolates suggests that the indigenous microbial communities in the oil-contaminated soils are very resilient, indicating adaptive resistance. The high resistance of isolates JD14 and JD16 represents an exceptional resistance in which they may have special genetic factors related to copper detoxification, and thus are considered as potential candidates for future biotechnological and bioremediation studies.

Conclusion

The present study showed that the crude oil contamination in Bere community caused significant changes in the soil physical and chemical properties. The heavy metal levels and hydrocarbon content and also affected the microbial community dynamics. The contaminated soils had higher concentrations of lead, cadmium, iron and copper than the control soil, indicating the long-term effects of petroleum-related activities. In spite of these unfavourable environmental conditions, the indigenous bacteria were detected in the polluted soils, with high counts of total heterotrophic bacteria and hydrocarbon-utilising bacteria. Considerable variation in hydrocarbon-degrading capacity among the bacterial isolates was detected using the DCPIP assay. *Salmonella spp*. (JD15) and *Proteus spp*. (JD16) exhibited the highest and most sustained hydrocarbon degradation activity, suggesting high metabolic adaptation to petroleum-contaminated environments. Additionally, the heavy metal tolerance assays showed that a few isolates had a remarkable tolerance to iron and copper stress, with JD14 and JD16 exhibiting exceptional copper tolerance at various concentrations. The ability of the indigenous bacteria from co-contaminated soils to be able to withstand the presence of heavy metals and degrade hydrocarbons demonstrates the adaptive resistance of these bacteria. Isolates JD15 and JD16 in particular, have great potential for use in future bioremediation programmes for the restoration of degraded ecosystems. Future research should focus on the development and evaluation of microbial consortia comprising hydrocarbon-degrading and heavy metal-tolerant bacteria to enhance bioremediation efficiency under complex contamination conditions. Comprehensive genetic and molecular characterization of promising isolates using whole-genome sequencing, metagenomics, and transcriptomics is necessary to identify the functional genes and metabolic pathways involved in hydrocarbon degradation and metal resistance. This is expected to lead to the development of more effective and sustainable bioremediation strategies.

Declaration of Interest

The authors declare that there are no known competing financial interests, personal relationships, or affiliations that could have appeared to influence the work reported in this manuscript.

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Table 1. Physical and chemical analysis of the soil used for the study.

Site	Sand (%)	Silt (%)	Clay (%)	Textural Class	Moisture content	O.C (%)	pH	TN (%)	Lead (mg/kg)	Cadmium (mg/kg)	Iron (mg/kg)	Copper (mg/kg)
CNTRL	72.22	18.13	1.75	S. L	11.4	3.2	5.6	0.04	10.4	12.6	22.4	12.4
BD 1	67.24	19.84	7.66	S. L	17.4	3.5	6.7	0.02	56.7	68.9	147.4	127.4
BD 2	72.35	17.81	9.84	L.S	23.5	4.3	6.3	0.02	112.5	94.2	124.6	265.4
BD 3	71.22	19.10	1.78	S. L	7.2	3.8	6.5	0.01	143.8	88.7	158.8	205.6
LSD $_{0.05}$					0.80	0.34	0.92	0.010	17.35	13.82	22.01	30.65

Note: CNTRL = Control; BD1, BD2, BD3= polluted samples; O.C = Organic carbon; TN = Total nitrogen; S.L = Sandy loam; L.S = Loamy sand.

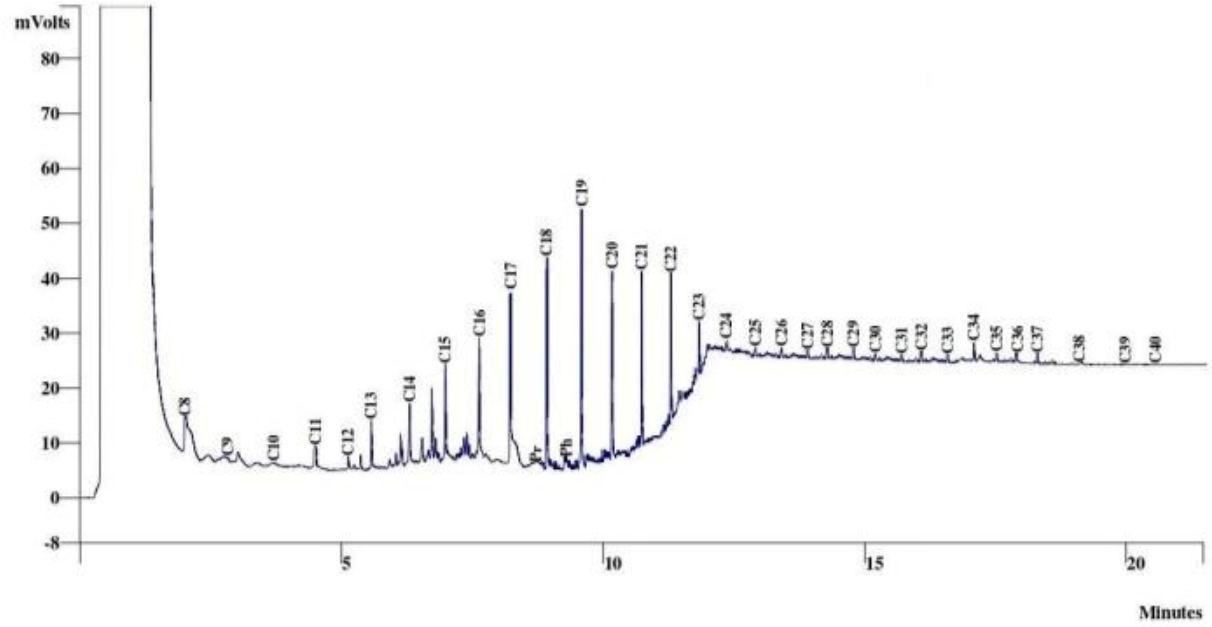

Fig. 2: Chromatograph of the TPH for the control soil

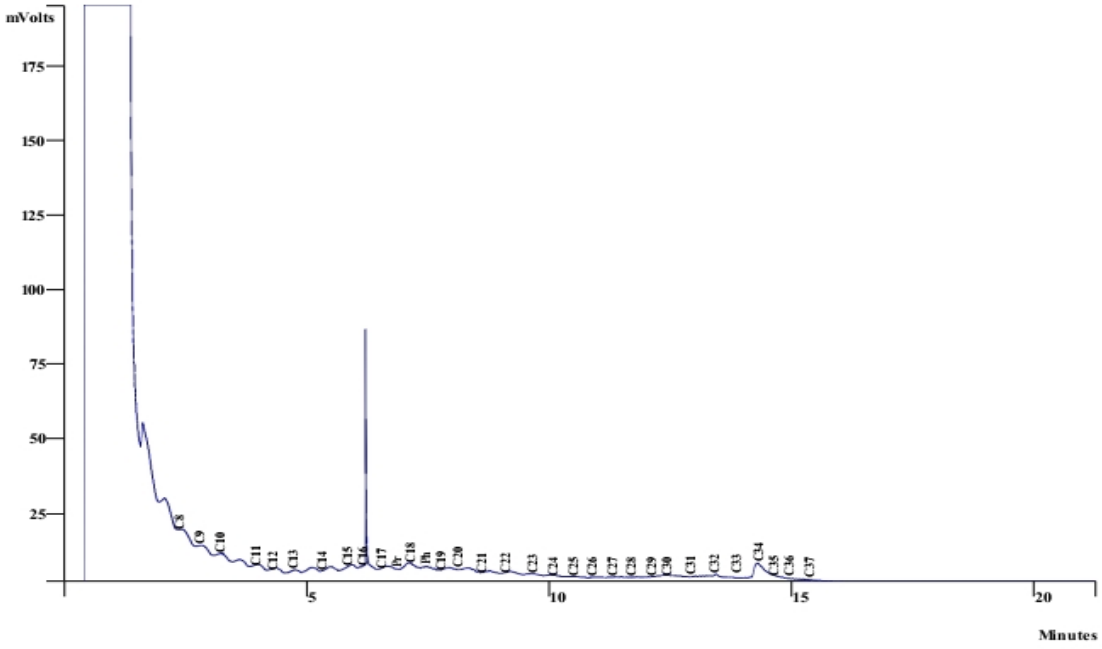

Fig. 3: Chromatograph of the TPH for BD1

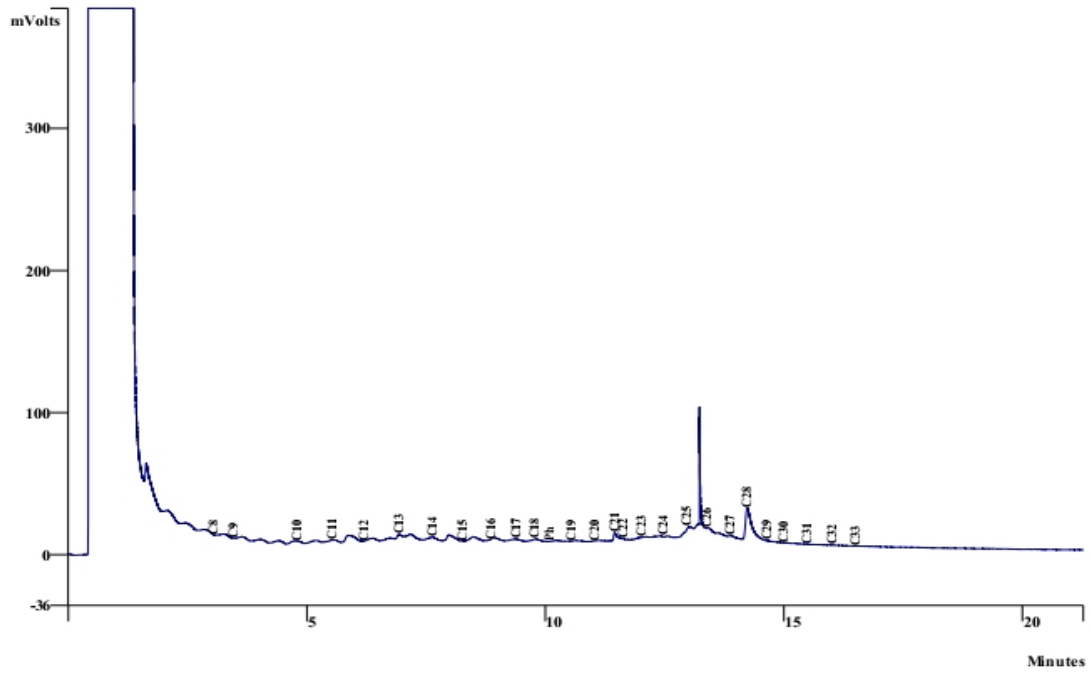

Fig.4: Chromatograph of the TPH for BD2

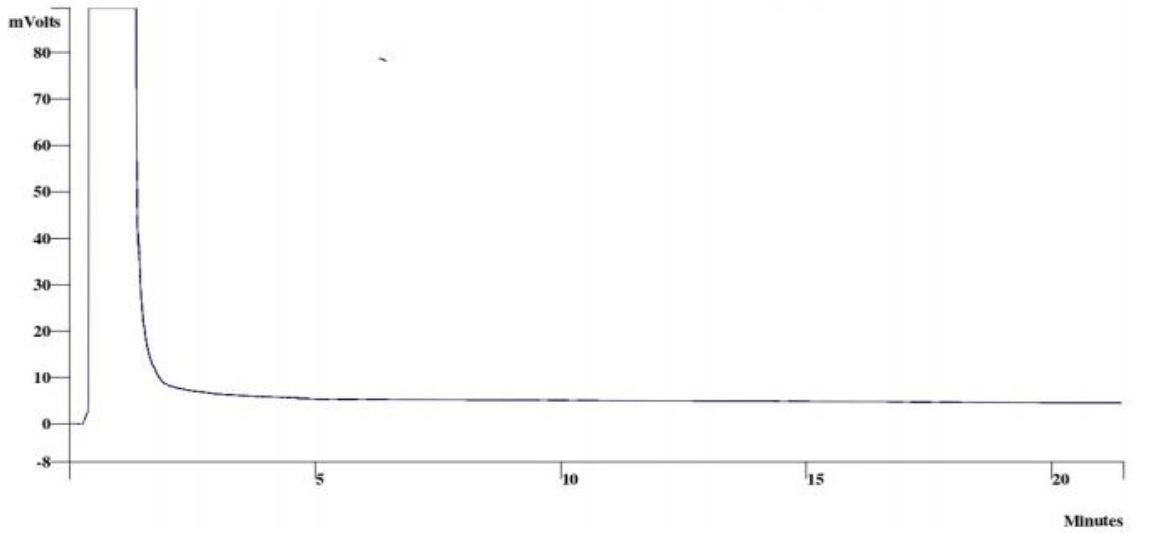

Fig. 5: Chromatograph of the TPH for BD3

Table 2: Mean Total Heterotrophic and Hydrocarbon-Utilizing Bacterial Counts in Soil Samples

Sample	**THB (CFU/g)** ± SD	**HUB(CFU/g)** ± SD
CNTRL	$1.9 \times 10^4 \pm 0.20$	$9.0 \times 10^4 \pm 0.35$
BD3	$3.4 \times 10^5 \pm 0.15$	$6.7 \times 10^5 \pm 0.17$
BD1	$3.0 \times 10^5 \pm 0.27$	$8.8 \times 10^5 \pm 0.19$
BD2	$2.8 \times 10^5 \pm 0.20$	$7.0 \times 10^5 \pm 0.33$

Note: CNTRL = control, THB = Total heterotrophic utilising bacteria, HUB = Hydrocarbon utilizing bacteria, CFU = colony forming unit, SD = Standard deviation

Table 2: Biochemical analysis and identification of bacterial isolates.

Code	Lactose	Glucose	Indole	Citrate	MR	VP	Butt	Slant	H_2S	Gas	Gram	Catalase	Tentative Organism
JD1	−	−	+	−	−	+	B	B	−	−	- rod	+	*Pseudomonas spp.*
D2	−	−	+	+	−	+	B	B	−	−	- rod	+	*Enterobacter spp.*
JD3	−	+	+	−	−	+	A	B	−	−	- rod	+	*Escherichia coli*
JD4	+	+	+	−	+	+	A	B	−	−	+cocci	+	*Staphylococcus spp*
JD5	−	+	+	−	−	+	A	A	−	−	- rod	−	*Proteus spp.*
JD6	−	+	+	−	+	+	A	B	−	−	- rod	+	*Escherichia coli*
JD7	−	+	+	−	−	+	A	B	−	−	+cocci	−	*Streptococcus spp.*
JD8	−	+	−	−	−	+	B	B	−	−	- rod	+	*Pseudomonas spp.*
JD9	+	+	−	−	−	+	B	B	−	−	- rod	−	*Klebsiella spp.*
JD10	−	+	−	−	−	−	A	B	−	−	- rod	+	*Pseudomonas spp.*
JD11	+	+	−	−	−	+	A	A	−	−	- rod	−	*Enterobacter spp.*
JD12	+	+	−	−	−	−	A	B	−	−	- rod	+	*Pseudomonas spp.*
JD13	−	+	−	+	−	+	B	B	−	−	- rod	−	*Enterobacter spp.*
JD14	+	+	−	−	−	+	B	B	−	−	- rod	+	*Klebsiella spp.*
JD15	+	+	+	+	−	−	B	B	−	−	- rod	+	*Salmonella spp.*
JD16	−	+	−	+	+	−	A	B	+	−	- rod	+	*Proteus spp.*

Note: JD = different isolates; + = Positive reaction; − = Negative reaction; A = Acid; B = Alkaline reaction; (Triple Sugar Iron Agar); +cocci = Gram-positive cocci; -rod = Gram-negative rod

Table 3: DCIP ASSAY (2,6- dichlorophenolindophenol)

Code	24 hrs	48 hrs	72 hrs	96 hrs	120 hrs	144 hrs
JD1	−	−	−	−	−	+
JD2	−	−	−	−	−	+
JD3	−	−	−	−	+	+
JD4	−	−	−	+	+	+
JD5	−	−	−	−	−	+
JD6	−	−	−	−	+	+
JD7	−	−	−	−	+	+
JD8	−	−	−	−	+	+
JD9	−	−	−	−	−	+
JD10	−	−	−	−	+	−
JD11	−	−	−	−	+	+
JD12	−	−	−	−	+	+
JD13	−	−	−	−	+	+
JD14	−	−	−	−	−	−
JD15	−	+	+	+	+	+
JD16	−	+	+	+	+	+

Note: hrs = hours;

Table 4: Tolerance assay for Fe (mm) growth diameter.

Code	Fe 0.1	Fe 0.2	Fe 0.3	Fe 0.4
JD1	1.4	1.5	1.5	1.5
JD2	1.6	2.0	1.5	1.5
JD3	1.5	1.3	1.5	1.2
JD4	2.0	2.0	2.0	1.5
JD5	1.2	2.0	1.5	1.5
JD6	1.5	1.5	2.0	2.0
JD7	2.0	1.5	2.0	1.5
JD8	1.5	2.0	1.0	1.5
JD9	1.5	1.5	1.5	1.5
JD10	2.0	2.0	2.0	2.0
JD11	1.9	2.0	1.3	1.5
JD12	1.5	1.7	1.0	1.0
JD13	1.5	1.5	1.5	2.0
JD14	1.4	1.5	1.5	1.5
JD15	2.0	2.0	2.0	2.0
JD16	1.5	1.0	1.5	1.0

Table 5: Tolerance assay for Cu (mm) growth diameter

Code	Cu 0.1	Cu 0.2	Cu 0.3	Cu 0.4
JD1	Resistant	2.0	2.0	2.0
JD2	1.5	1.5	1.5	1.5
JD3	1.0	1.5	1.5	1.5
JD4	1.0	1.5	1.5	1.5
JD5	1.0	1.5	1.5	1.5
JD6	Resistant	1.5	1.7	1.7
JD7	1.0	1.5	1.5	1.5
JD8	Resistant	1.5	1.5	1.5
JD9	1.0	1.5	1.5	1.5
JD10	Resistant	1.5	1.5	1.5
JD11	1.0	1.5	1.5	1.5
JD12	1.5	1.5	1.5	1.5
JD13	Resistant	Resistant	1.5	1.5
JD14	Resistant	Resistant	Resistant	1.0
JD15	1.5	1.5	1.5	1.5
JD16	Resistant	Resistant	Resistant	1.5

Note: JD 1 – JD 16 = Different bacterial strains; Resistant = No observable inhibition