FUDMA Journal of Sciences (FJS)
ISSN online: 2616-1370
ISSN print: 2645 - 2944
Vol. 10 No. 8, April, 2026, pp 76 – 86
DOI: https://doi.org/10.33003/fjs-2026-1008-5048

PRELIMINARY APPROACH TO REIMAGINING OF POLLUTED SOIL ECOSYSTEM: IDENTIFICATION AND UTILIZATION OF AUTOCHTHONOUS HYDROCARBON DEGRADING BACTERIA

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ABSTRACT

Crude-oil Pollution negatively impacts the ecosystems. This study sought to recover crude-oil impacted soil ecosystem, by isolating, screening and identifying indigenous bacteria in a microcosm experiment. Samples of agricultural soil, were thoroughly blended with crude-oil and allowed to equilibrate for three weeks. Physiochemical parameters (pH, Moisture Content, Electrical Conductivity, Heavy Metals, Polycyclic Aromatic Hydrocarbons (PAHs) and Total Petroleum Hydrocarbons (TPH) of the control and contaminated soils were assessed using standard laboratory methods and Gas Chromatography-Mass Spectrometry (GC-MS). Hydrocarbon-utilizing bacteria (HUB) were isolated from the impacted soil using Bacto Bushnell Haas broth supplemented with 1% crude-oil as the nutrient medium. Isolates' Morphological, Biochemical and molecular characterization, as well as degradation screening were done. Physical parameters for the control and impacted soils differed significantly ($P<0.05$). The soil sample had a mean value of 47.5 x 10^9 cfu/ml count for Total Heterotrophic Bacteria. Bacteria isolates characterized, belonged to the genera; *Klebsiella, staphylococcus, Enterobacter, Acinetobacter, Aeromonas and Kurthia.* The population of HUB consisted *Staphylococcus spp.* (30%), *Klebsiella spp.* (20%), *Aeronomas spp.* (10%), *Enterobacter spp.*, *Acinetobacter spp.* (10%), (10%) and *Kurthia spp.* (20%). *Acinetobacter spp.* showed the highest biodegradation capacity; with 2,6-dichlorophenol indophenols (DCPIP) indicator. *Kurthia spp*, a novel hydrocarbon degrader, was discovered. The study found that different isolates of native bacteria differ in degradation abilities and are effective for remediating polluted soils. The findings promote sustainable environmental management practices by harnessing naturally occurring bacteria for remediation, and supports the development of green technologies that align with global sustainability goals.

Keywords: Bioremediation, Biotechnology, Ecosystem Restoration, Hydrocarbon Utilizing Bacteria, Isolates' Characterization

INTRODUCTION

The primary constituents of crude oil are a complex mixture of non-hydrocarbon chemicals (resins and asphaltenes) and hydrocarbon molecules (aliphatic and aromatic) (Kanungo *et al*., 2024). These substances have drawn a lot of attention globally for some time due to their toxicity, hazardous nature and their effects on human health (Sayed *et al*., 2021). Environmental pollution from crude oil is one of the unavoidable effects of the growing need for energy sources. Significant ecological effects result from this type of oil pollution, which frequently happens during oil exploration, transportation, production, storage maintenance, and unintentional spills (Hassan *et al.*, 2025). Nigeria has one of the greatest numbers of oil spill accidents since 1970 and is the sixth-largest producer of crude oil worldwide (Akinwumiju *et al.*, 2020). Over 50 years, an estimated 1.5 million tons of oil have been leaked into the Niger Delta ecosystem. This amounts to fifty times greater than the estimated amount of oil that Exxon Valdez spilled in 1989, in Alaska (Faboya *et al*., 2023). The Niger Delta area continues to be one of the world's most significant hydrocarbon provinces, with estimated reserves of 180 trillion cubic feet of natural gas and 37 billion barrels of oil (Energy Information Administration, 2015). Oil refineries and some other processing industries are located there. There is no denying that crude oil contamination in the area is severe. The persistent oil spills in the area are the primary cause of contamination to both terrestrial and aquatic ecosystems (Ahiamadu *et al*., 2021). Despite the well-documented impacts of crude-oil pollution in the Niger Delta, there is still limited understanding of the specific bacterial strains in this region that can effectively degrade hydrocarbons.

The Niger Delta is home to some of the world's most important wetland and marine habitats, ranking among the top ten. However, due to unsustainable oil extraction activities, the region has suffered substantial petroleum damage, making it one of the most severely affected eco-systems worldwide (Numbere *et al*., 2024). In relation to their chemical makeup and the capacity of microorganisms to degrade them, hydrocarbons interact with both the environment and microorganisms to determine the fate of the contamination. There is an excellent probability that the environment will support a viable and active population of microorganisms that use hydrocarbons, provided that the contaminated site has the necessary features for environmental variables that affect microbial activities in the absence of metabolic inhibitors (Kebede *et al*., 2021). Necessity for the clean-up oil-polluted locations has emerged as a major environmental challenge, given the amount of oil that enters the Niger Delta ecosystem, particularly in farmlands and aquatic environment (Okoh *et al*., 2020). The capacity of bacteria to degrade hydrocarbons has drawn increasing attention in contemporary studies since some microbes are able to mineralize hydrocarbon components into environmentally benign molecules like carbon dioxide and water (Kalia *et al*., 2022). This makes them valuable for bioremediation efforts, where they can be employed in the remediation oil spills, contaminated soil, and other hydrocarbon-based pollutants. By breaking down these compounds, bacteria can help restore natural environments and mitigate the ecological damage caused by hydrocarbon pollution. The primary method of removing hydrocarbons from the environment is by biodegradation by microbes, which is regulated by the physicochemistry of the hydrocarbons, the environment, the existence of catabolically active bacteria and bioavailability (Kanwal *et al*., 2022). However, there are much bottle-necks in accessing the local communities and spillage sites for sampling, research and monitoring. This, was overcome by getting fresh soils from Niger Delta agricultural soils and mixing with crude-oil sourced from the government agency for the Petroleum Resources and set up a microcosm experiment following international established guide lines and best practices for bioremediation (Bala *et al*., 2022).

Soil microbial communities are essential for plant nutrition, ecosystem stability, and the transport of organic materials (Osburn *et al*., 2023). Diverse techniques and approaches have been used to remove petroleum pollutants from the environment. A growing amount of attention has been paid to bioremediation in the recent years as one of the more economical and environmentally beneficial methods (Alegbeleye *et al*., 2017; Umar *et al*., 2025). Petroleum-contaminated environments can be colonized by diverse microorganisms, including bacteria, yeasts, fungi, and archaea, which are capable of utilizing hydrocarbon pollutants as carbon and energy sources for growth. The study aimed at isolating, characterizing and identifying hydrocarbon utilizing bacteria from crude-oil contaminated soil using morphological, biochemical, molecular and biotechnological methods. Efficient HUB will be optimized for the amelioration of environmental damage in oil-spill regions and ecosystem restoration. This will offer a cost-effective and environmentally friendly method for bioremediation, reducing the need for expensive chemical treatments or physical removal of contaminated soil. Reducing pollutants in soil and water can decrease health risks associated with exposure to toxic compounds, leading to lower healthcare costs and improved quality of life for local populations as well as enhancing local economies dependent on agriculture and natural resources, ultimately leading to improved livelihoods for communities affected by oil pollution.

MATERIALS AND METHODS

Collection and Preparation of Samples

Soil was collected 0 to 15 cm below soil surface level with the aid of sterilized soil auger into clean zip-lock paper bags. Five samples were collected at different parts of the Agricultural land of Federal University of Petroleum Resources Effurun (FUPRE), as shown in figure 1, and mixed for randomisation, while the crude-oil used for this experiment was obtained from the Department of Petroleum resources (DPR), Delta State, Nigeria. The soil was sieved with 2mm mesh to remove debris and larger particles. The soil was then contaminated in the ratio of 3mls of crude-oil to 1kg of soil (0.3%v/w) and bulked for homogeneity for 3 weeks, under ambient conditions. The homogenised soil was carried to the laboratory for further analysis. However, the soil for the control was left uncontaminated. The soil was maintained under ambient temperature in the laboratory until further analysis. Sterile zip-lock paper bags were used to prevent contamination from bye-products of crude-oil like polythene. Polythene was eventually used as a water proof for the paper bags.

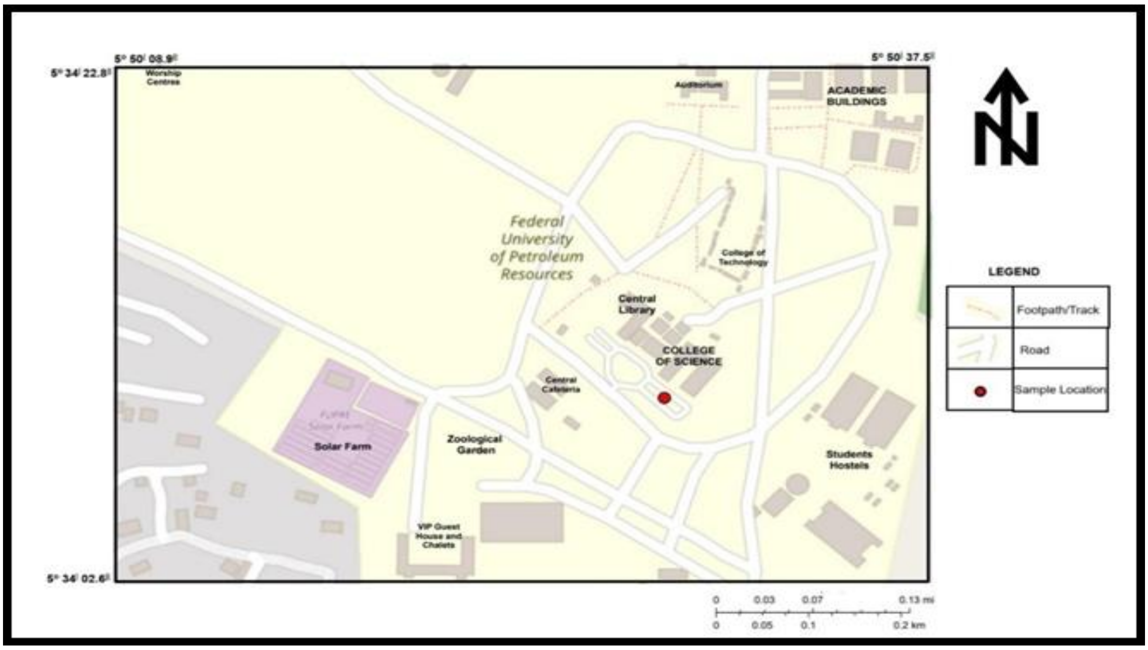

Figure 1: Map of the Study Site (Federal University of Petroleum Resources, Effurun)

Analytical Processes

Soil pH was measured using a Jenway 3510 pH benchtop meter, calibrated with standard buffer solutions. The soil sample was prepared in a 1:1 (w/v) soil-to-distilled water suspension (Hopton *et al*., 2025). This ratio was selected to minimize excessive dilution of hydrogen ions, thereby improving the accuracy of pH determination. As the water content increased beyond this ratio, the concentration of H$^+$ ions decreased, potentially resulting in artificially high pH values (Khadka *et al*., 2021). Measurement was then taken.

For the Electrical conductivity (EC), soil sample was mixed with distilled water (1:1) ratio. Myron L 512M5 DS Conductivity Meter was then employed in determining the ionic content and soil's salinity.

Moisture content (MC) was determined by oven drying the soil at 105^{o}C until constant weight was attained. MC was calculated using the formula below:

Moisture Content =$\left(\frac{Wet\ weight - Dry\ weight}{Dry\ \ weight} X\ 100\right)$

Soil Sample Extraction Procedure for TPH and PAHs

Extraction was done using an ultrasonic extractor (Elmasonic S40H, Germany) with hexane and dichloromethane in the ratio 1:1. Analysis and Quantification of TPH and PAHs were done according to Falodun *et al*. (2023). The GC-MS instrument used in the analyses was a GC-Agilent Technologies 7890A connected to a splitless injector with an HP-5MS UI capillary column (30 m long, 250 µmi.d ×0.25 µm film thickness) coupled with a mass spectrophotometer detector (Agilent 5975C VL MSD with Triple-axis Detector) for the determination of PAHs and TPH. Using an Agilent Technologies 7693auto sampler injector, the PAHs levels in the samples were measured. For quantification using the GC-MS, a five-point calibration curve was used. With a front inlet temperature of 250^{o}C, an initial oven temperature of 100 ^{o}C, and a final temperature of 320 ^{o}C, the samples were injected (1 µL) in the splitless mode. Helium was the carrier gas, and the flow rate was 1.5 mL/min.

Baseline Characterization of Soil Samples

The soil samples were analysed to characterize its physicochemical properties, like the EC, Total organic carbon, moisture content, pH, phosphate, using standard methods (APHA, 1998). The heavy metal content was determined with the aid of atomic absorption spectrophotometer (AAS) VGP 210 model. Gas chromatograph-mass spectrophotometers (GC-MS) were used for the quantification of polycyclic aromatic hydrocarbons (PAHs) and residual total petroleum hydrocarbons (TPH) from the samples.

Estimation of Heterotrophic Bacteria and Isolation of Degrading Bacteria

The pour plate method was adopted in estimation of the total heterotrophic bacteria (THB) on nutrient agar. Using a Heindolph vortexing machine, 1 g of soil sample was homogenized in 9 ml of sterile normal saline. One milliliter of each serial dilution, a 10-fold procedure, was placed on the agar medium. The plates were incubated for twenty-four hours at 37±2°C. After then, the colony-forming units were counted. This was then followed by the isolation of hydrocarbon degrading bacteria. Bushnell Hass-Agar medium (Sigma-Aldrich, USA) supplemented with 1% crude oil was prepared and sterilised at 121^{o}C and 15 psi for 15 minutes. One gram of each soil sample was suspended in 9 mL of sterile normal saline and agitated vigorously. A 10-fold serial dilution was done, and 1 ml inoculum from the dilutions was inoculated into the petri plates. Pour-plate method was employed and the petri plates were incubated at 37^{o}C for 7 days. The cultured plates were examined after incubation and bacterial colonies were counted and characterised morphologically, according to the method of Shekhar *et al*. (2015).

Testing Crude Oil Removal Efficiency of the Bacterial Isolates

Discreet colonies of various HUB were randomly selected using a sterile inoculating loop and subcultured for purification by streaking onto nutrient agar plates, which were then incubated at 30°C for 24 hours. The individual colonies were primarily identified through biochemical tests as outlined in Bergy's Manual for Determinative Bacteriology. Freshly prepared Bushnell-Hass broth medium supplemented with 1% (v/v) crude oil was sterilised in test tubes, and the isolated bacterial strains were inoculated. The tubes were then incubated for 14 days in a shaker incubator. The growth and crude oil degradation capacity of the individual bacterial strains was measured using UV spectrophotometer at 600 nm wavelength.

The degradation capacity was confirmed using 2, 6-dichlorophenolindophenol (DCPIP) indicator to evaluate the degradation of crude-oil by the different bacteria isolates. The DCPIP was prepared by dissolving 1 g of the DCPIP in 1 litre of sterile distilled water (Okoye *et al*., 2020). Biodegradation was evaluated by the turbidity of Bushnell Haas broth after 14-day incubation at 37 ^{o} C with simultaneous and gradual decolourization of the blue dye DCPIP to colourless. The percentage degradation from the decolourisation assay was calculated from optical density determined by spectrophotometer thus:

% degradation= $\frac{OD(control) - OD(sample)}{OD(control)} \times 100$

Where:

OD (*Control)* = the Optical Density of control,

OD (*sample*) = The Optical Density of the sample.

Identification of Hydrocarbon-degrading bacteria

According to Bergey's Manual for Determinative Bacteriology and related studies, the obtained bacterium isolates were defined based on certain morphological features. A systematic study of the isolated strains was then carried out based on physiological and biochemical properties (Gram reaction, catalase, Urease, Motility, Citrate, Hydrogen Sulphide, Starch, Methyl Red, Voges-Proskauer and sugar fermentation) (Mulet *et al*.,2018). Molecular characterization of the isolated bacterial strains was performed by 16S rRNA sequencing analysis. The forward and reverse primers used were:

5’GAGAGTTTGATCCTGGCTCAG3′; 5′-TACGGTTACCTTGTTACGACTT-3′;5′-AGAGTTTGATCCTGGCTCAG-3′;5′-GGTTACCTTGTTACGACTT-3′;5’AGAGTTTGATYMTGGCTCAG-3′;5′AAGGAGGTGATCCAGCC-3; 5′-AGAGTTTGATYMTGGCTCAG-3′; 5′-GGTTACCTTGTTACGACTT-3′; 5′-AGAGTTTGATCMTGGCTCAG-3′; 5′-TACGGYTACCTTGTTACGACTT-3′

The PCR was performed to amplify the hyper variable region within the 16S rRNA, and the subsequent parameters were applied: two minutes of initial denaturation at 95^{0}C, thirty cycles of denaturation at 95^{0}C for thirty seconds, one minute of annealing at 55^{0}C, one minute of beginning extension at 72^{0}C, ten minutes of final extension at 72^{0}C, and forty degrees Celsius for holding. Sanger sequencing was performed on the purified PCR products using an Applied Biosystems 3130xl genetic analyzer. Using sequencing data from the sequencer, computational analysis of the acquired nucleotide was performed. BioEdit Sequence Alignment Editor was used to identify the consensus sequence. The National Center for Biotechnology Information (NCBI) web platform's nucleotide Basic Local Alignment Search Tools (BLASTn) software was used to analyze sequence similarities. (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Molecular Evolutionary Genetics Analysis X was used to create the phylogenetic tree (MEGA X) (Kumar *et al*., 2018; Ogundolie, 2023).

Data Analysis

The descriptive analysis of the degradation capacities of the Bacterial isolates was presented using Microsoft Excel 2010. Mean of triplicate values, standard deviation, and *p*-values of the physicochemical properties of the soil were determined using the GraphPad Prism 8.0.2 (263). Welch's t-test was used to compare the physicochemical properties between contaminated and control soils due to unequal variances.

RESULTS AND DISCUSSION

Soil Characterisation

The physicochemical properties of the soil samples can be found in Table 1. The parameters evaluated revealed the organic and inorganic contaminants in the soil (Kebede *et al.*, 2021). The soil is classified as saline due to electrical conductivity measurements exceeding 120 µS/cm as prescribed by the Department of Petroleum Resources (DPR) (Ejairu & Okiotor, 2022). Crude-oil contamination affects the soil by increasing its electrical conductivity. Studies have shown that electrical conductivity of soil can significantly rise due to the introduction of high metal ion concentrations from hydrocarbon contamination. Alteration in the EC is a direct result of impact on the soil's physicochemical properties, leading to changes in soil's composition (Ejairu & Okiotor, 2022; Solomon *et al*., 2023). According to Li *et al.* (2016), EC plays a crucial role in the efficiency and activity of hydrocarbon-degrading bacteria, influencing both their metabolic processes and community dynamics. Crude oil contamination hikes the heavy metal concentration of the soil. It has been reported that heavy metal concentrations in oil polluted soils are often above recommended regulatory levels, indicating a high level of pollution and ecological risk (Bankole *et al*., 2024). Otugboyega *et al*. (2023) reported that if pollutants are not adequately remediated, they harm both people and the environment. Contamination might account for low pH of 5.86 recorded in the contaminated soil. Prior research has indicated that the ideal pH range for the biodegradation of hydrocarbons is between 5 and 8 (Samuel-Osamaka *et al.*, 2021). There is a significant difference between all the physicochemical parameters of the contaminated soil and the control; P<0.05, using Welch Test on Graphpad prism, this depicts the toxic effect crude-oil can have on an ecosystem. For hydrocarbon pollutants to biodegrade effectively, nutrients are essential.

Autochthonous Bacterial Characterization

The quantification of culturable total hydrocarbon-utilizing bacteria, as well as total heterotrophic bacteria, was used to ascertain the existence of microbial activity. Soil sample had a mean value of 47.5 x 10^9 cfu/ml. (Okoye *et al.*, 2020; Samuel-Osamaka *et al.*, 2021), noted similar findings. High concentration of organic matter, abundance of nutrients and other ecological parameters can affect optimal activities of soil microorganisms (Zhang *et al.*, 2024). They are crucial to the breakdown and recycling of nutrients and could largely be responsible for the high counts seen in the soil sample. Constant petroleum-based pollution input typically produces an enhanced microbial community that can endure hazardous contamination. The fact that there was a sizeable amount of HUB, indicates that the majority of the microorganisms in the samples are hydrocarbon degraders, able to both resist crude oil quantity and utilize them as carbon source (Yu *et al.*, 2020).

Using crude oil as a carbon source and mineral salt medium (Bushnell Haas Agar), ten pure cultures were able to develop and were identified by morphological (Table 2), biochemical (Table 3), phenotypic (Figures 2 to 7) and molecular (Figures 4 and 5) parameters. Molecular characterization was carried out after degradation screening and sorting of similar genera. PCR amplification was used to determine the taxonomic identities of the bacterial strains, and the resulting 16S rDNA sequences were compared to those in the database. Gene sequencing demonstrated a significant degree of amplicon homology (>85%). Fifty percent of the bacteria in the population of culturable hydrocarbon degraders from the examined soil samples were Gram positive, while the rest 50% were Gram negative; connoting 70% bacilli and 30% cocci. This is similar to autochthonous bacterial characterization report of Okoye *et al.* (2020). The isolates belonged to the general *Klebsiella, Staphylococcus, Enterobacter, Acinetobacter, Aeromonas and Kurthia* as shown in Table 3. This is a representative sampling of the typical heterotrophic bacteria found in the soil. It has been demonstrated that many oil-degrading species from oil-polluted areas are found in these prominent genera that have been isolated, which supports the reports and proof confirming the species to be the most likely the effective degraders in the ecosystem (Kebede *et al*., 2021). Other studies have also shown that these isolates breakdown hydrocarbons (Chattopadhyay *et al*., 2023; Azevedo *et al.*, 2023). According to Ali *et al.* (2018), *Klebsiella* spp. were able to degrade up to 99.53% of kerosene at 10,000ppm concentration under optimal conditions. *Klebsiella* spp., has the ability to degrade hydrocarbons using ortho-and-meta cleavage pathways (Rajkumari *et al*., 2018). Experiments conducted by Zhang *et al*. (2005), where crude oil was exposed to *Pseudomonas aeruginosa* with and without the presence of rhamnolipids, showed a high degradation capacity of the bacteria. Medic *et al.* (2020) and Das *et al.* (2023), reported high capacity of *Pseudomonas* spp. in the degradation of hydrocarbons. *Kurthia* spp. are good degraders of polycyclic aromatic hydrocarbons (Bisht *et al*., 2010). *Acinetobacter* spp. plays a significant role in crude-oil biodegradation. They can extensively degrade Bombay High crude-oil and utilize it as the sole carbon source, achieving a total degradation of 70% within 120 hours (Kumar & De, 2023). Additionally, Acinetobacter spp. SCYY-5, isolated from contaminated oil sludge, showed a high hydrocarbon degradation efficiency for C9-C34 alkanes. The degradation potential reached 69.17% on the third day of culture (Cai *et al*., 2021).

Acinetobacter spp. demonstrates superior hydrocarbon degrading capabilities compared to other bacteria in crude oil -contaminated soils due to several key factors. They have huge metabolic versatility; capable of utilizing wide range of hydrocarbon as carbon sources and effectively breaking down complex compounds found in crude-oil. The genus is known for high resilience to harsh environments; including high salinity and varying pH levels (Sharma *et al*., 2018). Acinetobacter can outcompete other bacteria for resources in contaminated environments due to its bioaugmentation potential (to accelerate the breakdown of contaminants by supplementing existing microbial populations with additional strains that possess specialized metabolic pathways.). Furthermore, *Acinetobacter* spp. are known to produce biosurfactants, which enhance the bioavailability of hydrophobic hydrocarbons, facilitating their uptake and degradation by the bacteria (Kumar & De, 2023).

Degradation Screening

The degradation capacity result is as shown in Figure 8, with SHUB 5 (*Acinetobacter* spp.) showing the highest degrading capacity. This also follows the same trend in colorimetric screening which was confirmed using 2, 6-

dichlorophenolindophenol (DCPIP) indicator in monitoring and evaluating the degree of crude-oil degradation by the various bacteria isolates.

The evaluated degradation capacity of *Acinetobacter* strain means well for its future use in bioremediation. Future studies should explore the scalability of using this bacteria species for remediation in various environmental conditions and investigate the potential for enhancing their degradation capacity through genetic engineering. Efforts can also be geared towards developing a consortium of bioremediation agents from the identified autochthonous bacteria for large scale use in oil spill areas, with potential applications in Niger-Delta region.

Table 1: Soil Characterization

Parameters Dtermined	Contaminated	Control
pH (1:1)	5.86±0.06	6. 34±0.05
Moisture (%)	15.61±0.12	13.47±0.03
EC (µS)	215.3±0.58	170.3±0.58
Heavy Metals (mg/kg)		
Chromium	2.71±0.01	0.34
Cadmium	1.23±0.05	BDL
Nickel	3.15±0.05	0.67±0.06
Iron	2466.3±0.58	1246±1
Copper	56.78±0.01	14.52±0.025
Lead	4.15±.05	0.28±0.01
Manganese	2.58±0.01	2.11±0.01
Zinc	124.33±0.58	72.44±0.04
TPH (mg/kg)	676.67±1.2	34±1
Total PAHs (mg/kg)	167	61

*Values presented represent mean and standard deviation

Table 2: Morphological Characterization of Isolates

Sample	Shape	Margin	Elevation	Colour	Surface	Size (mm)
SWHUB 1	Circular	Entire	Raised	Cream	Smooth	3
SHUB 1	Circular	Entire	Raised	Cream	Smooth	9
SHUB 2	Circular	Entire	Raised	Cream	Smooth	5
SHUB 3	Irregular	Undulate	Flat	Cream	Rough	4
SWHUB 3	Circular	Entire	Raised	Cream	Smooth	3
SWHUB 5	Circular	Entire	Raised	Cream	Smooth	3
SHUB 5	Irregular	Undulate	Raised	Cream	Rough	5
SHUB 7	Circular	Entire	Raised	Brown	Smooth	7
SW8B	Irregular	Undulate	Flat	Cream	Rough	3
SW11A	circular	entire	Raised	White	Smooth	4

Table 3: Biochemical Characterisation of Bacterial Isolates

Isolates	Gram rxn	Catalase	Indole	Motility	Urease	Citrate	H_2S	Starch	Mr	Vp	SUGAR FERMENTATION					Suspected organism
											Maano	Lact	Gluc	Mannit	Gas	
SWHUB 1	+/cocci	+	-	+	-	+	-	-	+	-	+	+	+	+	+	*Staphylococcus* sp.
SHUB1	-/bacilli	+	-	-	-	+	-	-	-	-	+	+	+	+	+	*Klebsiella* sp.
SHUB2	-/bacilli	+	+	-	+	+	-	+	-	-	+	+	+	+	+	*Klebsiella* sp.
SWHUB 3	+/cocci	+	+	+	+	+	-	+	+	-	+	+	+	+	+	*Staphylococcus* sp
SHUB3	-/bacilli	+	-	+	+	+	-	+	+	-	+	+	+	+	+	*Aeromonas* sp.
SHUB5	-/bacilli	+	-	-	-	+	-	-	-	-	+	+	+	-	-	*Acinetobacter* sp.
SWHUB 5	+/cocci	+	+	-	-	+	-	+	-	-	+	+	+	+	+	*Staphylococcus* sp
SHUB7	-/bacilli	+	-	+	+	+	-	-	-	-	+	+	+	+	+	*Enterobacter* sp.
SW8B	+/bacilli	+	+	+	+	+	-	+	-	-	+	+	+	+	+	*Kurthia* sp.
SW11A	+/bacilli	+	+	-	+	-	-	+	+	-	+	+	+	+	+	*Kurthia* sp.

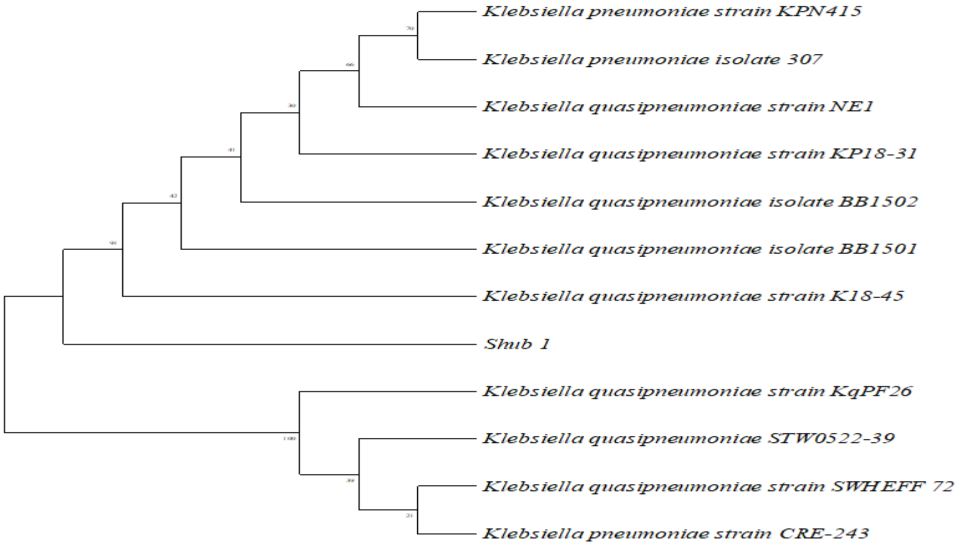

Figure 2: SHUB 1

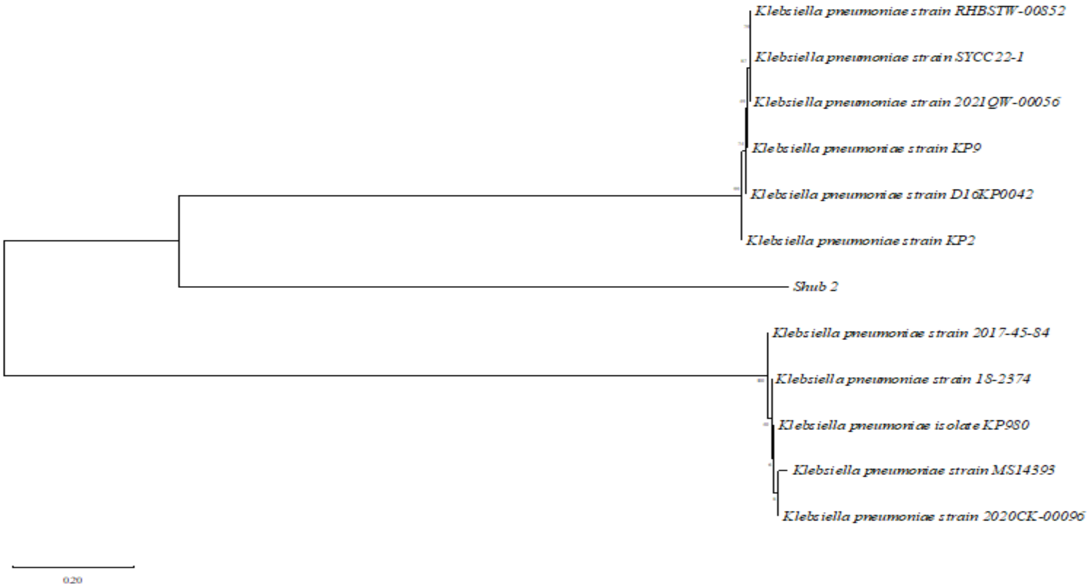

Figure 3: SHUB2

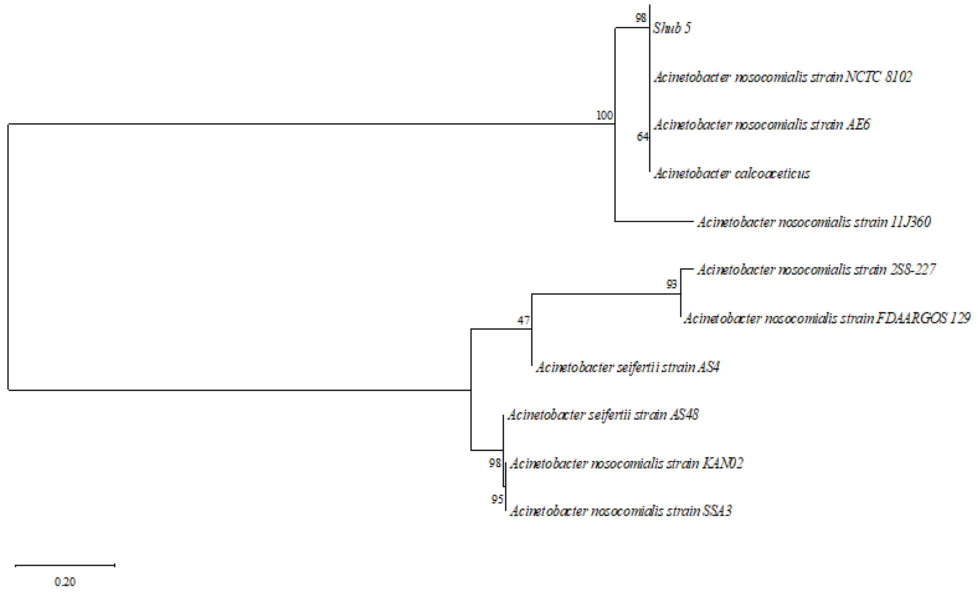

Figure 4: SHUB5

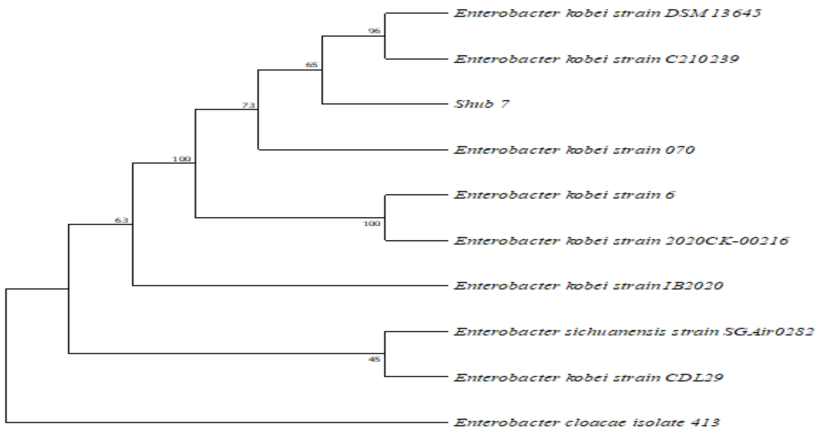

Figure 5: SHUB 7

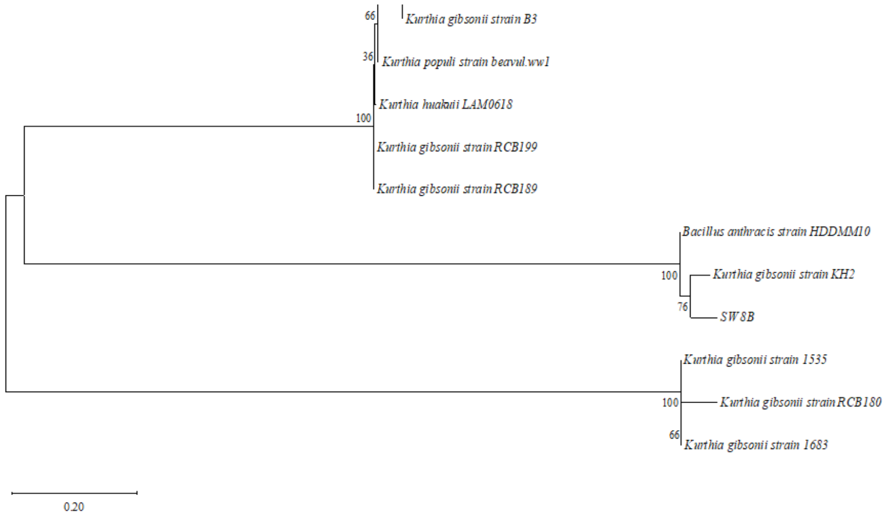

Figure 6: SW8B

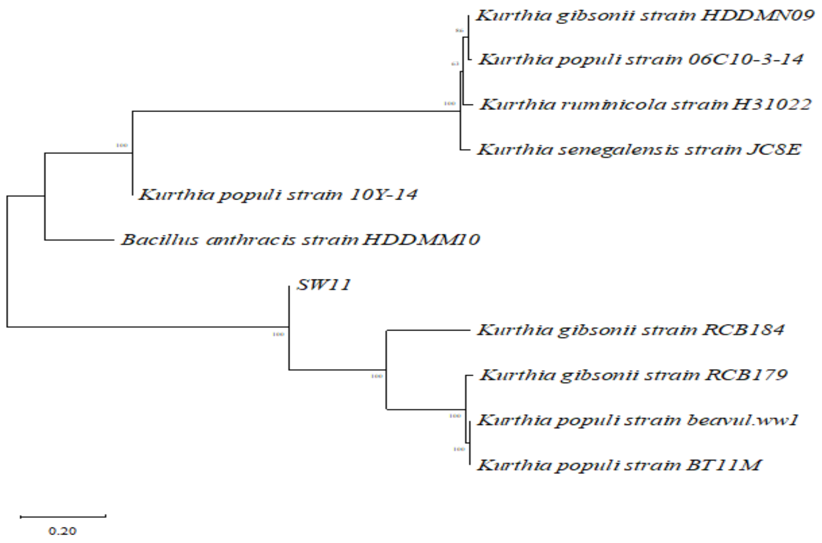

Figure 7: SW11A

Table 4: Result of Molecular Identification

ORGANISM	PERCENTAGE ID	PREDICTED ORGANISM	GENBANK ACCESSION
SHUB 1	99.46%	*Klebsiella quasipneumoniae*	CPO45641.1
SHUB 2	99.66%	*Klebsiella pneumoniae*	CPO54303.1
SHUB 5	100.00%	*Acinetobacter nosocomialis*	CPO45560.1
SHUB 7	99.45%	*Enterobacter kobei*	CPO50073.1
SW8b	99.45%	*Kurthia gibsonii*	EU881980.1
SW 11A	99.32%	*Kurthia gibsonii*	EU881980.1

Table 5: Blasting Prediction

SAMPLE	BLAST PREDICTION
SHUB 1	*Klebsiella quasipneumoniae*
SHUB 2	*Klebsiella pneumoniae*
SHUB 5	*Acinetobacter nosocomialis*
SHUB 7	*Enterobacter kobei*
SW8b	*Kurthia gibsonii*
SW11A	*Kurthia gibsonii*

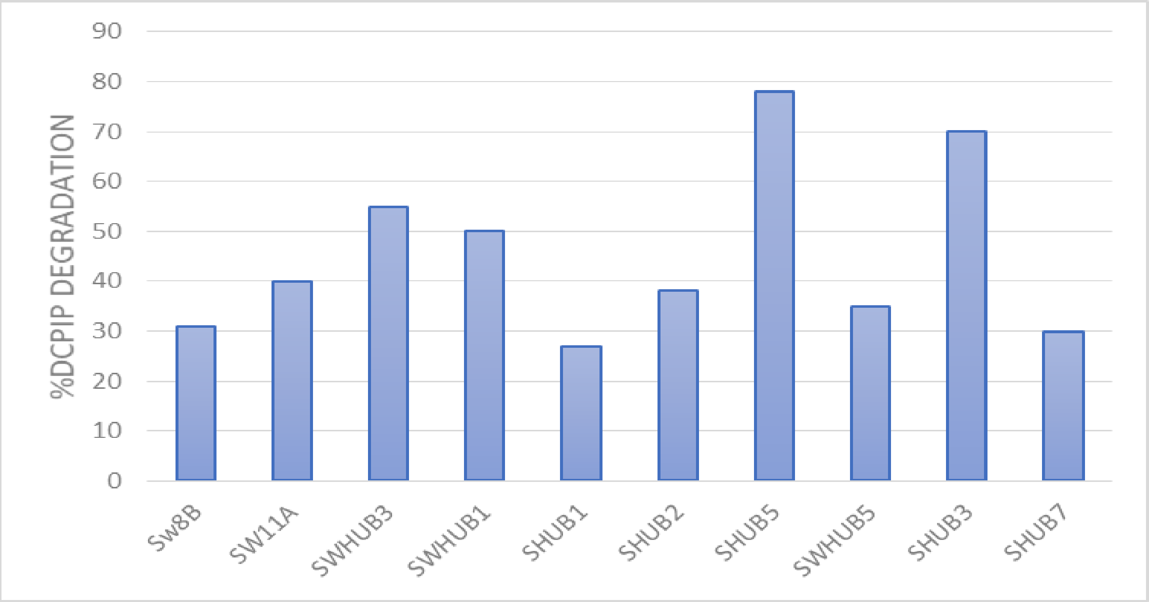

Figure 8: Degradation Screening

CONCLUSION

This study identifies key petrophilic bacterial species that can significantly degrade hydrocarbons in crude-oil contaminated soils, offering a potential solution for mitigating environmental damage in oil spill areas, justifying the aim of the research. Bacteria isolates identified belonged to the general *Klebsiella, staphylococcus, Enterobacter, Acinetobacter, Aeromonas and Kurthia*. Among all the bacteria isolated and identified using the crude oil enrichment technique, *Staphylococcus spp*. (30%) had the highest percentage of the population of degrading bacteria. *Acinetobacter spp*. showed the highest biodegradation capacity with highest Optical density and the best to decolourize 2,6 dichlophenol from blue to colourless. A novel specie of bacteria; *Kurthia,* was isolated from Niger-Delta soil, contributing to the field of biotechnology. This may lead to the development of new biotechnological applications and products aimed at environmental cleanup, creating opportunities for innovation and economic growth in the bioremediation sector. It is therefore recommended that further research should focus on optimizing the use of *Acinetobacter spp.* and its consortium in bioremediation processes and testing its effectiveness in diverse environmental settings. The study offers a cost-effective and environmentally friendly method for bioremediation, reducing the need for expensive chemical treatments or physical removal of contaminated soil. The findings promote sustainable environmental management practices by harnessing naturally occurring bacteria for remediation, and supports the development of green technologies that align with global sustainability goals. Effective bioremediation can lead to quicker recovery of contaminated lands, allowing for the restoration of agricultural and ecological functions. This can enhance local economies dependent on agriculture and natural resources, ultimately leading to improved livelihoods for communities affected by oil pollution.

REFERENCES

Kanungo, J., Sahoo, T., Swain, L. P., & Behera, I. D. (2024). Toxicity of persistent hydrocarbon pollutants, sources and sustainable remediation process. In *Impact of petroleum waste on environmental pollution and its sustainable management through circular economy* (pp. 39-65). Cham: Springer Nature Switzerland.

Sayed, K., Baloo, L., & Sharma, N. K. (2021). Bioremediation of total petroleum hydrocarbons (TPH) by bioaugmentation and biostimulation in water with floating oil spill containment booms as bioreactor basin. *International Journal of Environmental Research and Public Health, 18*(5), 2226. https://doi.org/10.3390/ijerph18052226

Hasan, A. M., Nassar, A. M., Nassar, I., Maysour, N., & Abdel-Raouf, M. E. (2025). Environmental impacts for the transportation of crude oil and refined products. *Environmental Science and Pollution Research, 32*(30), 17869–17896.

Akinwumiju, A. S., Adelodun, A. A., & Ogundeji, S. E. (2020). Geospatial assessment of oil spill pollution in the Niger Delta of Nigeria: An evidence-based evaluation of causes and potential remedies. *Environmental Pollution, 267*, 115545.

Faboya, O. L., Sojinu, O. S., & Otugboyega, J. O. (2023). Preliminary investigation of polycyclic aromatic hydrocarbons (PAHs) concentration, compositional pattern, and ecological risk in crude oil-impacted soil from Niger Delta, Nigeria. *Heliyon*, *9*(4), e15508. https://doi.org/10.1016/j.heliyon.2023.e15508

Energy Information Administration. (2015). *Country analysis brief: Nigeria*. http://www.eia.gov/beta/international/analysis_includes/countries_long/Nigeria/nigeria.pdf

Ahiamadu, N. M., Nwaogazie, I. L., & Momoh, Y. O. L. (2021). Assessment of polycyclic aromatic hydrocarbons in crude oil spill sites in Emohua Local Government Area of Rivers State, Nigeria. *American Journal of Engineering Research, 10*(6), 175–187.

Numbere, A. O., Jude, K., Chuku, S. B., Uzoma, M. C., Obanye, C., Ohia, P., ... & Dick-Abbey, I. W. (2024). The impact of unsustainable development and climate change on agriculture and forestry in Nigeria: Predictions, solutions, and management. In *Ecosystem management: Climate change and sustainability* (pp. 541–579).

Kebede, G., Tafese, T., Abda, E. M., Kamaraj, M., & Assefa, F. (2021). Factors influencing the bacterial bioremediation of hydrocarbon contaminants in the soil: Mechanisms and impacts. *Journal of Chemistry*, *2021*, Article 9823362. https://doi.org/10.1155/2021/9823362

Okoh, E., Yelebe, Z. R., Oruabena, B., *et al*. (2020). Clean-up of crude oil-contaminated soils: Bioremediation option. *International Journal of Environmental Science and Technology, 17*, 1185–1198. https://doi.org/10.1007/s13762-019-02605-y

Kalia, A., Sharma, S., Semor, N., *et al*. (2022). Recent advancements in hydrocarbon bioremediation and future challenges: A review. *3 Biotech*, *12*(6), 135. https://doi.org/10.1007/s13205-022-03199-y

Kanwal, M., Ullah, H., Gulzar, A., *et al*. (2022). Biodegradation of petroleum hydrocarbons and the factors effecting rate of biodegradation. *American Journal of Biomedical Science & Research, 16*(1). https://doi.org/10.34297/AJBSR.2022.16.002182

Bala, S., Garg, D., Thirumalesh, B. V., *et al*. (2022). Recent strategies for bioremediation of emerging pollutants: A review for a green and sustainable environment. *Toxics, 10*(8), 484. https://doi.org/10.3390/toxics10080484

Osburn, E. D., Yang, G., Rilling, M. C., & Strickland, M. S. (2023). Evaluating the role of bacterial diversity in supporting soil ecosystem functions under anthropogenic stress. SME Communications, 3(1). https://doi.org/10.1038/s43705-023-00273-1

Alegbeleye, O. O., Opeolu, B. O., & Jackson, V. (2017). Bioremediation of polycyclic aromatic hydrocarbon (PAH) compounds: (acenaphthene and fluorene) in water using indigenous bacterial species isolated from the Diep and Plankenburg rivers, Western Cape, South Africa. Brazilian Journal of Microbiology, 48, 314–325. https://doi.org/10.1016/j.bjm.2016.07.027

Umar, S. A., Dhakar, R., Sa'idu, M., Sulaiman, M., Wudil, A. A., Shitu, A. S., Abdullahi, A. R., Saminu, S. M., Teli, P. K., & Aliyu, U. A. (2025). Bioremediation of water pollutants from wastewater. *FUDMA Journal of Sciences*, *9*(1), 163–179. https://doi.org/10.33003/fjs-2025-0901-2988

Hopton, C. M., Nienow, P., & Cockell, C. S. (2025). Growth, physiology, and metabolism of Halomonas meridiana in aqueous ammonium sulfate with implications for icy moon astrobiology. *Frontiers in Microbiology*, *16*, 1642998.

Khadka, D., Amgain, R., Joshi, S., & Shrestha, S. (2021). Evaluation of distilled water pH measurement with electrolyte methods in cultivated soils of Nepal. AgroChemistry Soil Science, 92, 52–61. https://doi.org/10.31073/acss92-06

Falodun, T. O., Boisa, N., Ideriah, T. J. K., & Konne, J. L. (2023). Oral bioaccessibility of polycyclic aromatic hydrocarbons in fly ash derived from incineration of petroleum products and waste motor tyres. International Research Journal of Pure and Applied Chemistry, 24(3), 20–30

American Public Health Association. (1998). Standard methods for the examination of water and wastewater (20th ed.). American Public Health Association.

Shekhar, S. K., Godheja, J., & Modi, D. R. (2015). Hydrocarbon bioremediation efficiency by five indigenous bacterial strains isolated from contaminated soils. International Journal of Current Microbiology and Applied Sciences, 4(3), 892–905.

Okoye, A. U., Chikere, C. B., & Okpokwasili, G. C. (2020). Isolation and characterization of hexadecane degrading bacteria from oil-polluted soil in Gio Community, Niger Delta, Nigeria. Scientific African. https://doi.org/10.1016/j.sciaf.2020.e00340

Mulet, M., Sanchez, D., Rodriguez, A. C., Nogales, B., Bosch, R., Busquets, A., Gomila, M., Lalucat, J., & Garcia-Valdes, E. (2018). Pseudomonas gallaeciensis sp. nov., isolated from crude-oil-contaminated intertidal sand samples after the Prestige oil spill. Systematic and Applied Microbiology, 41(4), 340–347.

Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35, 1547–1549.

Ogundolie, F. A. (2022). Optimization and production of extracellular Bacillus megaterium (ISA 08) alpha-amylase isolated from cassava dumpsite soil. Archives of Science and Technology, 3(1), 92–104.

Ejairu, K. O., & Okiotor, M. E. (2022). Effect of oil spill on physicochemical properties of soil spilled sites in Kokori, Ethiope East Local Government Area, Delta State, Nigeria. Journal of Applied Science and Environmental Management, 26(11), 1869–1872.

Solomon, M. P., Agbagwa, I., & Osuji, L. C. (2023). Status of oil quality in the vicinity of artisanal refining sites in Rivers State. Asian Journal of Environment and Ecology, 22(4), 63–67.

Li, X., Wang, X., Zhang, Y., *et al*. (2016). Salinity and conductivity amendment of soil enhanced the bioelectrochemical degradation of petroleum hydrocarbons. Scientific Reports, 6, 32861. https://doi.org/10.1038/srep32861

Bankole, A. O., Ogunkeyede, A. O., Agboro, H., Ekhorutomwen, P. A., Otuomagie, O. I., Isimekhai, K. A., Fadairo, E. A., & Isuruku, E. J. (2024). Heavy metal levels and ecological risk in crude oil-contaminated soils from Okpare-Olomu, Niger Delta, Nigeria. Journal of Environmental Protection, 15, 415–438. https://doi.org/10.4236/jep.2024.154024

Otugboyega, J. O., Madu, F. U., Otugboyega, O. O., Ojo, A. M., Adeyeye, A. J., & Ajayi, J. A. (2023). Biomonitoring and biomathematical modeling of health risks associated with dumpsite grown vegetables in Lagos State. Biological Trace Element Research, 202(7), 3333–3348. https://doi.org/10.1007/s12011-023-03903-w

Samuel-Osamaka, F. C., Arotupin, D. J., Olaniyi, O. O., & Banat, I. M. (2021). Crude oil degrading bacteria obtained from unpolluted soils. Fuoye Journal of Physics and Applied Sciences, 6(2).

Zhang, H., Liu, W., Xiong, Y., Li, G., Cui, J., Zhao, C., & Zhang, L. (2024). Effects of dissolved organic matter on distribution characteristics of heavy metals and their interactions with microorganisms in soil under long-term exogenous effects. Science of the Total Environment, 947, 174565.

Yu, Y., Zhang, Y., Zhao, N., Guo, J., Xu, W., Ma, M., & Li, X. (2020). Remediation of crude-oil polluted soil by the bacterial rhizosphere community of Suaeda salsa revealed by 16S RNA genes. International Journal of Environmental Research and Public Health, 17(5), 1471. https://doi.org/10.3390/ijerph17051471

Chattopadhyay, S., & Ghosh Sachan, S. (2023). Biodegradation of polycyclic aromatic hydrocarbons and the impact of various genes for their enhanced degradation. Letters in Applied Microbiology, 76(2), ovac062.

Azevedo, M. A., do Nascimento, L. P., dos Remédios Vieira-Neta, M., & Duarte, I. C. S. (2023). Production of biosurfactant by bacteria from extreme environments: Biotechnological potential and applications. In Multifunctional microbial biosurfactants (pp. 129–156). Springer Nature Switzerland.

Ali, M. F., Ridha, M. J., & Taly, A. H. (2018). Optimization kerosene bio-degradation by a local soil bacterium isolate Klebsiella pneumoniae Sp. Pneumonia. Journal of Pure and Applied Microbiology, 12(4), 2049–2057.

Rajkumari, J., Singha, L. P., & Pandey, P. (2018). Genomic insight of aromatic hydrocarbon degrading Klebsiella pneumoniae AWD5 with plant growth promoting attributes: A paradigm of soil isolates with elements of biodegradation. 3 Biotech, 8(2). https://doi.org/10.1007/s13205-018-1341

Zhang, G., Wu, Y., Qian, X., & Meng, Q. (2005). Biodegradation of crude oil by Pseudomonas aeruginosa in the presence of rhamnolipids. Journal of Zhejiang University Science B, 6(8), 725–730. https://doi.org/10.1631/jzus.2005.B0725

Medić, A., Lješević, M., Inui, H., Beskoski, V., Kojic, I., Stojanovic, K., & Karadžić, I. (2020). Efficient biodegradation of petroleum n-alkanes and polycyclic aromatic hydrocarbons by polyextremophilic Pseudomonas aeruginosa SAN AI with multidegradative capacity. RSC Advances, 10(24), 14060–14070. https://doi.org/10.1039/C9RA1037F

Das, S., Das, N., Choure, K., & Pandey, P. (2023). Biodegradation of asphaltene by lipopeptide-biosurfactant producing hydrocarbonoclastic, crude-oil degrading Bacillus spp. Bioresource Technology, 382, 129198. https://doi.org/10.1016/j.biortech.2023.129198

Bisht, S., Pandey, P., Sood, A., Sharma, S., & Bisht, N. S. (2010). Biodegradation of naphthalene and anthracene by chemotactically active rhizobacteria of Populus deltoides. Brazilian Journal of Microbiology, 41(4), 922–930.

Kumar, R., & De, M. (2023). Enhanced degradation of petroleum hydrocarbons by Klebsiella michiganensis RK and Acinetobacter baumannii IITG19 isolated from local soil sources. International Journal of Environmental Science and Technology, 20, 13387–13398. https://doi.org/10.1007/s13762-023-04790-3

Cai, Y., Wang, R., Rao, P., Wu, B., Yan, L., Hu, L., Park, S., Ryu, M., & Zhou, X. (2021). Bioremediation of petroleum hydrocarbons using Acinetobacter sp. SCYY-5 isolated from contaminated oil sludge: Strategy and effectiveness study. International Journal of Environmental Research and Public Health, 18(2), 819. https://doi.org/10.3390/ijerph18020819

Sharma, A., Dubey, V., Sharma, R., Devnath, K., Gupta, V. K., Akhter, J., Bhando, T., Verma, A., Ambatipudi, K., Sarkar, M., & Pathania, R. (2018). The unusual glycine-rich C terminus of the Acinetobacter baumannii RNA chaperone Hfq plays an important role in bacterial physiology. Journal of Biological Chemistry, 293(35), 13377–13388. https://doi.org/10.1074/jbc.RA118.002921