



Characterisation of an Aged Crude Oil-Contaminated Soil from Nigeria's Niger Delta Environment: Physicochemical Properties, Heavy Metal Loading, Polycyclic Aromatic Hydrocarbon Content, and Indigenous Hydrocarbon-Degrading Microbiota

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Abstract

Decades of crude oil exploration and exploitation in the Niger Delta region of Nigeria have left agricultural soils heavily laden with hydrocarbons, posing serious ecological and public health risks. Before any bioremediation strategy can be designed, the contaminated soil itself needs to be properly characterised. In this study, the physicochemical, microbiological, and contaminant-loading properties of a composite soil assembled from five historically crude oil-impacted sites in Delta and Rivers States, with contamination histories ranging from 4 to over 40 years, were examined. The results show that the composite soil was strongly acidic ($\text{pH } 4.89 \pm 0.05$), with low electrical conductivity ($162.88 \mu\text{S/cm}$), low moisture content (2.25%), and a total organic carbon content of 3.33%. Benzo(a)pyrene and anthracene were the dominant polycyclic aromatic hydrocarbons (PAHs) detected, at mean concentrations of 20.36 mg/kg and 1.43 mg/kg, respectively. Both PAHs were found to be above the maximum permissible limits set by the Canadian Council of Ministers of the Environment (CCME). Heavy metal analysis by inductively coupled plasma-optical emission spectrometry (ICP-OES) revealed particularly high lead concentrations, reaching 456.67 mg/kg, nearly three times the 164 mg/kg limit set by Nigeria's National Environmental Standards and Regulations Enforcement Agency (NESREA). Four indigenous bacterial strains capable of utilising anthracene and benzo(a)pyrene as sole carbon sources were isolated and identified by 16S rRNA gene sequencing: *Lysinibacillus fusiformis*, a member of the *Bacillus cereus* group (*B. paramycoides/tropicus/cereus*), a member of the *Bacillus subtilis* group (*B. aerophilus/aerius/stratosphericus*), and *Streptomyces albidoflavus*. Together, these findings give a detailed picture of contamination at these sites and establish a baseline for future bioremediation efforts, particularly the development of locally derived biosurfactants.

Keywords: Crude Oil Contamination; Niger Delta; Soil Characterisation; polycyclic aromatic hydrocarbons; benzo(a)pyrene; anthracene

1. Introduction

The Niger Delta region of Nigeria occupies a unique and paradoxical position in global environmental discourse. It features the third-largest mangrove forest in the world, rich freshwater marshes, and extensive tropical rainforests, yet it sits atop one of Africa's most extensive crude oil reserves (Anejonu *et al.*, 2015; Chidumeje *et al.*, 2015). Decades of hydrocarbon exploration and exploitation have resulted in recurrent, often unmitigated, oil spills that have profoundly degraded the terrestrial and aquatic ecosystems of the region. Between 1976 and 2005 alone, Nigeria recorded over 9,100 oil spill incidents involving more than three million barrels of crude oil (Joseph *et al.*, 2021).

The persistent contamination of soils with polycyclic aromatic hydrocarbons (PAHs) is among the most toxicologically significant consequences of crude oil pollution. These fused-ring organic compounds are generated via the incomplete combustion and direct deposition of petroleum products and are classified by the United States Environmental Protection Agency (USEPA) as priority pollutants owing to their carcinogenic, mutagenic, and recalcitrant properties (Alegbeleye *et al.*, 2017). Benzo(a)pyrene, a high-molecular-weight (HMW) five-ring PAH, is considered the gold-standard cancer risk indicator among the USEPA's 16 priority PAHs, while anthracene, a three-ring low-molecular-weight (LMW) PAH, is notable for its exceptional hydrophobicity and environmental persistence (Alegbeleye *et al.*, 2017; Maletić *et al.*, 2019).

The high hydrophobicity of PAHs promotes their strong sorption to soil organic matter, combustion residues, and non-aqueous phase liquids, which makes them poorly bioavailable to indigenous microbial populations and limits the effectiveness of bioremediation approaches (Ismail *et al.*, 2022; Zhang *et al.*, 2024). This bioavailability limitation is further compounded by ageing processes, whereby PAHs increasingly accumulate within soil microaggregates over time, a phenomenon of particular relevance in the Niger Delta, where many oil-spill sites have remained unmitigated for decades (Lamichhane *et al.*, 2016; Lora *et al.*, 2010).

Despite the widespread recognition of crude oil pollution in the Niger Delta, comprehensive, site-specific characterisation data that integrate physicochemical soil properties, PAH concentrations, heavy metal loading, and indigenous microbial diversity remain sparse in the peer-reviewed literature. These baseline data are essential for understanding the multifactorial constraints on natural attenuation and for designing targeted bioremediation strategies. Therefore, this study presents a systematic characterisation of an aged composite crude oil-contaminated soil assembled from five historically impacted sites across the Niger Delta, with the overarching aim of informing the subsequent development of a locally derived biosurfactant-mediated bioremediation protocol.

2. Materials and Methods

2.1 Study Sites and Soil Sample Collection

Five sampling sites with documented histories of crude oil spillage and no prior remediation activity were selected across Delta and Rivers States, Nigeria. The sites encompassed a range of contamination ages, as follows: Agbarha (Delta; $5^{\circ}32'18.44''\text{N}$, $6^{\circ}6'28.34''\text{E}$; 2019), Iteregbi (Delta; $5^{\circ}34'23.43''\text{N}$, $5^{\circ}50'37.32''\text{E}$; 2017), Ogini (Delta; $5^{\circ}34'0.05''\text{N}$, $6^{\circ}16'9.08''\text{E}$; 2015), Eleme Site 1 (Rivers; $4^{\circ}46'11.58''\text{N}$, $7^{\circ}8'17.78''\text{E}$; >40 years), and Eleme Site 2 (Rivers; $4^{\circ}46'9.47''\text{N}$, $7^{\circ}8'18.16''\text{E}$; >40 years). Samples A–E corresponded to Iteregbi, Eleme 1, Ogini, Agbarha, and Eleme 2, respectively. All sites are located within a humid tropical climate zone.

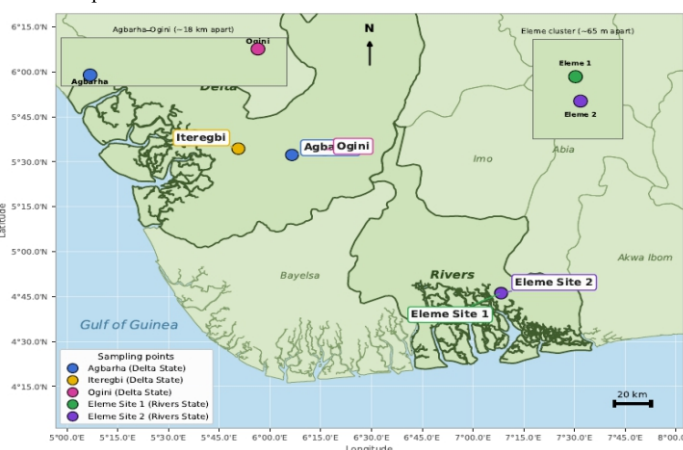


Figure 1: Map of the Niger Delta region, Nigeria, showing the sampling locations (Omoruwou *et al.*, 2023)

Soil samples were collected at depths of 0–20 cm from the surface using soil augers and shovels. The samples were placed in sterile polyethene bags, transported to the laboratory, and air-dried to reduce moisture content. Subsequently, they were sieved using a 500 μm mesh (No. 35) to remove debris and homogenise particle size. Equal masses of each sample were combined to obtain a representative composite (Sample F) used for all characterisation and remediation experiments.

2.2 Physicochemical Characterisation of the Composite Soil

2.2.1 Soil pH and Electrical Conductivity

Soil pH was determined using a calibrated digital pH meter (Cole-Parmer Digi-Sense, Model 5938-00) following the USEPA Method (2002). Air-dried 2 mm-sieved soil was suspended in deionised water (1:2.5 w/v) and equilibrated for 1 h, and the pH was measured after temperature correction. Electrical conductivity (EC) was measured in the same suspension using a Lasec Cond 61 conductivity meter.

2.2.2 Moisture Content

Soil moisture content was determined gravimetrically following ASTM D2974-00. Oven-dried moisture cans (105 °C; 24 h) were weighed before and after loading 5 g of soil, and the weight loss was expressed as a percentage of the initial soil mass.

2.2.3 Total Organic Matter and Total Organic Carbon

Total organic matter (TOM) was quantified via the loss-on-ignition (LOI) method. Oven-dried soil samples (105 °C, overnight) were combusted in a muffle furnace at 450 °C overnight, and the difference in mass before and after ignition was expressed as a percentage of oven-dry mass. Total organic carbon (TOC) was estimated based on TOM using the empirical relationship in Equation 1 reported by Abebe and Pathak (2022)

$$TOC = 1.703 + 0.502TOM \quad (1)$$

2.2.4 Total Nitrogen, Phosphorus, and Potassium

Total nitrogen content was determined using a LECO TruMac Carbon-Nitrogen-Sulphur Analyser (version 1.3). The contents of phosphorus, potassium, and selected heavy metals (Pb, Fe, Cu, Cr, As, Ni, Mn, Mo, Co, Cd, Zn, and V) were determined via inductively coupled plasma-optical emission spectrometry (ICP-OES), following acid digestion using 1M nitric acid (1:10 w/v) at 100°C, filtration using Whatman No. 40 filter paper, and further clarification using a 0.22-µm membrane filter. The obtained solutions were refrigerated at 4 °C before analysis.

2.3 Determination of PAH Concentrations

The target PAHs (anthracene and benzo(a)pyrene) were extracted from 3 g of air-dried soil via ultrasonic extraction (EPA Method 3550B) using a hexane–acetone solvent mixture (4:1 v/v) and a Celsius Scientific Ultrasonic Bath (Model PS-80, 50–60 Hz, 40°C, 10 min). After centrifugation at 2000 rpm for 5 min, the extraction was repeated twice, and the combined extracts were concentrated via rotary evaporation (Buchi R-114, 40°C). Solvent exchange was performed using acetonitrile, and the extract was filtered using a 0.22-µm PTFE syringe filter before HPLC analysis.

HPLC analysis was performed on a Waters 2695 Separation Module equipped with a Waters 2998 Photodiode Array Detector (PDA). Separation was performed on a Reverse Phase Waters C18 column (4.6 mm × 25 cm, 5 µm packing) at 35°C using a mobile phase of methanol and 1% acetic acid in water at a flow rate of 1.0 mL/min and an injection volume of 10 µL. Anthracene was

detected at 254 nm and benzo(a)pyrene at 267 nm. Quantification was performed against five-point external calibration curves prepared based on certified standard solutions (Sigma-Aldrich, South Africa).

2.4 Isolation and Identification of Indigenous PAH-Degrading Bacteria

Minimal media agar, prepared according to Sperotto *et al.* (2021), was used to select for indigenous bacteria capable of utilising anthracene and benzo(a)pyrene as sole carbon sources. M9 minimal salts (5× concentrate) were supplemented with 1 mM MgSO₄, 0.1 mM CaCl₂, and a mixture of anthracene (10 mg/L) and benzo(a)pyrene (5 mg/L). Serial dilutions of the composite soil (10⁻¹ to 10⁻³) were spread-plated onto the selective media and incubated at 37°C for 48–120 h.

Colonies exhibiting distinct morphologies were re-cultured to purity on Tryptone Yeast (TY) agar. DNA was extracted using a Zymo Research kit, and the 16S rRNA gene was amplified via PCR using universal primers (forward: 5'-G A G T T T G A T C M T G G C T C A G - 3'; reverse: 5'-CGGTTACCTTGTTACGGACTT-3'). PCR products were resolved on 0.9% agarose gel, purified using a QIAquick gel extraction kit (QIAGEN), and sequenced using the ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Kit on an ABI 3100 Automated Capillary DNA Sequencer. Sequences were edited in Chromas Lite 2.0 and BioEdit 5.09, aligned using ClustalW 1.83, and compared with GenBank entries via BLAST.

3. Results and Discussion

3.1 Physicochemical Properties of the Composite Soil

Table 1 summarises the physicochemical characterisation data for individual samples (A–E) and their composite (F).

The composite soil (sample F) exhibited a strongly acidic pH (4.89 ± 0.05), which was lower than the 5.63–6.47 range typically reported for pristine Niger Delta soils (Verla *et al.*, 2017; Ite *et al.*, 2018). This increased acidity is consistent with the generation of organic acids during crude oil weathering and microbial metabolism (Olusegun *et al.*, 2022; Joseph *et al.*, 2021). Soil pH is a key variable governing metal bioavailability, nutrient solubility, and microbial community structure. A one-unit decrease in pH results in a ten-fold increase in divalent metal solubility (Neina, 2019; Bünnemann *et al.*, 2018). Thus, the low pH of the composite soil indicated increased metal bioavailability and a microbial community restricted mainly to acidophilic taxa.

The electrical conductivity of the composite (162.88 µS/cm; 16.29 mS/m) was substantially below the 110–570 mS/m range indicative of nutrient-rich soils (Basumatary, 2021), ultimately reflecting a salt-depleted, nutrient-poor soil matrix consistent with the leaching of cations under the region's high annual rainfall. Total organic carbon (3.33%) was sufficient to sustain soil structural integrity (above the 2% critical threshold of Patrick *et al.*, 2013) and provide a substrate for indigenous microorganisms, yet low moisture content (2.25%)—attributed to extended air-drying during storage—indicated substantially suboptimal conditions for active microbial metabolism (Vidali, 2011).

Table 1: Physicochemical data of the soil samples

Parameter	Sample					
	A	B	C	D	E	F
Anthracene (mg/kg)	0.43	nd	0.78	0.89	nd	1.43
Benzo(a)pyrene (mg/kg)	18.30	28.91	19.24	17.23	21.31	20.36
pH±sd	6.10±0.1	4.38±0.08	5.41±0.08	5.16±0.18	4.18±0.03	4.89±0.05
TOC (%)	3.82	3.27	4.07	5.93	2.98	3.33
TOM (%)	4.22	3.11	4.71	8.41	2.53	3.25
EC (µS/cm ± sd)	212.67 ±2.52	85.60±2.44	74.27±1.40	164.63±3.71	102.20±1.76	160.50±2.38
MC (%)	2.74	3.30	2.94	1.86	1.50	2.25
Total Nitrogen (mg/kg)	1630.00	920.00	1190.00	1970.00	1210.00	1310.00
Phosphorus (mg/Kg)	8.90	32.55	nd	nd	7.36	7.59
Potassium (mg/Kg)	1.31	7.92	5.92	10.59	7.07	7.78
Iron (mg/Kg)	138.25	457.72	251.27	244.69	231.28	221.41
Copper (mg/Kg)	4.96	nd	nd	nd	nd	nd
Zinc (mg/Kg)	nd	nd	nd	nd	nd	nd
Lead (mg/Kg)	139.19	456.67	247.27	240.64	225.90	216.23
Chromium (mg/Kg)	5.32	1.64	1.02	1.12	1.09	1.06
Arsenic (mg/Kg)	13.91	10.33	10.23	10.23	10.32	10.21
Vanadium (mg/Kg)	nd	nd	nd	nd	nd	nd
Nickel (mg/Kg)	4.59	4.56	4.59	4.58	5.61	4.59
Manganese (mg/Kg)	0.45	0.72	2.85	0.18	0.09	1.27
Molybdenum (mg/Kg)	0.26	0.15	nd	0.08	nd	0.09
Cobalt (mg/Kg)	0.73	0.55	0.55	0.54	0.58	0.57
Cadmium (mg/Kg)	0.36	0.25	0.22	0.21	0.20	0.23

nd: Not Detected

Soil fertility parameters were indicative of poor arable land quality: total nitrogen, 1,310 mg/kg; phosphorus, 7.59 mg/kg; and potassium, 7.78 mg/kg. These values are consistent with findings from similar crude oil-impacted sites in the Niger Delta (Joseph *et al.*, 2021; Amaechi and Onwuka, 2021) and are substantially below the thresholds required to sustain microbial hydrocarbon degradation without exogenous nutrient supplementation.

3.1.1. Implications of Soil Acidity for Bioremediation

The strongly acidic pH of the composite soil (4.89) is below the optimum range of 6.5–8.0 reported for efficient hydrocarbon biodegradation by most neutrophilic bacteria (Koshlaf and Ball, 2017; Obi *et al.*, 2016; Obahiagbon *et al.*, 2014). Although the isolated strains were acidophilically adapted, the overall microbial community diversity is possibly constrained, thereby reducing the breadth of metabolic pathways available for PAH co-metabolism. From a bioremediation design perspective, pH control—for example, through the addition of agricultural lime or calcium carbonate—would be expected to broaden microbial community diversity and enhance degradation rates, although the specific pH optima for the identified isolates require further investigation. Low pH also directly affects PAH bioavailability. At reduced pH, soil surfaces exhibit increased positive charge, potentially enhancing the sorption of anionic PAH metabolites and reducing their aqueous-phase availability to degrading organisms. Furthermore, the increased solubility of heavy metals (particularly Pb) under acidic conditions leads to significant co-contaminant inhibition of microbial activity (Kuppusamy *et al.*, 2016; Olaniran *et al.*, 2013). Thus, strategies that simultaneously address pH control, metal immobilisation, and PAH solubilisation—such as the combined use of biosurfactants and organic amendments—are required for this contamination scenario.

3.2 PAH Concentrations in the Composite Soil

The mean benzo(a)pyrene (BaP) concentration in the composite soil was 20.36 mg/kg, while that of anthracene was 1.43 mg/kg, both exceeding the CCME maximum permissible levels for agricultural soil (CCME, 2010). A notable pattern emerged across the individual samples: BaP concentrations increased with contamination age, highest in Eleme Sites 1 and 2 (28.91 and 21.31 mg/kg, respectively; > 40-year history) and lowest in Agbarha (17.23 mg/kg; 2019 spill). This trend reflected the progressive sorption and sequestration of the recalcitrant HMW PAH in soil microaggregates over time (Lors *et al.*, 2010; CCME, 2010). In contrast, anthracene was undetectable in the two oldest-contaminated samples (Eleme Sites 1 and 2), consistent with the higher volatility and water solubility of the three-ring LMW PAH compared with the five-ring BaP (Ma *et al.*, 2010). The vapour pressure of anthracene (3.40×10^{-3} Pa) was four orders of magnitude greater than that of BaP (6.52×10^{-7} Pa), which ultimately promoted its progressive loss from aged soils through volatilisation, leaching, and biodegradation. These compositional differences between samples of differing contamination ages demonstrated the importance of molecular weight and vapour pressure as determinants of PAH fate and persistence in the soil environment (Ismail *et al.*, 2022; Maletić *et al.*, 2019).

3.2.1 PAH Ageing and Bioavailability Constraints

The differential distribution of BaP and anthracene across sites of varying contamination ages provided compelling evidence for the ageing-related sequestration of PAHs in this soil system. The progressive increase in BaP concentration with site age and the absence of detectable anthracene at the oldest sites are consistent with established models of PAH fate in soil: LMW PAHs are progressively lost through volatilisation and biodegradation, while HMW PAHs become increasingly bound to the soil organic matter matrix over time (Lors *et al.*, 2010; CCME, 2010; Lamichhane *et al.*, 2016).

For remediation purposes, aged BaP contamination presents a particularly difficult challenge. As PAHs age in soil, their desorption kinetics shift from rapid, linear processes to slow, non-linear diffusion from soil micropores, which in turn reduces the fraction available for microbial uptake to well below the total concentration (Ismail *et al.*, 2022; Lors *et al.*, 2010). This 'ageing effect' is the primary rationale for incorporating bioavailability-enhancement agents—such as surfactants—into bioremediation strategies for sites with long contamination histories (Adrian *et al.*, 2016; Lamichhane *et al.*, 2017).

3.3 Heavy Metal Contamination

Heavy metal analysis revealed significant contamination, particularly by lead (Pb) and arsenic (As). Lead concentrations across the five sites ranged from 139.19 to 456.67 mg/kg, with that of the composite at 216.23 mg/kg—substantially exceeding the NESREA maximum permissible level of 164 mg/kg for most samples (Omuruwou *et al.*, 2023; Amaechi and Onwuka, 2021). Consistent with the typically high Pb content of Bonny Light crude oil (Oti, 2016), these increased concentrations reflected the progressive accumulation of crude oil-derived metals in the soil matrix.

Arsenic concentrations (10.21–13.91 mg/kg; composite: 10.32 mg/kg) were within the WHO/EU acceptable limits for agricultural soil, indicating a lithogenic rather than petroleum-derived origin (Rahaman *et al.*, 2013). Iron concentrations (138.25–457.72 mg/kg) were high at two sites but within globally reported ranges for tropical soils for other sites (Mielki *et al.*, 2016). Nickel, chromium, manganese, molybdenum, and cobalt concentrations were all below

their respective NESREA, USEPA, and WHO regulatory thresholds, indicating that these elements, although present, did not constitute acute contamination concerns.

The co-occurrence of heavy metals and PAHs in the composite soil is of particular significance for bioremediation design. Previous research has demonstrated that metal co-contamination can inhibit the biodegradation of organic pollutants by suppressing microbial enzymatic activity and reducing microbial community diversity (Kuppusamy *et al.*, 2016; Zhang *et al.*, 2024). The strongly acidic conditions of the composite soil further amplified metal bioavailability, creating a dual stress scenario for indigenous microorganisms.

3.3.1 Heavy Metal Co-contamination and Microbial Resilience

The exceedance of NESREA limits for lead in the composite soil is a significant finding with implications for both ecological risk and bioremediation efficacy. Lead, at high bioavailable concentrations driven by low soil pH, can directly inhibit microbial enzyme systems, reduce cell membrane integrity, and alter community structure towards resistant, metabolically specialised taxa (Zhang *et al.*, 2024; Omuruwou *et al.*, 2023; Olaniran *et al.*, 2013). The identification of *Bacillus subtilis* group members and *Streptomyces albidoflavus* as constituents of the indigenous community is encouraging in this respect: both have been associated with heavy metal tolerance and biosorption (Cai *et al.*, 2018; Kaliyaraj *et al.*, 2019).

The co-occurrence of PAH and heavy metal contamination also creates potential for synergistic toxicity, where the combined effect of co-contaminants on microbial communities exceeds the sum of individual effects. Bioremediation strategies for such complex contamination scenarios must therefore address both contaminant classes simultaneously or in sequence, considering the potential for heavy metal-induced inhibition of PAH biodegradation pathways (Kuppusamy *et al.*, 2016).

3.4 Indigenous PAH-Degrading Bacterial Community

16S rRNA gene sequence analysis of four bacterial isolates cultured on anthracene and benzo(a)pyrene as sole carbon sources identified the following strains: *Lysinibacillus fusiformis* (99.5% sequence identity), *Bacillus paramycoides/tropicus/cereus*—*Bacillus cereus* group (99.8% identity), *Bacillus aerophilus/aerius/stratosphericus*—*Bacillus subtilis* group (100% identity), and *Streptomyces albidoflavus* (98.2% identity). All isolates were Gram-positive, spore-forming, rod-shaped bacteria widely distributed in soil environments.

The dominance of *Bacillus* species is consistent with previously reported PAH-contaminated soil microbial communities in the Niger Delta (Chikere *et al.*, 2020; Imam *et al.*, 2021; Bezza and Chirwa, 2017). *Bacillus* species tolerate increased hydrocarbon concentrations owing to their resistant endospores, and the *Bacillus cereus* group has been specifically associated with the simultaneous degradation of HMW PAH mixtures (Bezza and Chirwa, 2017). *Bacillus subtilis* produces the lipopeptide biosurfactant surfactin, which has been proven to significantly enhance PAH bioavailability and degradation (Ahmad *et al.*, 2018; Kotoky and Pandey, 2020).

Lysinibacillus fusiformis, formerly classified within *Bacillus*, has been reported as a degrader of heavy hydrocarbons, including asphaltene components (Kshirsagar *et al.*, 2020) and artisanally refined diesel (Chikere *et al.*, 2020). Its isolation in this study as a degrader of anthracene and benzo(a)pyrene extends the documented metabolic repertoire of this species. *Streptomyces albidoflavus*, an actinobacterium with established chitinolytic and antifungal activity (Swiontek Brzezinska *et al.*, 2013) and reported capacity for bioleaching of heavy metals from electronic waste (Kaliyaraj *et al.*, 2019), has, to the best of our knowledge, not previously been reported as a degrader of anthracene and benzo(a)pyrene. Its isolation in this study represents a novel finding that broadens the known diversity of PAH-degrading microorganisms and highlights the potential of long-term crude oil contamination to select for metabolically versatile actinobacteria.

The acidophilic character of the identified isolates is notable in the context of the composite soil's strongly acidic pH (4.89). *Lysinibacillus fusiformis* is known to produce acidogenic enzymes (Goud *et al.*, 2014), and *Bacillus aerophilus/aerius/stratosphericus* has been characterised as acidophilic, indicating that these taxa are well-adapted to the prevailing soil conditions and possibly represent the dominant functional guild for in situ PAH catabolism (Dar *et al.*, 2021).

3.4.1. Soil Nutrient Status and the Case for Biostimulation

The low concentrations of nitrogen, phosphorus, and potassium in the composite soil—well below levels required for unrestricted microbial growth—confirm that nutrient limitation is a critical constraint on natural attenuation at these sites (Omuruwou *et al.*, 2023). Carbon-to-nitrogen-to-phosphorus ratios far exceed the 100:10:1 optimum for hydrocarbon-degrading microbial communities (Kumari *et al.*, 2012; Mukred *et al.*, 2008). This nutrient deficiency, combined with suboptimal moisture content and low pH, creates a severely limiting environment for bioremediation.

The incorporation of organic amendments such as cow dung provides a cost-effective, locally available means of simultaneously addressing nitrogen and phosphorus deficiency, improving soil water retention, and stimulating

indigenous microbial populations (Adams *et al.*, 2015; Perfumo *et al.*, 2007). The present characterisation data support the use of cow dung as a biostimulant in subsequent remediation experiments, with the caveat that excessive organic matter addition may paradoxically reduce PAH bioavailability through enhanced sorption competition (Zhang *et al.*, 2005).

4. Conclusion

This study provided a comprehensive baseline characterisation of an aged crude oil-contaminated composite soil from five historically impacted sites in Nigeria's Niger Delta region. The composite soil was strongly acidic, nutrient-poor, and contaminated with benzo(a)pyrene and anthracene at concentrations exceeding international regulatory thresholds, alongside heavy metal loading—particularly lead—that posed additional ecological risks. Four autochthonous bacterial strains with documented or newly discovered capacity for PAH catabolism were isolated, among which *Streptomyces albidoflavus* was reported for the first time as a degrader of anthracene and benzo(a)pyrene.

The integrated characterisation revealed a convergence of multiple limiting factors—low pH, nutrient deficiency, metal co-contamination, and PAH ageing—that collectively limited natural attenuation and required a multifaceted bioremediation approach. The indigenous microbial community, although adapted to the prevailing harsh conditions, lacked the nutrient resources and bioavailability enhancement required for efficient PAH mineralisation. These findings provide a scientific rationale for the development and application of a locally derived, agro-based surfactant, combined with organic amendment, to enhance PAH bioavailability and support autochthonous microbial degradation, as will be demonstrated in subsequent experimental work.

AUTHOR CONTRIBUTIONS

Felix Omuruwou: Conceptualisation, Methodology, Investigation, Formal Analysis, Writing of Original Draft; Andrew O. Odeh: Writing, Review and Editing.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Abebe, G., Pathak, H. (2022). Relationship between organic matter and total organic carbon in different soil types of West Hararghe, Ethiopia. *Heliyon*, 8(6), e09691. <https://doi.org/10.1016/j.heliyon.2022.e09691>
- Adams, G.O., Fufeyin, P.T., Okoro, S.E., Ehinomen, I. (2015). Bioremediation, biostimulation and bioaugmentation: a review. *International Journal of Environmental Bioremediation & Biodegradation*, 3(1), 28–39. <https://doi.org/10.12691/ijebb-3-1-5>
- Adrian, A.C., Singleton, D.R., Nakamura, J., Shea, D., Aitken, M.D. (2016). Improving polycyclic aromatic hydrocarbon biodegradation in contaminated soil through low-level surfactant addition after conventional bioremediation. *Environmental Engineering Science*, 33(9), 659–670. <https://doi.org/10.1089/ees.2016.0128>
- Ahmad, Z., Imran, M., Qadeer, S., Hussain, S., Kausar, R., Dawson, L., Khalid, A. (2018). Biosurfactants for sustainable soil management. *Advances in Agronomy*, 150, 81–130. <https://doi.org/10.1016/bs.agron.2018.02.002>
- Alegbeleye, O.O., Opeolu, B.O., Jackson, V.A. (2017). Polycyclic aromatic hydrocarbons: a critical review of environmental occurrence and bioremediation. *Environmental Management*, 60(4), 758–783. <https://doi.org/10.1007/s00267-017-0896-2>
- Amaechi, M., Onwuka, S.U. (2021). Determination of the presence and level of heavy metals in soils of automobile workshops in Awka, Anambra State. *International Journal of Environment and Pollution Research*, 9(3), 18–32.
- Anejionu, O.C.D., Ahiamunnah, P.A.N., Nri-ezedi, C.J. (2015). Hydrocarbon pollution in the Niger Delta: geographies of impacts and appraisal of lapses in extant legal framework. *Resources Policy*, 45, 65–77. <https://doi.org/10.1016/j.resourpol.2015.03.012>
- Basumatary, B.K. (2021). Quality of soil in Gossaigaon sub-division and its impact on environmental degradation. *Current Agriculture Research Journal*, 9(3), 188–195. <https://doi.org/10.12944/CARJ.9.3.06>
- Bezza, F.A., Chirwa, E.M.N. (2017). The role of lipopeptide biosurfactant on microbial remediation of aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil. *Chemical Engineering Journal*, 309, 563–576. <https://doi.org/10.1016/j.cej.2016.10.055>
- Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Brussaard, L. (2018). Soil quality – a critical review. *Soil Biology and Biochemistry*, 120, 105–125. <https://doi.org/10.1016/j.soilbio.2018.01.030>
- Cai, Y., Li, X., Liu, D., et al. (2018). A novel Pb-resistant *Bacillus subtilis* bacterium isolate for co-biosorption of hazardous Sb(III) and Pb(II). *International Journal of Environmental Research and Public Health*, 15(4), 702. <https://doi.org/10.3390/ijerph15040702>
- CCME (Canadian Council of Ministers of the Environment). (2010). *Canadian soil quality guidelines for the protection of environmental and human health: polycyclic aromatic hydrocarbons*. Winnipeg, Canada.
- Chidumeje, N.P.O., Lalit, K., Subhashni, T. (2015). The Niger Delta wetland ecosystem: what threatens it and why should we protect it? *African Journal of Environmental Science and Technology*, 9(5), 451–463. <https://doi.org/10.5897/AJEST2014.1841>
- Chikere, C.B., Obieze, C.C., Chikere, B.O. (2020). Biodegradation of artisanally refined diesel and the influence of organic wastes on oil-polluted soil remediation. *Scientific African*, 8, e00385. <https://doi.org/10.1016/j.sciaf.2020.e00385>
- Dar, M.A., Dhole, N.P., Xie, R., et al. (2021). Valorization potential of a novel bacterial strain, *Bacillus altitudinis* RSP75, towards lignocellulose bioconversion. *Microorganisms*, 9(9), 1952. <https://doi.org/10.3390/microorganisms9091952>
- Goud, R.K., Sarkar, O., Chiranjeevi, P., Venkata Mohan, S. (2014). Bioaugmentation of potent acidogenic isolates: a strategy for enhancing biohydrogen production at elevated organic load. *Bioresour Technol*, 165, 223–232. <https://doi.org/10.1016/j.biortech.2014.03.049>
- Imam, A., Suman, S.K., Kanaujia, P.K., Ray, A. (2021). Biological approaches to polycyclic aromatic hydrocarbon bioremediation: a review. *Bioresour Technol*, 343, 126121. <https://doi.org/10.1016/j.biortech.2021.126121>
- Ismail, N.A., Kasmuri, N., Hamzah, N. (2022). Microbial bioremediation techniques for polycyclic aromatic hydrocarbon (PAHs) — a review. *Water, Air, & Soil Pollution*, 233(3), 1–18. <https://link.springer.com/article/10.1007/s11270-022-05598-6>
- Ite, A.E., Ibok, U.J., Ite, M.U., Petters, S.W. (2018). Petroleum exploration and production: past and present environmental issues in Nigeria's Niger Delta. *American Journal of Environmental Protection*, 1(4), 78–90. <https://doi.org/10.12691/env-1-4-2>
- Joseph, A., Iwok, E., Ekanem, S. (2021). Public health threats of heavy metals due to consumption of *Achachatina marginata* from a partially remediated site in Niger Delta, Nigeria. *Environmental Pollution*, 271, 116392. <https://doi.org/10.1016/j.envpol.2020.116392>
- Kaliyaraj, D., Rajendran, M., Angamuthu, V., et al. (2019). Bioleaching of heavy metals from printed circuit board (PCB) by *Streptomyces albidoflavus* TN10. *Bioresources and Bioprocessing*, 6(1), 43. <https://link.springer.com/article/10.1186/s40643-019-0283-3>
- Koshlaf, E., Ball, A.S. (2017). Soil bioremediation approaches for petroleum hydrocarbon-polluted environments. *AIMS Microbiology*, 3(1), 25–49. <https://doi.org/10.3934/microbiol.2017.1.25>
- Kotoky, R., Pandey, P. (2020). Rhizosphere mediated biodegradation of benzo(a)pyrene by surfactin producing soil bacilli applied through *Melia azadirachta* rhizosphere. *International Journal of Phytoremediation*, 22(4), 363–372.
- Kshirsagar, S.D., Mattam, A.J., Jose, S., Ramachandrarao, B., Velankar, H.R. (2020). Heavy hydrocarbons as selective substrates for isolation of asphaltene degraders. *Environmental Technology and Innovation*, 19, 100832. <https://doi.org/10.1016/j.eti.2020.100832>
- Kumari, B., Singh, S.N., Singh, D.P. (2012). Characterization of two biosurfactant producing strains in crude oil degradation. *Process Biochemistry*, 47(12), 2463–2471. <https://doi.org/10.1016/j.procbio.2012.10.010>
- Kuppusamy, S., Thavamani, P., Megharaj, M., Lee, Y.B., Naidu, R. (2016). Polyaromatic hydrocarbon (PAH) degradation potential of a new acid tolerant diazotrophic P-solubilizing and heavy metal resistant bacterium *Cupriavidus* sp. MTS-7. *Chemosphere*, 162, 31–39. <https://www.sciencedirect.com/science/article/abs/pii/S004565351630947X>
- Lamichhane, S., Bal Krishna, K.C., Sarukkalgir, R. (2016). Polycyclic aromatic hydrocarbons (PAHs) removal by sorption: a review. *Chemosphere*, 148, 336–353. <https://doi.org/10.1016/j.chemosphere.2016.01.036>
- Lamichhane, S., Bal Krishna, K.C., Sarukkalgir, R. (2017). Surfactant-enhanced remediation of polycyclic aromatic hydrocarbons: a review. *Journal of Environmental Management*, 199, 46–61.

- <https://doi.org/10.1016/j.jenvman.2017.05.037>
- Lors, C., Ryngaert, A., Périé, F., Diels, L., Damidot, D. (2010). Evolution of bacterial community during bioremediation of PAHs in a coal tar contaminated soil. *Chemosphere*, 81(10), 1263–1271. <https://doi.org/10.1016/j.chemosphere.2010.09.021>
- Ma, Y., Lei, Y. D., Xiao, H., Wania, F., & Wang, W. (2010). Critical Review and Recommended Values for the Physical-Chemical Property Data of 15 Polycyclic Aromatic Hydrocarbons at 25 ° C. *J. Chem. Eng.*, 55, 819–825. <https://doi.org/10.1021/jc900477x>
- Maletić, S.P., Beljin, J.M., Rončević, S.D., Grgić, M.G., Dalmacija, B.D. (2019). State of the art and future challenges for polycyclic aromatic hydrocarbons in sediments: sources, fate, bioavailability and remediation techniques. *Journal of Hazardous Materials*, 365, 467–482. <https://doi.org/10.1016/j.jhazmat.2018.11.020>
- Mielki, G.F., Novais, R.F., Ker, J.C., Vergütz, L., de Castro, G.F. (2016). Iron availability in tropical soils and iron uptake by plants. *Revista Brasileira de Ciência do Solo*, 40. https://www.rbcjournal.org/wp-content/uploads/articles_xml/0100-0683-rbcs-18069657rbcs20150174/0100-0683-rbcs-18069657rbcs20150174.x42865.pdf
- Mukred, A.M., Hamid, A.A., Hamzah, A., Yusoff, W.W.M. (2008). Enhancement of biodegradation of crude petroleum-oil in contaminated water by addition of nitrogen sources. *Pakistan Journal of Biological Sciences*, 11(17), 2122–2127. <https://doi.org/10.3923/pjbs.2008.2122.2127>
- Neina, D. (2019). The role of soil pH in plant nutrition and soil remediation. *Applied and Environmental Soil Science*, 2019, 5794869. <https://doi.org/10.1155/2019/5794869>
- Obahiagbon, K.O., Amenaghawon, A.N., Agbonghae, E.O. (2014). Effect of initial pH on the bioremediation of crude oil polluted water using a consortium of microbes. *International Journal of Scientific Research*, 3(10), 452–457.
- Obi, C.C., Adebuseye, S.A., Ugoji, E.O., Antai, S.P., Ogunshe, A.A., Frankenberger, W.T. (2016). Microbial communities in sediments of Lagos Lagoon, Nigeria: elucidation of community structure and potential impacts of contamination by municipal and industrial wastes. *Frontiers in Microbiology*, 9, 1922.
- Olaniran, A.O., Balgobind, A., Pillay, B. (2013). Bioavailability of heavy metals in soil: impact on microbial biodegradation of organic compounds and possible improvement strategies. *International Journal of Molecular Sciences*, 14(5), 10197–10228. <https://doi.org/10.3390/ijms140510197>
- Olusegun, S.J., Oluwaseun, A.C., Olawale, M.D., Moses, E.O. (2022). Environmental effects of crude oil spills in the Niger Delta: a review of physicochemical and microbial characteristics of impacted soils. *Environmental Science and Pollution Research*, 29(20), 29675–29692.
- Omoruwou, F., Obahiagbon, K.O., Amenaghawon, N.A., Daramola, M.O. (2023). Environmental risk analysis of aged soil in an environment impacted by Nigerian crude oil. *Direct Research Journal of Public Health and Environmental Technology*, 8(4), 53–65.
- Oti, W.O. (2016). Lead in Bonny light oil and its implication for Niger Delta pollution. *Nigerian Journal of Chemical Research*, 14, 23–29.
- Patrick, M., Tenywa, J.S., Ebanyat, P., et al. (2013). Soil organic carbon thresholds and nitrogen management in tropical agroecosystems. *Journal of Sustainable Development*, 6(12), 31–43. <https://doi.org/10.5539/jsd.v6n12p31>
- Perfumo, A., Banat, I.M., Marchant, R., Vezzulli, L. (2007). Thermally enhanced approaches for bioremediation of hydrocarbon-contaminated soils. *Chemosphere*, 66(1), 179–184. <https://doi.org/10.1016/j.chemosphere.2006.05.006>
- Rahaman, S., Sinha, A.C., Pati, R., Mukhopadhyay, D. (2013). Arsenic contamination: a potential hazard to the affected areas of West Bengal, India. *Environmental Geochemistry and Health*, 35(1), 119–132.
- Sperotto, G., Stasiak, L.G., Godoi, J.P.M.G., Gabiatti, N.C., De Souza, S.S. (2021). A review of culture media for bacterial cellulose production. *Cellulose*, 28(5), 2649–2673.
- Swiontek Brzezinska, M., Jankiewicz, U., Burkowska, A. (2013). Purification and characterization of *Streptomyces albidoflavus* antifungal components. *Applied Biochemistry and Microbiology*, 49(5), 451–457.
- Verla, A.W., et al. (2017). Physicochemical parameters of soils in Port-Harcourt City, Nigeria. *Journal of Environmental Science and Public Health*, 1(2), 1–15.
- Vidali, M.K. (2011). Bioremediation — an overview. *Journal of Industrial Pollution Control*, 27(2), 161–168.
- Zhang, C., Wu, D., Ren, H. (2024). Effects of heavy metals on microbial biodegradation of organic pollutants: mechanisms and future perspectives. *Journal of Hazardous Materials*, 469, 133940.
- Zhang, H., Ji, L., Wu, F., Tan, J. (2005). In situ ozonation of anthracene in unsaturated porous media. *Journal of Hazardous Materials*, 120(1–3), 143–148.