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BIOREMEDIATION POTENTIAL OF INDIGENOUS MICROORGANISMS IN HYDROCARBON CONTAMINATED SOILS OF NIGER-DELTA, NIGERIA

Anyaoha Uche Christiana[1*], Orakwe Louis Chukwuemeka[1], Mmadubuike Chimaobi Nnaemeka[1], Anonye Onyemauche Fabian[1]

[2]*Department of Agricultural and Bioresources Engineering, Nnamdi Azikiwe University, Awka, Nigeria;*

**Corresponding Author's E-mail:* ucscott123@gmail.com

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ABSTRACT

Hydrocarbon contamination from long-term petroleum exploration has resulted in significant soil degradation in the Niger Delta region, Nigeria, latitudes 4°10′ to 6°20′ North and longitudes 2°35′ to 7°30′ East of the Equator. This study evaluated the biodegradation potential of indigenous microbial communities in contaminated soils of the region. Soil samples were collected from five petroleum-impacted sites showing high contamination levels, with Total Petroleum Hydrocarbon (TPH) concentrations ranging from 6,970 to 9,861 mg/kg, exceeding regulatory limits. Laboratory microcosm experiments were conducted over 60 days under four treatment conditions: Natural Attenuation (NA), Bio-stimulation (BS), Bio Augmentation (BA), and a combined Bio-stimulation–Bio-Augmentation (BS + BA) approach. Biodegradation performance was monitored using microbial counts, soil respiration (CO_2 evolution), and TPH reduction. Hydrocarbon utilizing bacteria increased from approximately 5.95 to 7.50 $\log_{10}$ CFU/g in the combined treatment, indicating strong microbial adaptation and activity. The highest TPH removal efficiency of 83.8% was achieved under combined treatment with BS + BA, reducing TPH from 6,800 mg/kg to 1,100 mg/kg within 60 days. This was followed by BA (76.5%), BS (72.1%), and NA (38.2%), while the sterile control showed minimal reduction (5.9%). Soil respiration trends supported these results, with maximum CO_2 evolution reaching 74.5 mg CO_2/kg/day in the combined treatment. The results indicated that indigenous microbial communities can be effectively stimulated to achieve significant hydrocarbon degradation.

Keywords: Hydrocarbon, Contamination, Bio-Remediation, Bio-Augmentation, Bio-Stimulation.

1.0 INTRODUCTION

The Niger Delta region of Nigeria has experienced extensive environmental degradation caused by hydrocarbon contamination (Umar et al., 2021). The region being a crucial ecological zone has faced severe environmental challenges resulting from decades of crude oil exploration and production activities. These activities have left indelible marks on the ecosystem, with significant threats to biodiversity, soil health, water quality, and human well-being (Mnif *et al*., 2017). Nigeria's reliance on crude oil, which accounts for approximately 80% of its foreign earnings, has exacerbated the environmental impact of these activities (Chijioke and Olisah, 2023). Anejionu *et al*., (2015); Elum *et al*., (2016) hinted that pollution from oil spills affects all environmental compartments, including the hydrosphere, lithosphere, biosphere, and atmosphere. Alboiu and Walker (2019); Enuneku *et al*, (2020) stated that the activities can lead to persistent hydrocarbon pollutants that, if left unremediated, can pose long-term risks to human and ecological health. The oil spills can result from oil well blowouts, tanker accidents, intentional or accidental pipeline ruptures, and improper disposal of petroleum waste. Dong *et al*., (2022) hinted that global approximation of 0.08 to 0.46% of total oil production is spilled into the environment annually, which consequently compounds the challenge of environmental pollution. Illegal activities such

as pipeline vandalism, crude oil theft, and artisanal refining in recent years have intensified hydrocarbon pollution in the Niger Delta region of Nigeria (Yeeles and Akporiaye, 2016; Ezirim, 2018). Mnif *et al*. (2017) observed that Oil spill is not only an issue in Nigeria but a global concern due to the adverse effects on human health, biodiversity, and ecosystems. These activities often lead to accidental discharges of crude oil and refined petroleum products on the soil and in the water bodies. Microbial-enhanced remediation processes for removal of such contaminant can offer an eco-friendly alternative to conventional methods such as mechanical removal, evaporation, incineration, and chemical treatments. Juwarkar *et al*. (2010) specified that bioremediation employs microorganisms to naturally degrade hydrocarbons, making it a promising and widely accepted approach for restoring contaminated environments Ukaegbu-Obi and Mbakwem-Aniebo (2014) hinted that these traditional methods of bioremediation are effective but may often be cost-intensive and environmentally intrusive. The success of the process depends on enriching and sustaining microbial populations in the contaminated environment which involves optimizaion of organic pollutant levels and maintenance of favorable environmental conditions, such as temperature, pH, and nutrient availability, to help influence microbial activity and pollutant degradation rates.

Li *et al.* (2023) stated that crude oil, as a multi-component hydrocarbon mixture, poses unique challenges for biodegradation. Individual microorganisms can only metabolize a narrow range of hydrocarbon substrates, necessitating the use of microbial consortia to degrade a broader spectrum of compounds. Motteran *et al.* (2024) hinted that more soluble hydrocarbons are generally easier for microorganisms to degrade. There is a pressing need for sustainable and effective remediation strategies to address this threat and safeguard the livelihoods of local communities and the ecosystem.

2.0 Materials and Methods

The experimental design began with site selection, soil sampling, microbial and physicochemical characterization, then experimental setup for biodegradation analysis.

2.1 Sample Site Selection

Selection of sampling sites was conducted at petroleum-impacted sites in Rivers and Delta states, which are within the Niger Delta region of Nigeria, an area globally recognized for its long-standing hydrocarbon pollution and ecological sensitivity. The sites were selected based on:

i. **Accessibility and safety:** Sites where sampling could be conducted without compromising safety or violating local regulations.
ii. **Stakeholder cooperation:** Community consent and support were obtained to ensure ethical and practical sampling.
iii. **History of petroleum contamination:** Locations with confirmed records of hydrocarbon spills resulting from oil exploration activities, pipeline leakages, or artisanal refining
iv. **Ecological relevance:** Areas with natural vegetation and minimal prior remediation interventions to reflect relatively undisturbed microbial populations.

Five sites were selected across two states in the Niger Delta: Delta State and Rivers State. The selected communities included Effurun (DUE), Jesse (DEJ), Eleme-Ebubu (REE), Bori (RKB), and Tai-Pete (RTP) as summarized in Table (2.1). Figures A to E showed the various sampling sites.

Table 1 Description of the Sampling Site

S/N	State	Local Govt. Area	Town	Latitude (N)	Longitude (E)	Site Code
1.	Delta	Uvwie	Effurun	5º 34' 17.65"	5º 46' 33.67"	DUE
2.	Delta	Ethiope West	Jesse	5º 55' 11.86"	5º 43' 45.80"	DEJ
3.	Rivers	Eleme	Ebubu	5º 04' 60.00"	6º 38' 59.99"	REE
4.	Rivers	Khana	Bori	4º 40' 34.64"	7º 21' 54.68"	RKB
5.	Rivers	Tai	Pete	4º 42' 59.99"	7º 17' 60.00"	RTP

(a): Sampling Site at Effurun (DUE)

(b): Sampling Site at Jesse (DEJ)

(c): Sampling Site at Ebubu (REE)

(d): Source of contamination at Bori (RKB)

(e): Sampling Site at Bori (RKB)

Soil samples were collected following standard environmental microbiology protocols (ISO, 2017; USEPA, 2002). At each location, three to four subsamples were randomly collected using a sterilized stainless-steel auger from a depth of 0–15 cm, representing the biologically active zone most relevant for microbial processes. The subsamples from each site were composited into a single representative sample, placed into sterile polyethylene bags, and transported on ice to the laboratory. All samples intended for delayed analysis were stored at 4 ± 2°C in accordance with ISO (2017) preservation standards to prevent microbial shifts. The following physicochemical parameters were measured in the laboratory after sample collection:

i. **Soil Temperature:** This was measured using a calibrated soil thermometer to determine thermal conditions influencing microbial enzymatic activity.
ii. **Soil pH:** This was assessed using a portable pH meter (1:2.5 soil-to-water ratio), since pH influences microbial diversity and hydrocarbon degradation kinetics.
iii. **Moisture Content:** The moisture content was determined using the gravimetric method by weighing fresh and oven dried samples (105°C for 24 hrs) to assess water availability, which directly affects microbial respiration and nutrient diffusion.
iv. **Soil Texture and Color:** This was qualitatively observed and recorded to support interpretation of permeability, porosity, and potential contaminant retention, in which soil particles were dispersed in water using a dispersing agent such as sodium hexametaphosphate. Based on sedimentation principles, the percentages of sand, silt, and clay were calculated and classified using the USDA soil textural triangle.
v. **Total Organic Carbon (TOC):** The TOC was determined in the laboratory using the Walkley–Black wet oxidation method. The amount of oxidized carbon was calculated and expressed as a percentage of the soil sample.
vi. **Inorganic Nutrients (Nitrate and Phosphate):** Inorganic nutrients, particularly nitrate and phosphate, were analyzed because they are essential nutrients required for microbial growth and enzymatic activities during biodegradation. The concentrations obtained were expressed in mg/kg of soil
vii. **Water Holding Capacity (WHC):** The WHC was determined by saturating a known weight of dry soil sample with distilled water and allowing excess water to drain under gravity for a specified period. The saturated soil was weighed and subsequently oven dried at 105°C until constant weight was achieved. The difference between saturated and dry weights was used to calculate the amount of water retained by the soil

Control Sample: Uncontaminated reference soil was collected from pristine sites approximately 10 km away from each contaminated location which served as controls for comparing physicochemical parameters, microbial activity, and biodegradation potential.

Total Heterotrophic and Hydrocarbon Utilizing Bacterial Counts

The enumeration of total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) was performed using the spread plate technique. Serial dilutions of each composite soil sample were prepared in sterile saline solution (0.85% NaCl). Aliquots (0.1 mL) from appropriate dilutions were plated in triplicate on the following media:

Morphological and Biochemical Characterization

Purified isolates were subjected to Gram staining and standard biochemical tests including catalase, oxidase, citrate utilization, motility, and carbohydrate fermentation using the API 20E system. These tests assisted in preliminary taxonomic identification.

Biodegradation Experiment Design

This study evaluated the effectiveness of selected bioremediation strategies, including Natural attenuation, Bio-stimulation, and Bio augmentation, using controlled laboratory microcosm. This approach was to ensure that the findings are scientifically reliable and environmentally applicable.

Laboratory-Scale Microcosm Setup

Microcosms were established under controlled laboratory conditions to simulate biodegradation processes. Each treatment group was tested in triplicate using homogenized contaminated soil samples obtained from the field.

Experimental Treatments

In order to evaluate the effectiveness of various bioremediation strategies, a series of controlled treatments was implemented using hydrocarbon-contaminated soil mixtures collected from the study sites. These treatments simulated realistic remediation scenarios, ranging from natural attenuation to enhanced biodegradation through nutrient supplementation and microbial augmentation. The key components of each experimental setup were outlines in Table 2.

Table 2: Overview of Experimental Treatment

S/N	Treatment Code	Treatment Name	Description
1	NA	Natural Attenuation	No amendments; relies solely on the activity of indigenous microorganisms.
2	BS	Bio-stimulation	Addition of nutrient supplements (e.g., nitrogen, phosphorus) to enhance native microbial activity
3	BA	Bio-augmentation	Introduction of previously isolated hydrocarbon-degrading microorganisms from the study sites.
4	BS + BA	Combined Treatment	Synergistic application of nutrient supplements and microbial inocula to enhance biodegradation.
5	SC	Sterile Control	Autoclaved soil to serve as an abiotic control (no microbial activity).

Monitoring Parameters

The selected monitoring parameters were measured to assess the progress and efficiency of hydrocarbon degradation and were grouped into four broad categories, namely: microbial activity, contaminant reduction, soil health indicators and environmental variables.

Microbial Enumeration

Total heterotrophic and hydrocarbon-utilizing bacteria were enumerated using the spread plate technique on Nutrient Agar (NA) and Mineral Salt Agar (MSA) amended with crude oil. Colonyforming units (CFU) were recorded at defined intervals (Days 0, 15, 30, 45, and 60) to monitor population dynamics. Changes in CFU counts over time served as an indirect measure of microbial adaptation and growth in response to contaminant degradation

Total Petroleum Hydrocarbons (TPH)

TPH levels were quantified using the gravimetric method, in which hydrocarbons were extracted with dichloromethane and evaporated to dryness for residue measurement. Periodic TPH measurements (Days 0, 15, 30, 45, and 60) were used to determine the rate and extent of hydrocarbon degradation across treatments

Gas Chromatography–Mass Spectrometry (GC–MS) Analysis of Hydrocarbon Composition

This was employed as a supporting analytical technique to qualitatively assess the compositional characteristics of petroleum hydrocarbons present in the contaminated

Data Analysis

Data generated were subjected to both descriptive and inferential statistical analyses to assess treatment performance, quantify biodegradation trends, and estimate kinetic model parameters. All analyses were conducted using Microsoft Excel.

Inferential Statistics (ANOVA)

A One-Way Analysis of Variance (ANOVA) was used to test for statistically significant differences in:

i. Hydrocarbon degradation across different treatment groups
ii. Microbial count variations between contaminated and control soils
iii. Soil respiration rates across treatments

3.0 RESULTS AND DISCUSSION

3.1 Environmental Parameters of Sampling Sites

Microbiological assessments included counts of Total Heterotrophic Bacteria (THB) and Hydrocarbon-Utilizing Bacteria (HUB), followed by the isolation and preliminary characterization of dominant indigenous strains. Table 3 showed the site-by-site physicochemical summary (mean ± SD, n = 3) of the sampled soil.

Table 3: Site-by-site physicochemical summary (mean ± SD, n = 3)

Site Location	DUE		DEJ		REE		RKB		RTP	
Soil										
Characteristics	A	B	A	B	A	B	A	B	A	B
pH	6.75 ± 0.12	5.95 ± 0.18	6.88 ± 0.10	5.80 ± 0.15	6.92 ± 0.11	5.45 ± 0.20	6.70 ± 0.09	5.65 ± 0.16	6.98 ± 0.12	5.72 ± 0.17
Moisture (%)	16.0 ± 0.6	28.2 ± 1.1	15.6 ± 0.5	26.8 ± 1.0	16.3 ± 0.7	29.6 ± 1.2	15.8 ± 0.6	27.2 ± 0.9	16.1 ± 0.6	28.5 ± 1.0
Temperature (°C)	27.6 ± 0.3	28.4 ± 0.4	27.3 ± 0.3	28.1 ± 0.4	27.4 ± 0.3	28.7 ± 0.5	27.0 ± 0.3	28.2 ± 0.4	27.5 ± 0.3	28.5 ± 0.4
TOC (%)	1.20 ± 0.08	4.10 ± 0.35	1.25 ± 0.07	4.35 ± 0.40	1.18 ± 0.06	5.95 ± 0.45	1.10 ± 0.05	3.55 ± 0.30	1.22 ± 0.08	4.30 ± 0.38
Nitrate (mg/kg)	46.0 ± 2.5	8.6 ± 1.9	45.2 ± 2.3	9.2 ± 1.8	47.0 ± 2.6	7.9 ± 1.6	44.0 ± 2.2	8.8 ± 1.7	46.5 ± 2.4	8.9 ± 1.8
Phosphate (mg/kg)	11.6 ± 1.0	4.0 ± 0.6	11.2 ± 0.9	3.8 ± 0.5	12.0 ± 1.1	3.6 ± 0.6	10.8 ± 0.8	4.3 ± 0.7	11.8 ± 1.0	4.1 ± 0.6

WHC (%)	30.2 ± 1.4	38.1 ± 1.9	29.5 ± 1.6	37.4 ± 2.0	31.0 ± 1.5	40.2 ± 2.2	29.8 ± 1.7	36.5 ± 1.8	30.7 ± 1.6	39.0 ± 2.1
Texture	Sandy loam	Sandy loam	Silty loam	Silty loam	Clay loam	Clay loam	Loam	Loam	Silty clay loam	Silty clay loam

(Key: A = Control soil, B = Contaminated soil)

The baseline characterization of the Niger Delta study sites as shown in Table 3 establishes the environmental boundary conditions for the outcomes observed in this study. Control soils displayed near-neutral pH values (6.7–7.0), whereas contaminated soils exhibited slight acidity (5.4–6.0). This shift aligns with literature by Azuazu *et al*. (2023) which indicates that petroleum hydrocarbon contamination induces acidification through the production of acidic metabolites during oxidation. Optimal hydrocarbon degradation typically requires near-neutral conditions (pH 6.5–8.0), the observed acidity in contaminated samples presents a potential inhibitory factor for specific microbial taxa and enzyme systems, likely favoring acid tolerant degraders. Operationally, this necessitates continuous pH monitoring and potential amendment, such as liming, to sustain broad-spectrum microbial activity during the remediation window as indicated by Evans *et al.* (2024).

3.1.1 Moisture Content and Water Holding Capacity (WHC)

Contaminated samples recorded elevated moisture levels (26–30%) compared to control soils (15–16%), a trend mirrored in WHC values (36–40% in contaminated vs. 30% in control). The increase in hydrocarbon-impacted soils is attributable to the formation of oil films that reduce infiltration or soil compaction that restricts drainage which corresponds with the findings by Ogbeide and Henry (2024). While moderate moisture facilitates nutrient diffusion, the observed levels approach saturation points where oxygen diffusion becomes limited. This corresponds with the findings by Azuazu *et al* (2023) which hinted that at high moisture content levels, oxygen diffusion becomes limited.

3.1.2 Soil Temperature

Temperature readings across sampled sites ranged from 27.0ºC to 28.7°C. These values fall squarely within the tropical mesophilic range (25–35°C), which is optimal for microbial growth and enzymatic hydrocarbon breakdown. This corresponds with the findings by Kamranifar et al. (2025), who noted that ambient tropical temperatures are rarely a limiting factor for biodegradation.

3.1.3 Total Organic Carbon (TOC)

A sharp contrast exists between the TOC content of control soils (1.1–1.3%) and contaminated soils (3.6–5.95%). These significantly higher values in the contaminated plots quantify the petroleum loading and organic hydrocarbon accumulation, consistent with regional assessments of oil-impacted Niger Delta sites which agrees with the findings by Moses et al. (2023). While elevated TOC provides an abundant carbon source, it distorts the Carbon–Nitrogen–Phosphorus

(C:N:P) ratio. Evans et al. (2024) stated that without stoichiometric balancing, the excess carbon leads to rapid nutrient exhaustion and slowed mineralization rates.

3.1.4 Inorganic Nutrients (Nitrate and Phosphate)

Nutrient analysis confirmed severe depletion in the contaminated zones. Nitrate (7.9–9.2 mg/kg) and phosphate (3.6–4.3 mg/kg) concentrations in contaminated soils were markedly lower than those in control soils (44–47 mg/kg and 10–12 mg/kg, respectively). This reduction validates the hypothesis that nitrogen and phosphorus availability act as the primary constraint on microbial degradation in these soils as hinted by Akinkpelumi *et al*. (2025).

3.1.5 Soil Texture

Soil textures ranged from sandy loam to clay loam across the sites. Finer textures, such as clay loam, exhibit lower permeability, restricting oxygen and nutrient diffusion, which may promote localized anaerobic conditions as stated by Azuazu *et al*. (2023). Coarser/sandy loams facilitate aeration but are susceptible to rapid nutrient leaching.

3.1.6 Baseline Hydrocarbon Contamination

Establishing the baseline levels of petroleum contamination across the sample sites provides the necessary context for interpreting microbial responses and biodegradation performance. The data set in Table (3.2) serve as the benchmark for evaluating contamination severity and the reference point for calculating biodegradation efficiency. From the results, contaminated soil samples across all sites exceeded the acceptable limits for hydrocarbon contamination according to the Nigeria Upstream Petroleum Regulatory Commission, NUPRC (2023).

3.2.1 Contamination Severity and Environmental Implications

The TPH concentrations in contaminated soils ranged from 6,970.2 mg/kg at RKB to 9,860.7 mg/kg at REE, exceeding the Nigerian Upstream Petroleum Regulatory Commission (NUPRC) intervention limit of 5,000 mg/kg for impacted soils. These levels indicate substantial hydrocarbon loading consistent with chronic spill conditions common in the Niger Delta. Control soil sample recorded 148.2–175.1 mg/kg, which fall within background levels previously reported for uncontaminated soils in the region by Onianwah *et al.* (2022).

3.2.2 PAH Composition and Distribution

Table 4: Baseline Total Petroleum Hydrocarbon (TPH) and Selected PAH in Soil Samples (mg/kg, mean ± SD, n = 3)

Site (Code)	Condition	TPH (mg/kg)	PAH			Total PAHs
			Naphthalene	Phenanthrene	Pyrene	
DUE (Effurun)	Control	148.2 ± 6.1	0.72 ± 0.05	0.54 ± 0.03	0.39 ± 0.02	1.65 ± 0.10
	Contaminated	7,520.4 ± 165.2	19.3 ± 1.4	11.8 ± 1.0	9.2 ± 0.7	40.3 ± 2.6
DEJ (Jesse)	Control	165.7 ± 5.4	0.68 ± 0.04	0.51 ± 0.03	0.43 ± 0.03	1.62 ± 0.09
	Contaminated	8,135.6 ± 174.3	22.5 ± 1.5	13.7 ± 1.1	10.8 ± 0.9	47.0 ± 2.8
REE (Ebubu)	Control	175.1 ± 6.2	0.81 ± 0.05	0.57 ± 0.04	0.44 ± 0.03	1.82 ± 0.12
	Contaminated	9,860.7 ± 210.5	25.1 ± 1.8	15.6 ± 1.2	12.5 ± 0.9	53.2 ± 3.0
RKB (Bori)	Control	160.3 ± 5.8	0.76 ± 0.05	0.52 ± 0.03	0.42 ± 0.02	1.70 ± 0.09
	Contaminated	6,970.2 ± 142.4	17.2 ± 1.3	10.3 ± 0.8	8.7 ± 0.6	36.2 ± 2.4
RTP (Tai-Pete)	Control	172.5 ± 6.0	0.80 ± 0.04	0.55 ± 0.03	0.46 ± 0.02	1.81 ± 0.10
	Contaminated	8,740.3 ± 188.7	23.8 ± 1.6	14.6 ± 1.0	11.6 ± 0.8	50.0 ± 2.8
NUPRC Accepted contamination limits		<5000	<50mg/kg	<40mg/kg	<40mg/kg	

Total PAH concentrations (ΣPAHs) in contaminated soils ranged from its lowest 36.2 mg/kg (RKB) to its highest 53.2 mg/kg (REE), representing approximately 0.4–0.6% of the corresponding TPH. Naphthalene accounted for 45–50% of ΣPAHs, phenanthrene for approximately 30%, and pyrene for around 20%. This composition reflects contamination dominated by low-molecular-weight PAHs, which is typical of fresh or moderately weathered light crude contamination. The continued presence of phenanthrene and pyrene suggests long-term inputs and slower natural attenuation of heavier aromatic structures. Similar profiles have been documented in crude oil-impacted Niger Delta soils by Okoye *et al*. (2020).

3.2.3 Site-Specific Comparisons

The REE site recorded the highest hydrocarbon burden (TPH = 9,860.7 mg/kg; ΣPAHs = 53.2 mg/kg), consistent with persistent oil pollution historically associated with the area which agrees with earlier literature by Anyanwu *et al.* (2023). DUE and RTP also exhibited elevated TPH (>7,000 mg/kg), indicating active contamination or repeated exposure. RKB showed comparatively lower contamination, though still above regulatory limits, suggesting partial natural attenuation or reduced spill frequency. Across all uncontaminated samples, TPH values remained below the NUPRC target cleanup level of 50 mg/kg, confirming minimal background impact and providing a robust contrast for treatment evaluations.

3.2.4 Implications for Bioremediation Design

The high TPH and PAH concentrations reflect carbon-rich but nutrient-limited conditions, typical of oil-impacted Niger Delta soils. This imbalance supports the use of bio-stimulation (nitrogen and phosphorus amendments) and bio-augmentation strategies employed in the study. Faster mineralization is expected for naphthalene and other light aromatics, while phenanthrene and pyrene degradation will rely on slower oxygenase-mediated ring cleavage processes, consistent with observations by Kamranifar et al. (2025).

3.3 Microbial Enumeration and Diversity

Total heterotrophic bacteria (THB) and hydrocarbon-utilizing bacteria (HUB) were quantified using the standard plate count method on Nutrient Agar (THB) and Mineral Salt Agar supplemented with crude oil (HUB). The baseline counts show clear differences between contaminated and control soils across all five study locations as shown in Table (3.3). Control soils exhibited THB values between 5.75 and 5.88 $\log_{10}$ CFU/g, while contaminated soils ranged from 6.70 to 7.02 $\log_{10}$ CFU/g. HUB populations followed a similar trend, increasing from 4.28–4.35 $\log_{10}$ CFU/g in control soils to 5.86–6.12 $\log_{10}$ CFU/g in contaminated soils. The HUB/THB ratio provides an additional indicator of hydrocarbon-degrading capacity. Ratios in control soils were uniformly **0.07**, reflecting low background hydrocarbon exposure. Ratios in contaminated soils increased to **0.15–0.17**, with the highest value recorded at REE as shown in Table (5).

Table 5: Baseline Microbial Counts ($\log_{10}$ CFU/g)

Site	Sample	THB (mean ± SD)	HUB (mean ± SD)	HUB/THB Ratio
DUE	Control	5.82 ± 0.08	4.32 ± 0.06	0.07
	Contaminated	6.85 ± 0.11	5.97 ± 0.09	0.15
DEJ	Control	5.75 ± 0.07	4.28 ± 0.05	0.07
	Contaminated	6.78 ± 0.10	5.91 ± 0.08	0.15
REE	Control	5.88 ± 0.09	4.35 ± 0.07	0.07
	Contaminated	7.02 ± 0.12	6.12 ± 0.10	0.17
RKB	Control	5.79 ± 0.08	4.30 ± 0.06	0.07
	Contaminated	6.70 ± 0.11	5.86 ± 0.09	0.15
RTP	Control	5.83 ± 0.09	4.33 ± 0.06	0.07
	Contaminated	6.75 ± 0.10	5.90 ± 0.08	0.15

3.3.1 Microbial Population Dynamics over Time

Temporal changes in hydrocarbon-utilizing bacteria (HUB) in the samples combined were monitored over a 60-day period under five treatment conditions: Natural Attenuation (NA), Bio-stimulation (BS), Bio-augmentation (BA), Combined Bio-stimulation and Bio-augmentation (BS + BA), and Sterile Control (SC). The HUB counts, expressed as $\log_{10}$ CFU/g of dry soil, are presented in Table 6.

Table 6: Hydrocarbon-Utilizing Bacteria (HUB) Counts ($\log_{10}$ CFU/g) Across Treatments and Time

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60
Natural					
Attenuation (NA)	5.95	6.1	6.25	6.3	6.35
Bio-stimulation					
(BS)	5.95	6.45	6.85	7.05	7.1
Bio-augmentation					
(BA)	5.95	6.6	7	7.2	7.3
Combined BS + BA	5.95	6.75	7.2	7.4	7.5
Sterile Control					
(SC)	5.95	5.85	5.7	5.5	5.4

3.4. Hydrocarbon-Utilizing Bacteria (*HUB)*

The HUB populations increased across all biologically active treatments. Under NA, counts rose from **5.95** at Day 0 to **6.35** at Day 60, demonstrating the capacity of indigenous microbes to respond to hydrocarbon inputs without external amendments. BS and BA produced stronger responses, reaching **7.10** and **7.30**, respectively, by Day 60. The combined BS + BA treatment produced the highest HUB counts throughout the study, increasing from **5.95** at Day 0 to **7.50** at Day 60. The sterile control exhibited a continuous decline from 5.95 at Day 0 to 5.40 at Day 60, pointing the absence of active microbial contribution.

3.5 Total Petroleum Hydrocarbon (TPH) Degradation Trends

Total petroleum hydrocarbon (TPH) degradation was monitored over a 60-day period under five treatment conditions: Natural Attenuation (NA), Bio-stimulation (BS), Bio-augmentation (BA), Combined Bio-stimulation + Bio-augmentation (BS + BA), and Sterile Control (SC (Table 7). Sampling was conducted at 15-day intervals to evaluate both biotic and abiotic contributions to hydrocarbon removal. TPH concentrations were quantified gravimetrically following solvent extraction with dichloromethane.

Table 7: TPH Concentrations (mg/kg) Over Time under Different Treatments

Treatment	Day 0	Day 15	Day 30	Day 45	Day 60	% Reduction
Natural Attenuation (NA)	6800 ± 150	6100 ± 140	5200 ± 130	4700 ± 120	4200 ± 110	38.20%
Bio-stimulation (BS)	6800 ± 150	5000 ± 130	3600 ± 120	2500 ± 110	1900 ± 100	72.10%
Bio-augmentation (BA)	6800 ± 150	4800 ± 120	3400 ± 110	2200 ± 100	1600 ± 90	76.50%
Combined BS + BA	6800 ± 150	4300 ± 120	2800 ± 100	1600 ± 90	1100 ± 80	83.80%
Sterile Control (SC)	6800 ± 150	6700 ± 140	6600 ± 140	6500 ± 130	6400 ± 120	5.90%

3.5.1 Natural Attenuation (NA)

The NA treatment achieved a 38.2% reduction within 60 days, driven solely by indigenous hydrocarbon degrading microorganisms and natural nutrient recycling. The modest degradation rate reflects known nutrient and oxygen limitations commonly encountered in Niger Delta soils as stated by Kuppusamy et al., (2020).

3.5.2 Bio-stimulation (BS)

The addition of Nitrogen and Phosphorus significantly accelerated degradation, achieving a 72.1% reduction by Day 60. This trend mirrors the elevated microbial respiration described in Section 4.5 and reinforces that nutrient deficiency is a primary bottleneck in natural hydrocarbon attenuation. Similar nutrient-enhanced biodegradation was reported in tropical soils by Abioye and Ikhumetse (2022); Ite *et al*. (2023).

3.5.3 Bio-augmentation (BA)

The BA treatment achieved a 76.5% reduction slightly outperforming BS reflecting the strong degradation capabilities of the introduced *Pseudomonas* and *Bacillus* isolates. Their known enzyme diversity, bio-surfactant production, and resilience in hydrocarbon-rich environments as indicated by Dutta et al. (2023), contributed to the robust degradation pattern, consistent with observed trends in microbial proliferation and CO_2 evolution

3.5.4 Combined Bio-stimulation + Bio-augmentation (BS + BA)

The BS + BA treatment recorded the highest TPH reduction at 83.8%, demonstrating a clear synergistic effect between nutrient supplementation and microbial inoculation. The most rapid decline occurred between Days 15 and 45, coinciding with the exponential microbial growth phase as indicated by Ogunbiyi et al. (2023), showing that combined amendments significantly enhance hydrocarbon degradation in tropical soils.

3.5.5 Sterile Control (SC)

The minimal reduction (5.9%) observed in the SC treatment confirms that abiotic losses such as volatilization, sorption, or chemical oxidation, play only a minor role. This suggests that the substantial reductions in other treatments were driven by biological activity.

3.6 Statistical Analysis of Treatment Effects

Statistical analyses were conducted to evaluate whether differences in treatment performance (NA, BS, BA, BS + BA, and SC) were significant and to quantify relationships among the measured biodegradation indicators. The results were presented using analysis of variance (ANOVA), Tukey's HSD post-hoc comparisons, and correlation/regression outputs.

3.6.1 Analysis of Variance (ANOVA)

A one-way ANOVA was used to compare mean TPH concentrations among the treatment groups at Day 60. The analysis revealed a statistically significant difference in TPH levels across treatments ($F(4, 10) = 148.26$, $p < 0.001$). This outcome demonstrates that the remediation strategies produced distinct removal efficiencies and that these differences were not attributable to random variation. The Sterile Control maintained the highest residual TPH concentration, while the BS + BA treatment achieved the lowest value, consistent with the degradation patterns previously documented.

3.6.2 Correlation Analysis of Degradation Indicators

Pearson correlation analysis was used to evaluate the relationships among TPH concentration, HUB counts, and CO_2 evolution. A strong inverse relationship was observed between TPH concentration and HUB abundance ($r = –0.95$, $p < 0.001$), confirming that reductions in hydrocarbons corresponded directly to increases in microbial populations. TPH concentration also

showed a very strong negative correlation with CO_2 evolution ($r = –0.99$, $p < 0.001$), reflecting the increase in respiration associated with active hydrocarbon mineralization.

4.0 CONCLUSION

Indigenous hydrocarbon-utilizing bacteria were present in all soils samples, but their activity differed sharply between uncontaminated and contaminated samples. The findings indicated that microbial degradation of crude oil in Niger Delta soils is driven less by the presence of degraders and more by the constraints imposed by low pH, depleted inorganic nutrients, and high organic loading.

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