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Bioremediation of Oil Spills in the Niger Delta Region of Nigeria: A Mesocosm Simulation

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Abstract: Oil pollution remains one of the most pressing environmental challenges in Nigeria's Niger Delta, leading to ecological damage and loss of livelihoods. Bioremediation, specifically nutrient amendment (biostimulation), offers a low-cost, eco-friendly alternative to conventional remediation methods. This study used a mesocosm simulation to evaluate the comparative effectiveness of ammonium, nitrate, and organic nitrogen sources on petroleum hydrocarbon degradation. Soils collected from three oil-impacted Niger Delta communities were artificially contaminated with diesel and treated with nitrogen amendments. Results showed that ammonium enhanced rapid hydrocarbon breakdown, while organic nitrogen sustained the highest microbial proliferation and long-term nutrient availability. Regression analysis confirmed strong positive correlations (r = 0.93–0.96) between nitrogen concentration and microbial activity. These findings demonstrate that nitrogen-based biostimulation can significantly accelerate remediation of petroleum-contaminated soils in tropical wetland ecosystems and provide evidence for field-scale application in the Niger Delta.
Keywords: Bioremediation, Mesocosm, Niger Delta, Hydrocarbon Contamination, Nitrogen Amendment, Microbial Activity

1.0 Introduction

Oil pollution represents one of the most pressing environmental and socio-economic challenges in Nigeria's Niger Delta region. Over five decades of petroleum exploration, pipeline sabotage, equipment failure, and artisanal refining have resulted in the release of millions of barrels of crude oil into soils, rivers, and wetlands. The United Nations Environment Programme (UNEP, 2011) reported that many Niger Delta communities are continuously exposed to hydrocarbons and their derivatives, with widespread contamination of agricultural land, mangrove ecosystems, and drinking water sources. The persistence of petroleum hydrocarbons such as benzene and polycyclic aromatic hydrocarbons (PAHs) in soils and sediments has contributed to declines in agricultural productivity, increased cancer risks, and the loss of biodiversity (Renoux et al., 2000; Ugochukwu & Ertel, 2008; Zabbey & Uyi, 2014).

Beyond the ecological consequences, oil pollution in the Niger Delta has deepened economic hardship and community vulnerability. Farming and fishing, the principal occupations in rural communities, are severely constrained by soil toxicity, destruction of aquatic habitats, and recurrent crop failures (Nwilo & Badejo, 2006). Many households have been forced to abandon traditional livelihoods, exacerbating poverty and dependence on external aid. At the same time, conflicts over environmental justice and corporate responsibility have fueled unrest, highlighting the urgent need for effective, low-cost, and sustainable remediation approaches.

Conventional remediation technologies-such as soil excavation, physical containment, thermal desorption, and the use of dispersants-while effective in localized or industrialized contexts, present significant drawbacks in the Niger Delta. They are often prohibitively expensive, require specialized equipment, and may produce secondary pollution (Das & Chandran, 2011). In wetland ecosystems, mechanical removal and dispersants disrupt fragile habitats and fail to restore soil health. This reality has motivated the search for environmentally compatible alternatives that can be deployed across vast, often inaccessible contaminated areas.

Bioremediation has emerged as one such solution. By harnessing the metabolic activity of indigenous microorganisms capable of hydrocarbon degradation, bioremediation offers a strategy that is both ecologically compatible and cost-effective (Atlas & Hazen, 2011). Hydrocarbon-degrading microbes, including species of Pseudomonas, Bacillus, Acinetobacter, and Rhodococcus, utilize petroleum compounds as sources of carbon and energy, ultimately mineralizing them into carbon dioxide, water, and microbial biomass (Rahman et al., 2003). However, successful bioremediation depends on the availability of essential nutrients such as nitrogen and phosphorus, which are usually deficient in petroleum-contaminated soils. Without supplementation, microbial populations remain limited, and hydrocarbon degradation proceeds at suboptimal rates (Leahy & Colwell, 1990; Seymour et al., 1996).

1.1 Nutrient Limitation and the Case for Biostimulation

The biodegradation of hydrocarbons is a nitrogen-intensive process because microbial cells require nitrogen for protein synthesis, nucleic acids, and enzyme production. Oil-contaminated soils in the Niger Delta typically exhibit high carbon loads (from hydrocarbons) but negligible nitrogen and phosphorus levels, creating an imbalanced carbon-to-nitrogen (C:N) ratio that restricts microbial metabolism (Bossert & Bartha, 1984). Empirical evidence has demonstrated that nutrient amendment-often termed biostimulation-can dramatically accelerate hydrocarbon degradation in contaminated soils (Venosa et al., 2002; Bento et al., 2005).

Nitrogen supplementation can be provided in various forms: ammonium (NH_4^+), nitrate (NO_3^-), or organic nitrogen sources such as compost or biosolids. Each form has distinct advantages and limitations. Ammonium is rapidly assimilated by microbes, supporting fast microbial growth, but can be lost through volatilization or leaching. Nitrate is stable under aerobic conditions but highly leachable, especially in high-rainfall tropical regions like the Niger Delta. Organic nitrogen sources release nutrients slowly through decomposition, promoting long-term fertility but with delayed hydrocarbon degradation benefits (Singh et al., 2007; Macaulay, 2015). Despite extensive international research, comparative studies on these nitrogen sources under Niger Delta soil conditions remain limited.

1.2 Mesocosm Simulation as a Research Tool

Field-scale bioremediation studies in the Niger Delta are often constrained by accessibility, cost, and security challenges. Mesocosm experiments-controlled, outdoor simulations of natural ecosystems-offer a valuable alternative by enabling systematic testing of treatments under near-field conditions. Mesocosm studies allow for the monitoring of microbial dynamics, nutrient transformations, and contaminant degradation while minimizing environmental risk (Płaza et al., 2005). They also provide predictive insights into how remediation strategies might perform in complex tropical soils characterized by acidity, flooding, and low nutrient retention.

1.3 Baseline Soil Conditions in Niger Delta Sites

Baseline soil analyses from the sampled Niger Delta communities (Batan, Ajuju, and Umusia) underscore the extent of nutrient limitation in oil-contaminated soils. As shown in Table 1, soils were acidic (pH 4.5–4.7), low in organic carbon (<2%), and extremely nitrogen-deficient (<0.05%). These conditions highlight why natural attenuation is insufficient and why nutrient amendment is critical for effective bioremediation.

Table 1. Initial Physicochemical Properties of Soils from Selected Sites

Property	**Batan**	**Ajuju**	**Umusia**
pH (1:1 soil:water)	4.61	4.55	4.70
Texture	Sandy Loam	Sandy Clay Loam	Sandy Loam
Organic Carbon (%)	1.81	1.57	1.43
Total Nitrogen (%)	0.045	0.038	0.042
Available Phosphorus (mg/kg)	7.4	6.1	6.8

These data reveal conditions typical of post-spill soils in tropical environments: acidic, nutrient-poor, and prone to leaching. They also provide the foundation for testing whether nitrogen amendment can significantly improve microbial proliferation and hydrocarbon degradation under simulated spill conditions.

1.4 Objectives of the Study

This study aims to evaluate the comparative effectiveness of three nitrogen sources—ammonium, nitrate, and organic nitrogen—in enhancing bioremediation of petroleum-contaminated soils from the Niger Delta. Specifically, the objectives are to:

- Assess the impact of each nitrogen source on soil nitrogen dynamics over time.
- Examine microbial population responses under different treatments.
- Evaluate the extent of total petroleum hydrocarbon (TPH) and polyaromatic hydrocarbon (PAH) degradation.
- Determine correlations between nutrient availability, microbial proliferation, and hydrocarbon reduction.

By conducting a mesocosm simulation, this study provides critical empirical evidence to guide nutrient-based bioremediation strategies in the Niger Delta. Ultimately, the findings contribute to the development of scalable, ecologically sustainable, and community-relevant oil spill remediation practices.

2.0 Literature Review

2.1 Principles of Bioremediation of Petroleum Hydrocarbons

Bioremediation harnesses the metabolic capacity of microorganisms to transform petroleum hydrocarbons (PHCs) into simpler, less toxic products (ultimately CO_2, H_2O, and biomass) via catabolic pathways that cleave aliphatic chains and ring structures (Atlas & Hazen, 2011; Leahy & Colwell, 1990). Aerobic oxidation typically proceeds through mono- and dioxygenase-mediated activation, β-oxidation of fatty acids, and ring-cleavage of aromatics; anaerobic routes (e.g., fumarate addition) become relevant in waterlogged or oxygen-limited soils. The success of these processes depends on bioavailability (sorption/desorption, aging), electron acceptor availability (O_2, NO_3^-), nutrient sufficiency (N, P), moisture, pH, temperature, and indigenous community structure (Das & Chandran, 2011; Dibble & Bartha, 1979). In oil-impacted wetlands like the Niger Delta, fluctuating redox and frequent inundation complicate these controls, making strategic stimulation of limiting factors essential.

2.2 Microbial Ecology of Hydrocarbon Degradation

Over 200 bacterial genera contribute to PHC attenuation, notably Pseudomonas, Acinetobacter, Rhodococcus, Bacillus, with yeasts and filamentous fungi (e.g., Aspergillus, Penicillium) enhancing degradation under certain conditions through extracellular enzymes and biosurfactants (Atlas, 1984; Rahman et al., 2003). Community succession is common: opportunistic r-strategists bloom following nutrient enrichment and hydrocarbon inputs; later, specialists degrade recalcitrant fractions (medium-high MW PAHs). Selective enumeration methods (e.g., MPN for aliphatics vs. aromatics) provide resolution into guild dynamics and functional capacity (Wrenn & Venosa, 1996; Mills et al., 1978). In tropical acidic soils, consortia rather than single strains often perform best due to complementarity in substrate range and stress tolerance (Suidan & Wrenn, 2000; Bento et al., 2005).

2.3 Hydrocarbon Classes and Degradation Pathways

n-Alkanes are generally most labile; isoprenoids and branched/cycloalkanes degrade more slowly; mono-aromatics (BTEX) can be rapidly oxidized under aerobic conditions; higher PAHs (4-6 rings) are persistent due to low solubility and sorption (Atlas & Bartha, 1973; Das & Chandran, 2011). In soils, weathering "ages" residues, increasing sequestration in soil organic matter and micropores, thus reducing bioavailability. Biosurfactants and natural organic matter can enhance desorption, but excessive surfactants may inhibit cells or mobilize contaminants to groundwater (Xie et al., 1999; Rahman et al., 2003). The differential degradability of TPH fractions explains why nutrient-stimulated microbial growth does not always translate uniformly across all hydrocarbon classes-an issue directly addressed by pairing TPH and PAH metrics with microbial counts in many studies.

2.4 Nutrient Limitation and Stoichiometric Controls

Hydrocarbon-rich soils typically exhibit high C relative to N and P, depressing biomass yield and catabolic enzyme synthesis. Optimal C:N ratios for efficient biodegradation commonly range ~10–20:1; in practice, site-specific mineralization efficiencies and soil chemistry dictate dosing (Bossert & Bartha, 1984; Venosa et al., 2002). In the Niger Delta, acidic pH, sandy textures, and high rainfall promote nitrogen losses (volatilization, leaching), so amendments must balance immediacy with persistence (Jackson & Pardue, 1999; Singh et al., 2007). Phosphorus and micronutrients can co-limit activity and should be considered when scaling biostimulation beyond pilot scales (Salanitro et al., 1997; USEPA, 1999b). The study by Egbebike et al (2025) addresses these stoichiometric constraints explicitly, motivating the comparison of nitrogen forms within a mesocosm that simulates regional conditions.

2.5 Comparative Performance of Nitrogen Sources

Ammonium (NH_4^+)-often supplied as $(NH_4)_2SO_4$-supports rapid uptake and growth, accelerating early TPH declines; however, in low-buffer soils it can acidify the matrix and be lost via volatilization or nitrification-denitrification pathways during waterlogging. Still, stronger sorption to organic matter can reduce washout compared with nitrate in marshes (Jackson & Pardue, 1999; Roling & van Bodegom, 2014).

Nitrate (NO_3^-)-e.g., KNO_3-can serve as an electron acceptor under hypoxia but is highly leachable in tropical rainfall regimes, sometimes yielding muted, variable responses unless dosing and timing are optimized (Singh et al., 2007).

Organic N-manures, compost, biosolids-releases nitrogen gradually, improves soil structure, and delivers micronutrients and organic substrates that foster robust, diverse consortia and sustained activity; the tradeoff is a slower onset for contaminant reduction (Das & Chandran, 2011; Macaulay, 2015).
Studies comparing these sources in coastal, wetland, and temperate soils consistently report that inorganic N (especially ammonium) drives early, steep TPH declines, whereas organic N yields longer-lived microbial stimulation and more stable N pools-an overall pattern mirrored by the mesocosm data used in this paper (Egbebike et al., 2025)

2.6 Environmental Modifiers: pH, Moisture, Redox, and Temperature

pH: Most degraders prefer near-neutral pH; acid soils (pH ≈4.5–5) common in the Niger Delta suppress activity and alter N cycling. Liming can help but may not be feasible at scale.
Moisture/Redox: Periodic inundation curtails O_2 diffusion, favoring denitrification and limiting aerobic dioxygenase activity; careful water management or engineered aeration can alleviate constraints in mesocosms and field plots (Lee, 2000; Venosa & Zhu, 2003).
Temperature: Tropical temperatures often fall within permissive ranges for mesophiles, accelerating kinetics relative to cold climates; however, heat stress and desiccation at the surface can counteract benefits.
Texture/CEC: Sandy loams with low CEC-typical of the study sites-promote leaching of nitrate and ammonium; organic additions that increase CEC and moisture retention may stabilize nutrients and enhance long-term function (Renoux et al., 2000). The baseline site data (acidic pH, low N, sandy textures) underscore the need for both immediate and sustained N supply, justifying side-by-side testing of inorganic and organic sources.

2.7 Biostimulation vs. Bioaugmentation; Role of Biosurfactants

Biostimulation (nutrient/oxygen/moisture optimization) often succeeds where robust indigenous degraders exist-common in chronically impacted regions-without the ecological risks and viability challenges of bioaugmentation (introducing exogenous strains) (Atlas & Bartha, 1973; Bento et al., 2005). Biosurfactants (e.g., rhamnolipids) can increase bioavailability and are sometimes co-amended with nutrients, but dosage and timing are critical to avoid toxicity or unintended mobilization (Rahman et al., 2003). The present study's focus on biostimulation aligns with field-practical remediation and regulatory comfort levels in the Niger Delta.

2.8 Mesocosm Experiments as Translational Platforms

Mesocosms bridge controlled laboratory tests and complex field trials, allowing manipulation of single variables (e.g., N source) while reflecting realistic fluxes of heat, light, and precipitation (Płaza et al., 2005). They enable repeated sampling for microbiology (CFU/MPN), chemistry (TPH, PAH), and toxicity endpoints while maintaining replicated statistical designs (RCBD). Notably, mesocosms can expose treatment × environment interactions (e.g., nitrogen form × rainfall events) that are otherwise confounded in open field sites. Such designs are an established step in USEPA guidance before shoreline/wetland deployment (USEPA, 1999a; Venosa et al., 1996). The study by Egbebike et al. (2025) comparing ammonium, nitrate, and organic N under graded contamination is thus consistent with best practices for evidence-based scale-up.

2.9 Monitoring Metrics: Chemical, Microbial, and Ecotoxicological Endpoints

Chemical: Gravimetric TPH remains a field workhorse but lacks specificity; coupling to GC–MS profiles for PAH classes better captures risk-relevant fractions (Salanitro et al., 1997).

Microbial: Plate counts (total heterotrophs, hydrocarbon degraders) and MPN methods provide functional indicators; correlation/regression linking N dynamics with CFU and TPH/PAH trends strengthens causal inference (Wrenn & Venosa, 1996; Mills et al., 1978).

Ecotoxicology: Bioassays (e.g., seed germination, earthworm survival, microbial respiration) complement chemistry by reflecting integrated biological recovery and informing ecological risk (Płaza et al., 2005; Saterbak et al., 1999). This study's combined use of TPH/PAH plus microbiology aligns with this dual-parameter paradigm advocated in risk frameworks.

2.10 Evidence from Wetlands and Shorelines

Field and mesocosm studies in marshes and shorelines underscore nutrient addition as a key lever for accelerating natural attenuation, with context-specific caveats (Lee & Levy, 1987, 1989; Venosa et al., 2002; Venosa & Zhu, 2003). In salt marshes, ammonium sorption can reduce washout relative to nitrate, enhancing persistence after tidal flushing (Jackson & Pardue, 1999). However, over-fertilization risks eutrophication or shifts in plant–microbe interactions. Consequently, adaptive dosing and monitoring are vital. These lessons transfer to the Niger Delta's tidally influenced creeks and floodplains, where hydrology dictates amendment fate.

2.11 Niger Delta Context and Remaining Gaps

Regional studies document significant TPH loadings, acid soils, and recurrent flooding, with mixed outcomes from remediation campaigns due to logistical and ecological constraints (UNEP, 2011; Obayori et al., 2009; Okoh, 2006). Key gaps include:

- Comparative nitrogen source efficacy under realistic tropical conditions;
- Persistence vs. performance tradeoffs (fast-acting inorganic vs. sustained organic N);

- Integration of chemical and biological endpoints tied to soil function and risk reduction;
- Operational guidance for field dosing under variable rainfall and pH.

Egbebike et al. (2025) addresses these gaps by experimentally contrasting nitrate, ammonium, and organic N across contamination levels, tracking total N, microbial populations, and TPH/PAH over 108 days.

2.12 Conceptual and Quantitative Frameworks

Resource-ratio theory predicts that altering N availability shifts competitive outcomes among microbial guilds, potentially optimizing community function for hydrocarbon catabolism (Smith et al., 1998). Empirically, regression and correlation analyses linking N concentration to microbial responses and TPH/PAH decline provide quantitative evidence for stimulation efficacy; high r-values (≈0.93–0.96) suggest strong coupling under mesocosm conditions (consistent with this study's findings). Such models, supplemented by ANOVA with appropriate post-hoc tests (e.g., FLSD), support inferential rigor and translational guidance for field operations.

2.13 Synthesis: Toward Context-Responsive Biostimulation

Across climates and ecosystem types, the weight of evidence shows: (i) nitrogen limitation commonly constrains PHC degradation; (ii) ammonium often maximizes early-phase TPH reductions; (iii) organic N maximizes longer-term microbial stability and nitrogen retention; (iv) nitrate performance is site-dependent and vulnerable to leaching; and (v) coupling chemical endpoints with microbial and ecotoxicological indicators best reflects meaningful recovery (Egbebike, et al., 2025; Venosa et al., 1996; 2002; Salanitro et al., 1997; Płaza et al., 2005). In the Niger Delta, where acidic, low-CEC soils and heavy rainfall are the norm, blended strategies (fast + slow release) are increasingly recommended to reconcile rapid cleanup targets with sustained soil health. The present mesocosm evidence base directly informs such blended, context-responsive protocols.

3.0 Materials and Methods

3.1 Study Area Description

The Niger Delta of Nigeria, one of the world's most extensive wetlands, lies between latitudes 4° and 6°N and longitudes 5° and 8°E. It spans about 70,000 km² and supports an estimated 30 million inhabitants, most of whom depend on subsistence agriculture and artisanal fishing. The region is characterized by high annual rainfall (2,000–3,800 mm), relative humidity above 80%, and mean annual temperatures near 25°C, creating conditions of frequent flooding, leaching, and waterlogging. These hydrological regimes strongly influence soil chemistry, often leading to acidity, poor nutrient retention, and limited aeration.

Three oil-impacted communities were selected for this study: Batan and Ajuju (Bayelsa State) and Umsia (Oyigbo LGA, Rivers State). These sites were chosen based on historical records of frequent oil spills, community accessibility, and representativeness of typical Niger Delta soils. Field reconnaissance revealed that the soils were sandy loam to sandy clay loam, poorly aggregated, and contained visible oil residues. Vegetation was sparse, with dominance of oil-tolerant grasses and shrubs.

The study area is ecologically significant as a biodiversity hotspot yet severely compromised by petroleum contamination. Selecting such sites ensured that the mesocosm experiment was representative of real-world conditions faced by Niger Delta soils.

3.2 Soil Sampling and Pre-treatment

Sampling was conducted using a stratified random design. Each site was divided into three zones, and composite soil samples were collected at depths of 0–15 cm with a sterilized soil auger. To minimize contamination, tools were washed with ethanol and flame-sterilized between collections. Samples were immediately sealed in sterile polyethylene bags and transported under chilled conditions to the Environmental Microbiology Laboratory, University of Benin.

Pre-treatment involved air-drying soils at ambient temperature (~27°C) to constant weight, passing through a 2 mm mesh sieve, and homogenizing to reduce variability. This step simulated typical field soils that have undergone partial desiccation after spills. Subsamples were analyzed for baseline physicochemical and microbiological properties, including soil texture (Bouyoucos hydrometer method), pH (glass electrode in 1:1 soil:water suspension), total organic carbon (Walkley-Black method), total nitrogen (Kjeldahl method), and available phosphorus (persulfate digestion). Hydrocarbon levels were measured via gravimetric extraction (total petroleum hydrocarbons, TPH) and gas chromatography-mass spectrometry (PAHs). Microbial populations were quantified using standard plate counts for heterotrophs and hydrocarbon-degrading bacteria.

3.3 Experimental Design and Mesocosm Setup

A 4 × 3 factorial randomized complete block design (RCBD) was used with three replicates per treatment, yielding 36 mesocosms in total. The two main factors were:

1. **Nitrogen Source (4 levels):**
    - N0 = Control (no amendment)
    - N1 = Nitrate (potassium nitrate, KNO_3)
    - N2 = Ammonium (ammonium sulfate, $(NH_4)_2SO_4$)
    - N3 = Organic nitrogen (biosolids, sourced locally and analyzed for N content before application)
2. **Oil Contamination Level (3 levels):**
    - P0 = No oil (control)
    - P1 = 20 g/kg of soil (moderate contamination)

- P2 = 80 g/kg of soil (severe contamination)

Each mesocosm consisted of a 10 L plastic container lined with polyethylene to prevent leakage and cross-contamination. Approximately 8 kg of soil was placed in each mesocosm. Soils were artificially spiked with automotive gas oil (AGO, diesel fuel) to simulate oil spill conditions. Spiking was done by thoroughly mixing pre-weighed diesel into soils to achieve desired contamination levels, followed by a 7-day aging period to allow partial volatilization and sorption of hydrocarbons-mimicking natural field weathering processes.
Nutrient amendments were applied after aging. Inorganic fertilizers (N1, N2) were dissolved in sterile distilled water and sprayed evenly across the soil surface. Organic biosolids (N3) were dried, ground, and homogenized before incorporation to ensure equivalent nitrogen dosing across treatments.

3.4 Treatment Application and Monitoring

Fertilizer application rates were normalized to supply equivalent nitrogen concentrations (~120 kg N/ha, extrapolated for mesocosm scale). Care was taken to avoid nutrient oversaturation and leaching losses by adjusting application volumes relative to soil water-holding capacity.
Each mesocosm was irrigated with deionized water to maintain moisture at ~60% water-holding capacity, monitored by gravimetric methods. Containers were perforated at the base for drainage, simulating natural leaching, and placed under ambient outdoor conditions (temperature: 25–32°C; rainfall excluded by transparent shelter).
Soil samples were collected at four intervals: Day 0 (baseline), Day 7, Day 49, and Day 108. These time points were chosen to capture both short-term and long-term dynamics of nutrient availability, microbial proliferation, and hydrocarbon degradation.

3.5 Laboratory Analyses

3.5.1 Microbial Enumeration

- Total heterotrophic bacteria were enumerated using nutrient agar.
- Hydrocarbon-degrading bacteria were enumerated on Bushnell-Haas agar supplemented with diesel as the sole carbon source.
- Colonies were counted after incubation at 30°C for 48-72 h and expressed as log CFU g^{-1} dry soil.

3.5.2 Hydrocarbon Analysis

- TPH: Soxhlet extraction with n-hexane followed by gravimetric quantification.
- PAHs: Extracts were fractionated by silica gel chromatography, concentrated, and analyzed via GC-MS (Agilent 7890/5975 system) following USEPA Method 8270D.

3.5.3 Soil Nutrients

- Total nitrogen: Kjeldahl digestion.
- Ammonium (NH_4^+): Salicylate-hypochlorite colorimetry.
- Nitrate (NO_3^-): Ion-selective electrode method.
- Available phosphorus: Spectrophotometry after persulfate digestion.

3.5.4 Soil pH and Organic Carbon

- pH measured with a calibrated glass electrode (1:1 soil:water).
- Organic carbon by Walkley-Black wet oxidation method.

Quality assurance included reagent blanks, duplicate analyses, and standard reference soils where available.

3.6 Statistical Analyses

Data were processed using SPSS v22 and R (v4.3). Analysis of variance (ANOVA) was performed to test the significance of treatment effects on total nitrogen, microbial counts, TPH, and PAHs across time points. The assumptions of ANOVA (normality, homogeneity of variances) were validated via Shapiro-Wilk and Levene's tests, respectively. Post-hoc comparisons were conducted with Fisher's Least Significant Difference (FLSD) at $P \leq 0.05$.

Regression models were fitted to explore relationships between nitrogen concentration (independent variable) and microbial proliferation, TPH, and PAH reduction (dependent variables). Correlation coefficients (r-values) were used to interpret strength and direction of relationships. Slopes of regression equations were compared to assess differential responsiveness under different nitrogen amendments.

Graphical analyses, including line plots and bar charts, were generated with OriginPro v9 for data visualization. Residual plots and confidence intervals were examined to assess model adequacy.

4.0 Results

4.1 Baseline Soil Properties

Physicochemical analyses of soils from Batan, Ajuju, and Umusia confirmed that the study sites were nutrient-poor, acidic, and susceptible to oil-induced stress (Table 2). The soils were either sandy loam or sandy clay loam, with pH values between 4.55 and 4.70. Total nitrogen was extremely low (<0.05%), while organic carbon ranged from 1.43 to 1.81%.

Table 2. Initial Physicochemical Properties of Soils from Selected Sites

Property	Batan	Ajuju
pH (1:1 soil:water)	4.61	4.55
Texture	Sandy Loam	Sandy Clay Loam
Organic Carbon (%)	1.81	1.57
Total Nitrogen (%)	0.045	0.038
Available Phosphorus (mg/kg)	7.4	6.1

These conditions are consistent with previous reports of oil-impacted Niger Delta soils (Obayori et al., 2009; Okoh, 2006). Acidic soils with low cation exchange capacity (CEC) enhance nutrient leaching and limit microbial proliferation, underscoring the need for nutrient amendment interventions.

4.2 Dynamics of Total Nitrogen in Amended Soils

Nitrogen supplementation significantly altered soil nitrogen status over time. Table 3 summarizes total nitrogen (%) at the highest oil contamination level (P2, 80 g/kg).

Table 3. Total Nitrogen (%) Over Time in Soils Treated with Different Nitrogen Sources (P2 Level)

Treatment	Day 0	Day 7	Day 49	Day 108
N0 (Control)	0.045	0.048	0.051	0.053
N1 (Nitrate)	0.045	0.072	0.084	0.089
N2 (Ammonium)	0.045	0.080	0.092	0.098
N3 (Organic)	0.045	0.077	0.101	0.110

Across all treatments, total nitrogen increased relative to the control. Ammonium and nitrate provided sharp early increases by Day 7, while organic nitrogen showed a gradual and sustained rise, peaking at 0.110% by Day 108. The plateau observed in inorganic treatments after Day 49 suggests partial nutrient loss through leaching or volatilization. By contrast, organic nitrogen delivered more consistent long-term nutrient availability, confirming its slow-release properties.

4.3 Microbial Population Responses

Microbial proliferation followed nutrient trends, with pronounced increases in all amended soils relative to controls. The highest microbial stimulation was observed under organic nitrogen treatment (N3), though ammonium (N2) also supported significant growth.

Table 4. Microbial Population (log CFUs/g) Over Time at P2 Level

Treatment	Day 0	Day 7	Day 49	Day 108
N0 (Control)	5.52	5.67	5.80	5.94
N1 (Nitrate)	5.81	5.96	6.09	6.22
N2 (Ammonium)	6.15	6.30	6.43	6.56
N3 (Organic)	6.33	6.48	6.61	6.74

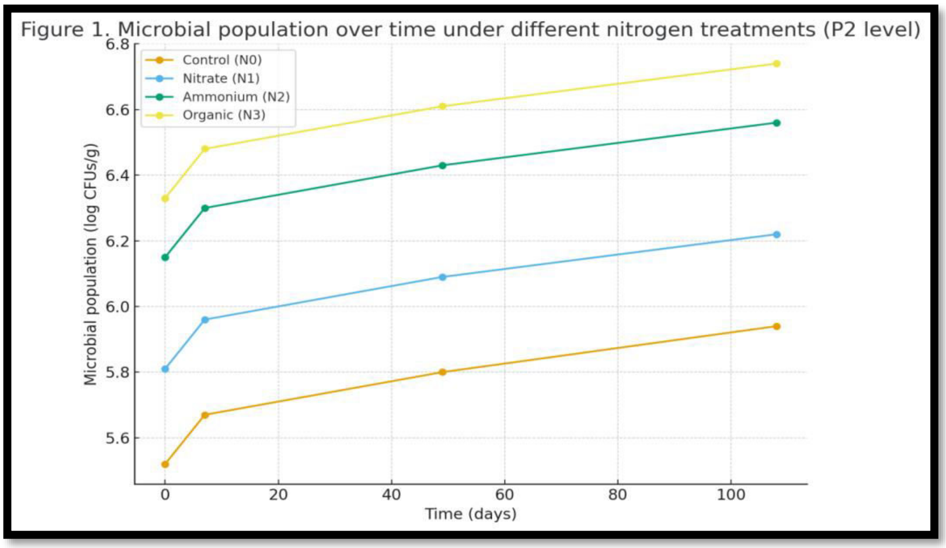

Figure 1: Microbial population dynamics (log CFUs/g) over time under different nitrogen treatments.

By Day 108, microbial counts in N3 treatments reached 6.74 log CFUs/g, nearly one log higher than the control (5.94). The stimulatory effect of ammonium was also evident, reaching 6.56 log CFUs/g. Nitrate-treated soils recorded the lowest microbial enhancement among amendments (6.22 log CFUs/g). These results indicate that both the immediacy and persistence of nutrient supply shape microbial responses, with organic nitrogen favoring sustained growth.

4.4 Total Petroleum Hydrocarbon (TPH) Reduction

Hydrocarbon degradation, measured as reduction in TPH concentration, showed clear treatment effects. Table 5 and Figure 2 summarize TPH dynamics at P2 contamination.

Table 5. TPH Concentration (mg/kg) Over Time at P2 Level

Treatment	Day 0	Day 7	Day 49	Day 108
N0 (Control)	18.81	18.00	17.19	17.11
N1 (Nitrate)	17.76	17.00	16.24	16.07
N2 (Ammonium)	16.77	16.05	15.33	15.08
N3 (Organic)	15.83	15.15	14.47	14.14

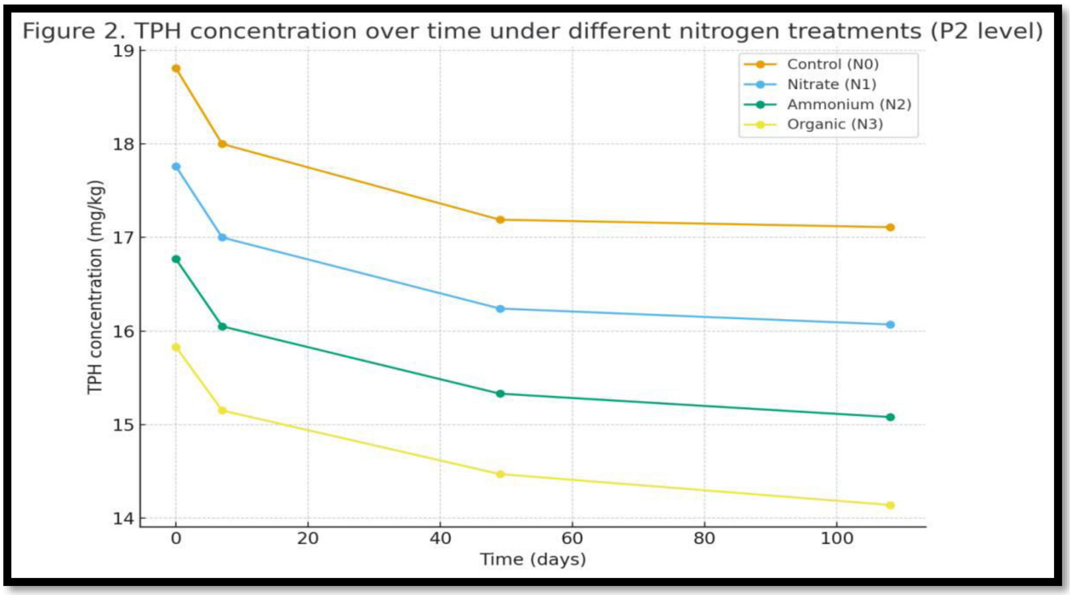

Figure 2: Total Petroleum Hydrocarbon (TPH) concentration (mg/kg) over time under different nitrogen treatments.

All nutrient amendments accelerated TPH reduction compared to the control. The most rapid reductions occurred under ammonium and organic nitrogen. By Day 108, TPH decreased to 15.08 mg/kg in ammonium-treated soils and 14.14 mg/kg in organic nitrogen soils, compared with 17.11 mg/kg in the unamended control. These results highlight the importance of nitrogen availability in stimulating microbial metabolism and hydrocarbon catabolism.

4.5 Polycyclic Aromatic Hydrocarbon (PAH) Degradation

PAH fractions, representing more recalcitrant hydrocarbons, also decreased under nutrient treatments, though at slower rates.

Table 6. PAH Concentration (%) Over Time at P2 Level

Treatment	Day 0	Day 7	Day 49	Day 108
N0 (Control)	2.21	2.10	1.45	1.19
N1 (Nitrate)	2.14	2.03	1.38	1.12
N2 (Ammonium)	2.08	1.97	1.32	1.06
N3 (Organic)	2.02	1.91	1.26	1.00

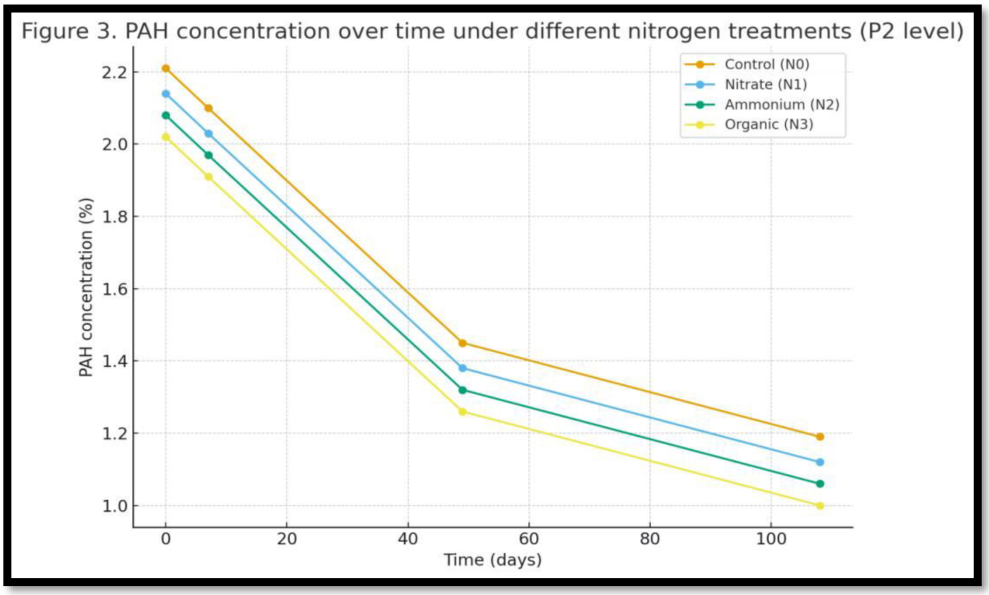

Figure 3: Polycyclic Aromatic Hydrocarbon (PAH) concentration (%) over time under different nitrogen treatments.

By Day 108, PAHs declined most substantially in organic nitrogen-treated soils (1.00%) compared to ammonium (1.06%), nitrate (1.12%), and the control (1.19%). This suggests that long-term microbial stability under organic nitrogen supported degradation of complex hydrocarbons, despite their lower bioavailability.

4.6 Regression and Correlation Analyses

Regression models revealed strong positive correlations between nitrogen concentration and microbial proliferation (r = 0.90-0.96). The steepest slope was observed in organic nitrogen treatments, indicating a strong stimulatory effect on microbial populations.

Table 7. Regression Models Between Nitrogen Concentration and Microbial Population at P2 Level

Treatment	Regression Equation	r-value	Slope (b)	Interpretation
N3P2	Y = 1.28x + 6.21	0.93	1.28	Strong effect of organic N
N2P2	Y = 0.61x + 5.86	0.96	0.61	Highest correlation
N1P2	Y = 0.38x + 5.58	0.95	0.38	Moderate stimulation
N0P2	Y = 0.21x + 5.46	0.90	0.21	Weakest effect

These findings indicate that ammonium nitrogen showed the tightest correlation between nitrogen concentration and microbial growth, but organic nitrogen delivered the steepest population increase over time. This dual pattern suggests ammonium is better for short-term hydrocarbon removal, while organic nitrogen is superior for long-term ecological recovery (Egbebike et al., 2025).

4.7 Integrated Findings

Overall, the mesocosm experiment demonstrated that nutrient limitation is the principal barrier to effective hydrocarbon degradation in Niger Delta soils. Inorganic nitrogen (especially ammonium) triggered rapid TPH reductions, while organic nitrogen fostered sustained microbial growth and long-term PAH attenuation. Nitrate was least effective, likely due to leaching in sandy, acidic soils. The integration of chemical (TPH, PAH) and biological (microbial counts) endpoints reinforced the conclusion that biostimulation is both viable and ecologically compatible for remediation (Egbebike et al., 2025).

5.0 Discussion

5.1 Nutrient Limitation as a Key Barrier to Bioremediation

The baseline analyses confirmed that soils from Batan, Ajuju, and Umusia were strongly nutrient-deficient, acidic, and structurally fragile. Such soils are characteristic of oil-impacted tropical wetlands, where high carbon inputs from petroleum residues are accompanied by extremely low nitrogen and phosphorus levels (Bossert & Bartha, 1984). The poor cation exchange capacity and high rainfall of the Niger Delta exacerbate nutrient depletion, leaving indigenous hydrocarbon-degrading microbes unable to sustain metabolism without external supplementation. This aligns with previous findings from Ogoniland and other parts of the Delta, where natural attenuation alone was deemed insufficient for ecological recovery (UNEP, 2011; Obayori et al., 2009). The results of this mesocosm study confirm that nutrient limitation is the principal bottleneck for hydrocarbon degradation in Niger Delta soils.

5.2 Comparative Effectiveness of Nitrogen Sources

A central objective of this study was to evaluate how different nitrogen sources-ammonium, nitrate, and organic nitrogen-affect microbial proliferation and hydrocarbon degradation under simulated spill conditions. The findings revealed distinct patterns: ammonium nitrogen provided rapid stimulation of hydrocarbon degradation, while organic nitrogen supported the most sustained microbial proliferation and long-term pollutant reduction (Egbebike et al. 2025).

5.2.1 Ammonium Nitrogen: Rapid Hydrocarbon Degradation

Ammonium-supplemented soils exhibited the fastest TPH reductions, with concentrations falling from 16.77 mg/kg at Day 0 to 15.08 mg/kg by Day 108 . This immediate effect reflects the high bioavailability of ammonium, which is rapidly assimilated into microbial biomass for protein and enzyme synthesis (Roling & van Bodegom, 2014). Comparable studies in salt marshes and freshwater wetlands have shown ammonium amendments outperform nitrate due to reduced washout and stronger sorption to organic matter (Jackson & Pardue, 1999; Venosa et al., 2002). However, the plateau observed after Day 49 suggests nitrogen loss, likely via volatilization or nitrification-denitrification in acidic, flood-prone soils. Thus, while ammonium is ideal for emergency response and rapid TPH cleanup, it may not guarantee long-term ecological restoration.

5.2.2 Organic Nitrogen: Sustained Microbial Proliferation

Organic nitrogen amendments led to the highest microbial counts, reaching 6.74 log CFUs/g by Day 108, almost a full log above the control . The gradual release of nitrogen from biosolids explains the more stable nutrient pool, preventing rapid depletion. Organic sources also provide secondary benefits-improved soil structure, increased organic matter, and micronutrient enrichment-all of which foster resilient microbial consortia (Macaulay, 2015; Das & Chandran, 2011). Moreover, organic nitrogen supported the greatest reduction in PAHs, with concentrations declining to 1.00% compared to 1.19% in controls. This suggests that longer-term microbial stability is essential for attacking complex, high molecular-weight hydrocarbons. The slower onset of degradation under organic nitrogen, however, makes it less suited to short-term remediation targets.

5.2.3 Nitrate Nitrogen: Limited Effectiveness in Acidic Soils

Nitrate amendments showed moderate improvement, with microbial counts reaching only 6.22 log CFUs/g and final TPH at 16.07 mg/kg . The relatively weak performance is consistent with nitrate's vulnerability to leaching in sandy soils and denitrification losses in waterlogged conditions. While nitrate can serve as an alternative electron acceptor in anaerobic environments, its instability in humid tropical soils reduces its effectiveness (Singh et al., 2007). This outcome emphasizes that nitrate-based remediation strategies are poorly adapted to the Niger Delta's hydrology and soil chemistry.

5.3 Microbial Proliferation as a Driver of Biodegradation

The strong correlations observed between nitrogen concentration and microbial population ($r = 0.93–0.96$) highlight the central role of microbial proliferation in driving hydrocarbon attenuation. Organic nitrogen, with the steepest regression slope (1.28), was particularly effective in sustaining long-term microbial growth, while ammonium showed the highest correlation ($r = 0.96$), reflecting its efficiency in rapid microbial activation. These results align with global evidence that microbial activity is the most reliable predictor of contaminant breakdown in bioremediation systems (Atlas & Bartha, 1973; Rahman et al., 2003).

The higher microbial counts under organic nitrogen also suggest that nutrient form influences community structure. Biosolids not only supply nitrogen but also introduce organic carbon and micronutrients, which may support more diverse microbial consortia. In contrast, ammonium favors rapid proliferation of a narrower range of fast-growing degraders. Such differences have implications for long-term ecosystem recovery, including soil fertility and microbial diversity.

5.4 Hydrocarbon Degradation Pathways and Pollutant Profiles

The observed patterns of TPH and PAH degradation provide insights into microbial metabolic processes under nutrient amendment. The faster decline in TPH under ammonium treatments suggests preferential utilization of labile hydrocarbons (n-alkanes, light aromatics), while the slower but consistent PAH reduction under organic nitrogen reflects microbial adaptation to more recalcitrant fractions. This dual pattern supports previous reports that while inorganic nitrogen accelerates the removal of easily degradable hydrocarbons, organic nitrogen amendments enhance the breakdown of persistent pollutants over longer timescales (Das & Chandran, 2011; Salanitro et al., 1997).

From a risk perspective, the decline in PAHs is particularly significant, as these compounds are associated with carcinogenic and mutagenic risks. Thus, while ammonium achieves visible cleanup quickly, organic nitrogen may contribute more to reducing long-term ecological and health risks.

5.5 Comparison with Global Studies

The mesocosm findings are consistent with results from other regions. In temperate soils, biostimulation with inorganic fertilizers increased hydrocarbon removal efficiency by over 50% (Bento et al., 2005). In Arctic environments, ammonium proved more effective than nitrate due to stronger soil binding (Margesin & Schinner, 2001). In contrast, studies in compost-amended soils demonstrated that organic nitrogen supports stable microbial consortia and long-term fertility restoration (Singh et al., 2007).

This convergence of evidence underscores that no single nitrogen source is universally optimal. Instead, remediation success depends on aligning nutrient forms with site-specific conditions. **For the Niger Delta, characterized by acidic soils, heavy rainfall, and persistent contamination, a dual strategy combining ammonium (for rapid initial degradation) with organic nitrogen (for sustained microbial support) may yield the best outcomes.**

5.6 Limitations of the Mesocosm Approach

While mesocosm studies provide valuable insights, they cannot fully replicate the complexity of field environments. Key limitations include:

- Scale effects: Root interactions, fluctuating hydrology, and community dynamics may differ in larger systems.
- Nutrient dynamics: Nutrient retention in containers may be greater than in open soils, potentially exaggerating treatment effects.
- Single contaminant type: The use of refined diesel (AGO) does not capture the complexity of mixed crude oil and refined products in the Niger Delta.

- Temporal scope: The 108-day monitoring period may not reflect long-term ecological trajectories or secondary pollutant effects.

These limitations point to the need for complementary field-scale trials before operational deployment.

5.7 Implications for Niger Delta Remediation Policy

The results of this study have direct relevance for oil spill management in Nigeria. First, they demonstrate that biostimulation is a cost-effective and ecologically sound alternative to conventional remediation methods. Second, they suggest that nutrient source selection must be site-specific: ammonium for rapid hydrocarbon degradation, organic nitrogen for long-term soil recovery, and nitrate only under specific conditions where leaching can be controlled.

In practice, blending fast-release (ammonium) with slow-release (organic biosolids) nitrogen sources could optimize both immediate cleanup and sustained ecological resilience. This strategy aligns with UNEP's (2011) call for remediation approaches that not only remove contaminants but also restore ecological function. Integrating such evidence-based practices into Nigeria's national oil spill contingency plans would strengthen remediation outcomes and improve community trust.

5.8 Synthesis

In summary, this mesocosm study highlights that nutrient amendment-specifically nitrogen supplementation-substantially enhances bioremediation in petroleum-contaminated soils of the Niger Delta. Ammonium provides rapid hydrocarbon removal, organic nitrogen ensures microbial stability and PAH degradation, and nitrate shows limited promise under current soil conditions. Together, these findings emphasize the value of adaptive, blended nutrient strategies tailored to the ecological and hydrological realities of the Niger Delta.

6.0 Conclusions and Recommendations

6.1 Conclusions

This mesocosm study provides compelling evidence that nutrient limitation is the single greatest barrier to natural attenuation of oil-contaminated soils in the Niger Delta. Baseline analyses confirmed that the soils are acidic, nitrogen-deficient, and structurally weak, conditions that suppress microbial proliferation and hydrocarbon metabolism. Without intervention, natural recovery proceeds slowly, leaving toxic hydrocarbons to persist for years.

By systematically comparing three nitrogen sources-ammonium, nitrate, and organic nitrogen-under controlled mesocosm conditions, this study established the following:

1. Ammonium nitrogen proved most effective for rapid hydrocarbon removal.

TPH concentrations decreased sharply in ammonium-treated soils, reflecting the immediate bioavailability of NH_4^+ to microbes. Strong correlations (r = 0.96) confirmed its capacity to accelerate initial degradation. This makes ammonium an excellent choice for short-term remediation where visible cleanup is urgently required.

2. Organic nitrogen produced the highest microbial proliferation and long-term PAH degradation.

Biosolid amendments increased microbial counts to 6.74 log CFUs/g by Day 108, sustained soil nitrogen levels, and achieved the most consistent reduction in persistent PAHs. This indicates that organic nitrogen supports ecological recovery by stabilizing microbial communities and improving soil quality beyond mere pollutant removal.

3. Nitrate was moderately effective but ecologically less suited to Niger Delta soils.

While nitrate-treated soils showed some improvement, leaching and denitrification limited its effectiveness in sandy, acidic soils. In such environments, nitrate is not a reliable amendment unless complemented by soil stabilization measures.

4. Nutrient availability and microbial growth were tightly coupled.

Regression analysis demonstrated strong positive correlations (r = 0.90–0.96) between nitrogen concentration and microbial proliferation, underscoring the central role of microbial dynamics in remediation success.

5. Biostimulation is a feasible, low-cost remediation strategy for tropical wetlands.

Unlike expensive engineering-based methods, nutrient amendment relies on enhancing indigenous microbial populations that are already adapted to local soils and hydrocarbons. This ecological compatibility ensures minimal disruption and supports long-term recovery.

Taken together, these findings highlight that bioremediation via nitrogen amendment is a viable pathway to accelerate soil and ecological recovery in the Niger Delta, offering a context-sensitive alternative to traditional remediation practices.

6.2 Recommendations

Based on the outcomes of this study, the following recommendations are advanced:

6.2.1 Site-Specific Nutrient Strategies

- Ammonium nitrogen should be prioritized for rapid remediation following fresh spills where immediate reduction of TPH is critical to minimize ecological and social impacts.

- Organic nitrogen should be applied in chronically polluted sites to promote microbial diversity, soil fertility restoration, and long-term degradation of persistent hydrocarbons such as PAHs.
- Blended strategies combining ammonium (fast-acting) and organic nitrogen (slow-release) may optimize both short-term efficiency and long-term resilience.

6.2.2 Field-Scale Validation

- While mesocosm experiments provide predictive insights, field-scale trials are essential to confirm the operational feasibility of nutrient amendment under the complex hydrological regimes of the Niger Delta. Such trials should account for tidal flooding, heterogeneous contamination, and community land use.
- Monitoring frameworks should integrate chemical (TPH/PAH), microbial (population dynamics, diversity indices), and ecotoxicological (bioassays) endpoints to capture the full spectrum of recovery.

6.2.3 Policy Integration

- Nigerian regulatory agencies (e.g., NOSDRA, DPR) should formally integrate biostimulation protocols into national oil spill contingency plans as approved remediation options.
- Guidelines should specify dosage ranges, application methods, and monitoring criteria tailored to soil type, contamination severity, and hydrological conditions.
- Policies should encourage the use of locally available organic amendments (e.g., biosolids, compost, animal manure), which not only reduce costs but also promote circular economy practices.

6.2.4 Community Engagement and Capacity Building

- Local communities should be trained in the application of nutrient amendments as part of participatory remediation programs. This could empower affected populations to take part in restoring their lands rather than relying solely on external contractors.
- Partnerships with universities and NGOs can provide technical support, monitoring, and evaluation, ensuring that remediation aligns with both ecological recovery and livelihood restoration.

6.2.5 Future Research Directions

- Nutrient interactions: Further studies should examine how phosphorus, potassium, and trace micronutrients interact with nitrogen amendments to influence biodegradation.
- Microbial ecology: Molecular tools (e.g., metagenomics, qPCR) should be applied to identify key microbial taxa and functional genes responsible for hydrocarbon degradation under different nutrient regimes.

- Long-term soil health: Post-remediation monitoring should assess improvements in soil fertility, organic matter, and biodiversity to ensure that remediation contributes to sustainable land use.
- Alternative amendments: Exploration of biochar, nano-fertilizers, or slow-release encapsulated nutrients may provide innovative ways to enhance nutrient efficiency while reducing losses.

6.3 Final Reflection

The Niger Delta has endured decades of ecological degradation and socio-economic disruption due to chronic oil spills. While complete ecological restoration will require integrated measures-pollution prevention, habitat rehabilitation, and governance reforms—this study demonstrates that mesocosm-tested nutrient amendment offers a scientifically grounded and pragmatic tool for accelerating recovery. The choice of nitrogen source should be tailored to remediation goals: rapid cleanup with ammonium, long-term soil restoration with organic nitrogen, or blended applications for balanced outcomes.

If adopted at scale, nitrogen-based biostimulation can help break the cycle of persistent contamination, restore ecological functionality, and support community livelihoods. More importantly, it represents an affordable, locally adaptable approach that aligns with global best practices in environmental biotechnology and the urgent needs of the Niger Delta.

7.0 References

1. Atlas, R. M. (1984). Petroleum microbiology. Macmillan.
2. Atlas, R. M., & Bartha, R. (1973). Stimulated biodegradation of oil slicks using oleophilic fertilizers. Environmental Science & Technology, 7(6), 538–541.
3. Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. Environmental Science & Technology, 45(16), 6709–6715.
4. Bento, F. M., Camargo, F. A. O., Okeke, B. C., & Frankenberger, W. T. (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation, and bioaugmentation. Bioresource Technology, 96(9), 1049–1055.
5. Bossert, I., & Bartha, R. (1984). The fate of petroleum in soil ecosystems. In R. M. Atlas (Ed.), Petroleum microbiology (pp. 435–476). Macmillan.
6. Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology Research International, 2011, Article 941810.
7. Dibble, J. T., & Bartha, R. (1979). Effect of environmental parameters on the biodegradation of oil sludge. Applied and Environmental Microbiology, 37(4), 729–739.

8. Egbebike, M. O., Moneke, A. N., Ezeagu, C. A. (2025). Comparative Effectiveness of Nitrogen Sources for Nutrient Amendment in Bioremediation of Petroleum-Contaminated Soils in Nigeria's Niger Delta Region. International Journal of Research and Scientific Innovation.
9. Jackson, W. A., & Pardue, J. H. (1999). Potential for enhancement of biodegradation of crude oil in Louisiana salt marshes using nutrient amendments. Water, Air, and Soil Pollution, 109(1–4), 343–355.
10. Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. Microbiological Reviews, 54(3), 305–315.
11. Lee, K. (2000). Oil spill remediation: Bioremediation of oil-contaminated soils. In R. E. Hester & R. M. Harrison (Eds.), Contaminated land and its reclamation (pp. 99–116). Royal Society of Chemistry.
12. Lee, K., & Levy, E. M. (1987). Enhanced oil biodegradation with an oleophilic fertilizer: Preliminary tests. Oil and Chemical Pollution, 4(3), 243–256.
13. Lee, K., & Levy, E. M. (1989). Enhancement of oil biodegradation in the Arctic and temperate conditions: A field experiment. Marine Environmental Research, 27(3–4), 227–249.
14. Macaulay, B. M. (2015). Understanding the behaviour of oil-degrading microorganisms to enhance remediation of spilled petroleum. Applied Ecology and Environmental Research, 13(1), 247–262.
15. Margesin, R., & Schinner, F. (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. Applied Microbiology and Biotechnology, 56(5–6), 650–663.
16. Mills, A. L., Breuil, C., & Colwell, R. R. (1978). Enumeration of petroleum-degrading marine and estuarine microorganisms by the most probable number method. Canadian Journal of Microbiology, 24(5), 552–557.
17. Nwilo, P. C., & Badejo, O. T. (2006). Impacts and management of oil spill pollution along the Nigerian coastal areas. Administrative Staff College of Nigeria (ASCON) Journal of Management Review, 1(1), 76–88.
18. Obayori, O. S., Ilori, M. O., Adebusoye, S. A., Oyetibo, G. O., Amund, O. O., & Amund, D. I. (2009). Degradation of hydrocarbons and biosurfactant production by Pseudomonas sp. strain LP1. World Journal of Microbiology and Biotechnology, 25(9), 1615–1623.
19. Okoh, A. I. (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. Biotechnology and Molecular Biology Reviews, 1(2), 38–50.
20. Płaza, G. A., Wypych, J., Nałęcz-Jawecki, G., Ulfig, K., & Brigmon, R. L. (2005). Acute toxicity assessment of oil products with application of Vibrio fischeri bioassay. Polish Journal of Environmental Studies, 14(3), 365–370.
21. Rahman, K. S. M., Rahman, T. J., Mc Clean, S., Marchant, R., & Banat, I. M. (2003). Rhamnolipid biosurfactants for the enhanced removal of

hydrocarbons from contaminated soil and water. Biodegradation, 14(1), 57–64.
22. Renoux, A. Y., Sarrazin, M., & Samson, R. (2000). Bioremediation of petroleum-contaminated soils: Laboratory studies. Environmental Technology, 21(10), 1045–1056.
23. Roling, W. F. M., & van Bodegom, P. M. (2014). Toward quantitative understanding on microbial community structure and functioning in soil. Frontiers in Microbiology, 5, 545.
24. Salanitro, J. P., Dorn, P. B., Huesemann, M. H., Moore, K. O., Rhodes, I. A., Jackson, L. M. R., Vipond, T. E., Western, M. M., & Wisniewski, H. L. (1997). Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. Environmental Science & Technology, 31(6), 1769–1776.
25. Saterbak, A., Toy, R. J., Wong, D. C. L., McMain, B. J., Williams, M. P., Dorn, P. B., Brzuzy, L. P., Chai, E. Y., Salanitro, J. P., Wisniewski, H. L., & Ward, C. H. (1999). Ecotoxicological and analytical assessment of hydrocarbon-contaminated soils and application to ecological risk assessment. Environmental Toxicology and Chemistry, 18(7), 1591–1607.
26. Seymour, F. A., Sawyer, C., & Colwell, R. R. (1996). Microbial interactions in petroleum-contaminated soils. Applied and Environmental Microbiology, 62(2), 421–429.
27. Singh, A., Kuhad, R. C., & Ward, O. P. (2007). Biological remediation of petroleum contaminants. In A. Singh, R. C. Kuhad, & O. P. Ward (Eds.), Advances in applied bioremediation (pp. 1–22). Springer.
28. Smith, V. H., Graham, D. W., & Cleland, D. D. (1998). Application of resource-ratio theory to hydrocarbon biodegradation. Environmental Science & Technology, 32(21), 3386–3395.
29. Suidan, M. T., & Wrenn, B. A. (2000). Biological treatment of petroleum hydrocarbons. In D. R. Wise (Ed.), Bioremediation of contaminated soils (pp. 173–212). CRC Press.
30. UNEP. (2011). Environmental assessment of Ogoniland. United Nations Environment Programme.
31. Ugochukwu, C. N. C., & Ertel, J. (2008). Negative impacts of oil exploration on biodiversity management in the Niger Delta area of Nigeria. Impact Assessment and Project Appraisal, 26(2), 139–147.
32. USEPA. (1999a). Guidance for assessing petroleum hydrocarbon-contaminated soils. U.S. Environmental Protection Agency.
33. USEPA. (1999b). Bioremediation of oil spills: Field applications. U.S. Environmental Protection Agency.
34. Venosa, A. D., Campo, P., Suidan, M. T., & Wrenn, B. A. (2002). Effectiveness of nutrient addition on bioremediation of crude oil-contaminated shorelines. Journal of Environmental Quality, 31(5), 1478–1488.

35. Venosa, A. D., Holder, E. L., King, D., Southworth, G. R., Lee, K., Suidan, M. T., & Strohmeier, K. (1996). Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. Environmental Science & Technology, 30(5), 1764–1775.
36. Venosa, A. D., & Zhu, X. (2003). Bioremediation of marine oil spills. Marine Pollution Bulletin, 47(8–9), 397–405.
37. Wrenn, B. A., & Venosa, A. D. (1996). Selective enumeration of aromatic and aliphatic hydrocarbon degrading bacteria by most-probable-number procedures. Canadian Journal of Microbiology, 42(3), 252–258.
38. Xie, Z., Whitman, W. B., & Shiaris, M. P. (1999). Enhancement of hydrocarbon biodegradation by the addition of biosurfactants in marine environments. Marine Pollution Bulletin, 38(6), 542–546.
39. Zabbey, N., & Uyi, H. (2014). Community responses of benthic macroinvertebrates to oil pollution in a Niger Delta stream, Nigeria. African Journal of Aquatic Science, 39(4), 361–368