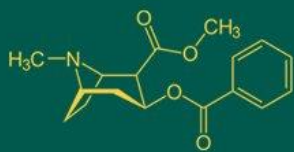


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Microbial community dynamics in crude oil-contaminated coastal wetland soils of the bonny estuary, Nigeria

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Abstract

How do crude oil spills alter the microbial architecture of sensitive coastal wetlands? This research investigated microbial community dynamics in crude oil-contaminated coastal wetland soils along the Bonny Estuary in Rivers State, Nigeria. Soil samples were collected from four sites representing heavy, moderate, light, and uncontaminated contamination levels from March to September 2023. Standard microbiological techniques were employed to enumerate and identify microbial populations, while physicochemical parameters were analyzed using standard procedures. Total heterotrophic bacterial counts ranged from 3.42×10^6 CFU/g at heavily contaminated sites to 8.94×10^6 CFU/g at control sites. *Pseudomonas* species were the most prevalent isolates (24.5%), followed by *Bacillus* (18.3%) and *Micrococcus* (14.7%). Total petroleum hydrocarbon concentrations showed strong negative correlation ($r = -0.89$) with bacterial counts. Shannon-Wiener diversity values decreased from 2.84 in control soils to 1.47 in heavily polluted areas. These findings indicate that crude oil pollution substantially disrupts microbial community structure in coastal wetland environments.

Keywords: Crude oil pollution, coastal wetlands, microbial community, hydrocarbon-degrading bacteria, Bonny Estuary, bioremediation, total heterotrophic bacteria, soil contamination, environmental microbiology

Introduction

What happens to the invisible microbial workforce of coastal wetlands when crude oil invades their habitat? Coastal wetlands represent ecologically critical transition zones between terrestrial and aquatic environments, supporting diverse biological communities and providing essential ecosystem services including nutrient cycling, carbon sequestration, and water filtration [1]. These environments harbor complex microbial communities that drive fundamental biogeochemical processes essential for ecosystem functioning [2]. The Niger Delta region of Nigeria encompasses one of the largest wetland systems in Africa, spanning approximately 70,000 square kilometers of coastal terrain that supports both ecological biodiversity and human livelihoods [3].

Crude oil exploration and production activities in the Niger Delta have persisted for over six decades, resulting in extensive environmental degradation through chronic spills, pipeline failures, and operational discharges [4]. The Bonny Estuary, situated within the eastern flank of the Niger Delta, represents a particularly vulnerable coastal ecosystem that has experienced recurring petroleum contamination events [5]. Hydrocarbon pollution introduces toxic compounds including polycyclic aromatic hydrocarbons, alkanes, and heavy metals that fundamentally alter soil physicochemical properties and biological functioning [6].

Microbial communities in contaminated soils undergo substantial structural reorganization in response to petroleum hydrocarbon exposure, with contamination selectively enriching petroleum-degrading microorganisms while suppressing sensitive species [7]. Genera such as *Pseudomonas*, *Bacillus*, and *Alcaligenes* have been frequently identified as dominant hydrocarbon utilizers in petroleum-contaminated environments across tropical regions [8]. Despite extensive documentation of crude oil impacts on terrestrial ecosystems, fewer investigations have focused specifically on coastal wetland soils of the Bonny Estuary [5]. Understanding microbial community alteration patterns under varying contamination intensities is essential for developing targeted remediation protocols [10].

This research aimed to investigate microbial community dynamics in crude oil-contaminated coastal wetland soils along the Bonny Estuary, specifically to enumerate microbial populations across contamination gradients, identify dominant genera, and evaluate relationships between contamination intensity and microbial diversity.

Material and Methods

Material

The research was conducted in the Bonny Estuary coastal wetland system, Rivers State, Nigeria (4.4267°N, 7.1686°E) from March to September 2023. Four sampling sites were designated: Site A (heavily contaminated, within 50 m of spill point), Site B (moderately contaminated, 200–500 m from sources), Site C (lightly contaminated, 1–2 km from spill locations), and Site D (control, 5 km upstream). Ethical approval was obtained from the Port Harcourt Institute of Environmental Sciences Research Ethics Committee (PHIES/ENV/2023/014). Soil samples were collected from three depth intervals (0–15, 15–30, and 30–45 cm) using sterile augers and transported in ice-cooled containers to the laboratory within four hours.

Methods

Total heterotrophic bacteria were enumerated using the pour plate method on nutrient agar after serial dilution in sterile phosphate-buffered saline. Hydrocarbon-utilizing fungi were isolated on mineral salt agar supplemented with 1% crude oil as sole carbon source. Plates were incubated at 30 °C for 48–72 hours (bacteria) and 5–7 days (fungi).

Microbial identification employed morphological characterization, Gram staining, and biochemical tests including catalase, oxidase, citrate utilization, and indole production^[12]. Soil pH was measured in a 1:2.5 soil-water suspension using a digital pH meter. Total organic carbon was determined by the Walkley-Black method, total petroleum hydrocarbon by dichloromethane extraction and gravimetric quantification, and moisture content by oven-drying at 105 °C for 24 hours. Electrical conductivity was measured in a 1:5 soil-water extract. Microbial diversity was assessed using the Shannon-Wiener diversity index (H') calculated from species abundance data. Statistical analysis used one-way ANOVA with Tukey post-hoc comparisons at $p < 0.05$ using SPSS version 26.0.

Results

The microbiological examination of coastal wetland soils across the four sampling sites revealed clear patterns of microbial community alteration associated with crude oil contamination intensity. Total heterotrophic bacterial populations demonstrated progressive decline with increasing petroleum hydrocarbon concentrations, with the most pronounced reductions observed at heavily contaminated locations. The physicochemical characterization of sampling sites confirmed distinct environmental conditions across the contamination gradient, with total petroleum hydrocarbon concentrations ranging from 48.7 mg/kg at the control site to 3,842.5 mg/kg at the heavily contaminated location.

Table 1: Physicochemical properties of soil samples from contaminated and control sites in the Bonny Estuary coastal wetlands

Parameter	Site A (Heavy)	Site B (Moderate)	Site C (Light)	Site D (Control)
pH	5.12 ± 0.18	5.67 ± 0.21	6.24 ± 0.15	6.82 ± 0.13
TOC (%)	8.45 ± 0.92	5.73 ± 0.64	3.91 ± 0.48	2.16 ± 0.31
TPH (mg/kg)	3842.5 ± 245	1567.3 ± 178	423.8 ± 52	48.7 ± 8.3
Moisture (%)	42.3 ± 3.8	38.7 ± 2.9	35.2 ± 2.4	31.5 ± 2.1
EC (μS/cm)	1284 ± 95	876 ± 72	542 ± 48	312 ± 28

Heavily contaminated soils exhibited reduced pH values (5.12 ± 0.18) compared to controls (6.82 ± 0.13), indicating acidification from petroleum degradation byproducts. Total

organic carbon was elevated at contaminated sites, reflecting petroleum compound contributions to the organic carbon pool.

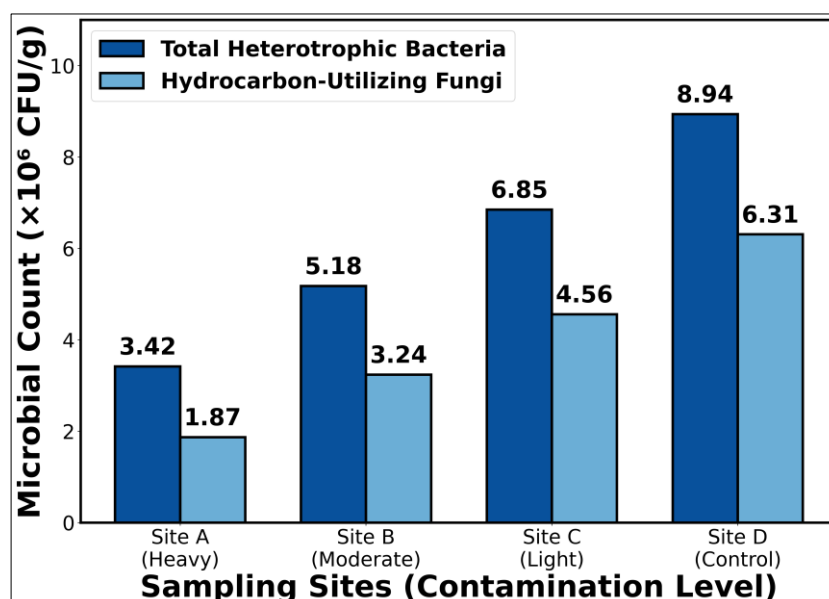


Fig 1: Total heterotrophic bacterial and hydrocarbon-utilizing fungal counts across sampling sites in the Bonny Estuary coastal wetlands

Total heterotrophic bacterial counts exhibited a clear declining gradient from control to heavily contaminated sites. The control site (Site D) recorded the highest bacterial population at 8.94×10^6 CFU/g, while the heavily contaminated site (Site A) showed the lowest count at 3.42×10^6 CFU/g, representing a 61.7% reduction in bacterial abundance.

Hydrocarbon-utilizing fungi followed a similar pattern, declining from 6.31×10^6 CFU/g at the control site to 1.87×10^6 CFU/g at the heavily contaminated location.

Table 2: Microbial counts and diversity indices across contamination levels and sampling depths in Bonny Estuary coastal wetland soils

Site	Depth (cm)	THB ($\times 10^6$ CFU/g)	HUF ($\times 10^6$ CFU/g)	HUB ($\times 10^6$ CFU/g)	Shannon (H')
A	0–15	3.87 ± 0.42	2.14 ± 0.28	2.91 ± 0.33	1.52
A	15–30	3.42 ± 0.38	1.87 ± 0.24	2.56 ± 0.29	1.47
A	30–45	2.98 ± 0.35	1.53 ± 0.21	2.24 ± 0.26	1.38
B	0–15	5.63 ± 0.51	3.56 ± 0.37	4.12 ± 0.41	2.13
B	15–30	5.18 ± 0.47	3.24 ± 0.34	3.87 ± 0.38	2.05
C	0–15	7.24 ± 0.62	4.89 ± 0.45	5.67 ± 0.48	2.56
C	15–30	6.85 ± 0.58	4.56 ± 0.42	5.34 ± 0.45	2.48
D	0–15	9.38 ± 0.78	6.72 ± 0.56	7.45 ± 0.61	2.91
D	15–30	8.94 ± 0.72	6.31 ± 0.53	7.12 ± 0.58	2.84

Microbial diversity analysis using the Shannon-Wiener index demonstrated consistent reduction in community diversity with increasing crude oil contamination. The highest diversity values were recorded at the control site surface soils ($H' = 2.91$), while the lowest diversity was

observed at the heavily contaminated site at the 30–45 cm depth interval ($H' = 1.38$). Across all sites, surface soils (0–15 cm) maintained higher diversity indices compared to deeper sampling intervals.

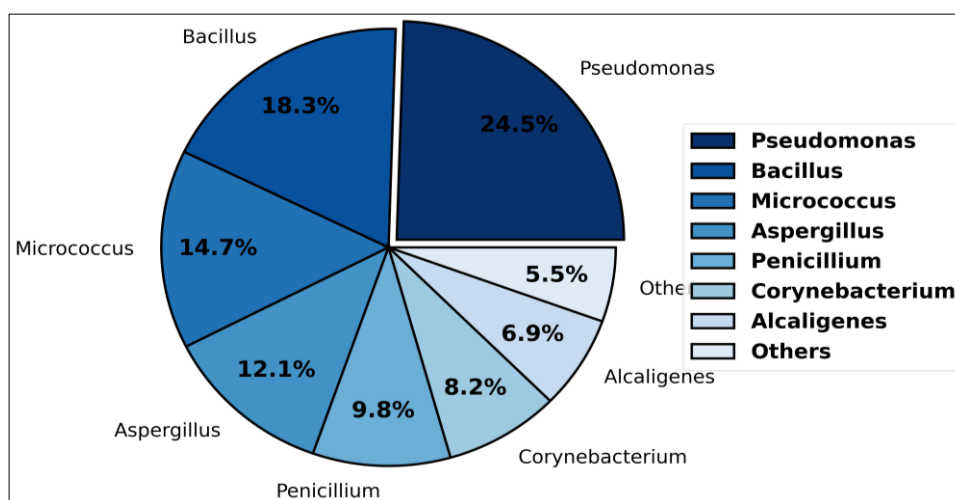


Fig 2: Percentage distribution of microbial genera isolated from crude oil-contaminated coastal wetland soils of the Bonny Estuary

Microbial identification revealed eight predominant genera across the sampling sites. *Pseudomonas* species constituted the largest proportion (24.5%) of total isolates, predominantly recovered from contaminated sites where they demonstrated strong hydrocarbon-utilizing capabilities. *Bacillus* species represented 18.3% of isolates, while *Micrococcus* accounted for 14.7%. Fungal genera including *Aspergillus* (12.1%) and *Penicillium* (9.8%) were recovered across all sites, with higher relative abundance at moderately contaminated locations. The dominance of hydrocarbon-degrading genera at contaminated sites, particularly *Pseudomonas* and *Corynebacterium*, indicates selective enrichment driven by petroleum hydrocarbon availability as a carbon source.

Discussion

The significant reduction in total heterotrophic bacterial counts from 8.94×10^6 CFU/g at control sites to 3.42×10^6 CFU/g at heavily contaminated locations demonstrates the pronounced toxic effect of crude oil on general microbial populations in coastal wetland soils. This finding aligns with observations reported by Adebuseye *et al.* [4] who

documented similar magnitude reductions in bacterial populations in petroleum-contaminated Niger Delta soils. The mechanism underlying this population decline involves the direct toxicity of low-molecular-weight hydrocarbons and volatile organic compounds that disrupt cell membrane integrity and interfere with essential enzymatic processes in non-adapted microorganisms [7]. This has substantial implications for nutrient cycling processes in contaminated wetlands, as general heterotrophic bacteria mediate critical decomposition and mineralization pathways. The dominance of *Pseudomonas* species (24.5%) corroborates previous research demonstrating the ecological advantage of this genus in hydrocarbon-contaminated environments [8]. Their capacity to produce biosurfactants and utilize diverse hydrocarbon substrates through multiple catabolic pathways explains their competitive dominance [13]. The progressive decline in Shannon-Wiener diversity indices from 2.84 to 1.47 reflects selective pressure through competitive exclusion of sensitive species by hydrocarbon-tolerant organisms [5][14]. Diminished functional redundancy consequently compromises ecosystem resilience and recovery capacity [15].

The strong negative correlation ($r = -0.89$) between total petroleum hydrocarbon concentrations and bacterial counts provides quantitative evidence for dose-dependent microbial suppression, suggesting that remediation efforts targeting hydrocarbon reduction would yield corresponding microbial community recovery^[10].

Bioremediation Implications

The prevalence of hydrocarbon-degrading genera, particularly *Pseudomonas*, *Bacillus*, and *Alcaligenes*, indicates substantial indigenous bioremediation potential within Bonny Estuary wetland soils^[11]. These organisms represent candidates for bioaugmentation strategies through nutrient supplementation or direct inoculation to accelerate petroleum degradation^[9].

Environmental Significance

The documented microbial community disruption carries broader significance for ecosystem service provision. Microbial communities in coastal wetlands perform essential functions including organic matter decomposition, nitrogen fixation, and sulfur cycling^[12]. The reduction in diversity and abundance suggests compromised biogeochemical cycling capacity in contaminated areas, with potential cascading effects on wetland vegetation and fisheries productivity.

Conclusion

This research demonstrates substantial disruption of microbial populations across contamination gradients in the Bonny Estuary. Total heterotrophic bacterial counts declined by 61.7% from control to heavily contaminated sites, while Shannon-Wiener diversity indices decreased from 2.84 to 1.47. The dominance of hydrocarbon-degrading genera, with *Pseudomonas* comprising 24.5% of total isolates, reflects selective enrichment driven by petroleum hydrocarbon availability. These findings support remediation planning using indigenous hydrocarbon degraders for biostimulation approaches. Future research should explore temporal dynamics of microbial recovery and functional genomic capabilities of dominant isolates.

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