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Microbial Dynamics and Hydrocarbon-Degrading Bacteria in Water and Bacterial Populations from Different Sources

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Abstract

This study evaluated microbial communities and physicochemical properties of three petroleum-contaminated water samples, Alpha, Bar, and Eleko, focusing on total heterotrophic and hydrocarbon-degrading bacteria to assess biodegradation potential. Eleko exhibited the highest total bacterial load (4.00×10^{12} cfu/mL) but the lowest proportion of hydrocarbon degraders (0.0005%), indicating dominance of generalist bacteria. Bar sample showed the highest percentage of hydrocarbon degraders (1.37%), reflecting microbial specialization to hydrocarbon substrates, while Alpha had intermediate values. Isolates included *Pseudomonas*, *Bacillus*, *Proteus*, *Staphylococcus*, and *Escherichia coli*, with enzymatic activities such as catalase and oxidase, salt tolerance, and diverse carbohydrate utilization, indicative of both Gram-positive and Gram-negative bacteria with hydrocarbon-degrading capabilities. Physicochemical analyses revealed neutral to slightly alkaline pH (7.6–7.9), warm temperatures (29.5–31°C), and high conductivity (5,580–5,980 µS/cm), total solids (2,790–2,990 ppm), and hardness (6,830–7,330 ppm $CaCO_3$), suggesting significant dissolved materials. Elevated chloride, biochemical oxygen demand (63.64–77 ppm), and chemical oxygen demand (140–170 ppm) confirmed organic pollution, while trace toxic metals including lead (0.47–0.69 ppm), chromium (0.59–0.61 ppm), and cadmium (up to 0.13 ppm) posed ecological risks. Although Bar demonstrated the greatest hydrocarbon-degrading potential, elevated pollution levels could inhibit microbial efficiency, highlighting the necessity for continuous environmental monitoring and remediation efforts.

Keywords: Heterotrophic bacteria, Hydrocarbon-degrading bacteria, Biodegradation, Water quality, Petroleum pollution

Introduction

Aquatic environments harbor diverse microbial communities, including heterotrophic bacteria that play vital roles in nutrient cycling, organic matter decomposition, and maintaining ecosystem balance. Among these, certain bacteria possess the unique metabolic capability to degrade hydrocarbons, making them essential agents in the natural attenuation and bioremediation of oil-contaminated waters [1]. In

regions affected by petroleum pollution from industrial discharges, runoff, or accidental spills understanding the presence and activity of hydrocarbon-utilizing bacteria (HUB) is crucial for assessing ecosystem health and designing effective remediation strategies.

Profiling both total heterotrophic bacteria and hydrocarbon-degrading bacterial populations in contaminated water bodies provides valuable insights into the natural biodegradation potential of these environments. Despite this importance, there remains a knowledge gap regarding how combined environmental stressors particularly heavy metals and high organic loads—impact these microbial communities and their functional capacity. Elevated levels of biochemical oxygen demand (BOD), chemical oxygen demand (COD), chloride, and water hardness, alongside toxic heavy metals such as lead (Pb), cadmium (Cd), and chromium (Cr), are frequently reported in petroleum-impacted sites. These physicochemical stressors can inhibit bacterial enzymatic activity, reduce microbial metabolic rates, and potentially limit biodegradation efficiency [2–4].

The water bodies sampled in this study Alpha, Bar, and Eleko are situated in regions experiencing industrial and urban pollution pressures, with known incidences of petroleum contamination and elevated heavy metal concentrations. These environmental stressors pose challenges to microbial survival and activity, potentially reducing the intrinsic capacity of microbial communities to degrade hydrocarbons.

This study aims to characterize the microbial communities, focusing on total heterotrophic and hydrocarbon-degrading bacteria, in petroleum-contaminated water samples, evaluate key physicochemical parameters influencing microbial activity and investigate the relationship between microbial degradation potential and environmental inhibitors such as heavy metals and organic pollutants. Addressing these objectives will fill critical knowledge gaps on how multiple contaminants interact to affect biodegradation in polluted aquatic ecosystems, thereby guiding effective environmental monitoring and remediation strategies.

Materials and Methods

Sampling and Enumeration of Microbial Populations

Water samples were aseptically collected from three distinct sites designated as Alpha, Bar, and Eleko, following standard environmental sampling protocols [5]. Samples were immediately placed on ice and transported to the laboratory for prompt processing within 4 hours to minimize changes in microbial composition [6]. Total heterotrophic bacterial counts (THBC) were determined using the standard plate count method as described by APHA [7]. Serial ten-fold dilutions of each water sample were prepared using sterile saline solution. Aliquots (0.1 mL) of appropriate dilutions were spread on nutrient agar plates and incubated aerobically at 30°C for 24–48 hours. Colony-forming units (cfu) were counted on plates with 30–300 colonies, and results were expressed as cfu/mL of sample. Hydrocarbon-utilizing bacteria (HUB) were isolated using mineral salt agar (MSA) supplemented with crude oil or aromatic

hydrocarbons as the sole carbon source, adapting methods outlined by Atlas and Bartha [8]. Plates were incubated at 30°C for 5–7 days to allow growth of slow-growing hydrocarbon degraders. Colonies exhibiting growth were enumerated and expressed as cfu/mL. The percentage of hydrocarbon utilizers was calculated relative to THBC to assess biodegradation potential [9].

Cultural and Biochemical Characterization of Isolates

Representative isolates from hydrocarbon-enriched plates were purified by repeated streaking on nutrient agar. Cultural characteristics, including colony morphology (colour, shape, margin, elevation), were recorded following guidelines by Cappuccino and Sherman [10].

Biochemical characterization employed standard microbiological tests to identify genus-level traits and metabolic capabilities [11]: Gram staining to differentiate Gram-positive and Gram-negative bacteria [9]. Spore staining to detect endospore formation [12]. Catalase and oxidase tests for enzymatic activity relevant to oxidative metabolism [13]. Citrate utilization to assess ability to use citrate as sole carbon source [14]. Growth at 50°C and in 6.5% NaCl to determine thermotolerance and salt tolerance respectively [15]. Growth on selective media: Mannitol Salt Agar (MSA) for salt-tolerant staphylococci and starch agar for amylase activity [16]. Kligler Iron Agar (KIA) test to detect glucose and lactose fermentation, and hydrogen sulfide production [17]. Indole and urease production tests for metabolic profiling [18]. Oxidation/fermentation of glucose to distinguish metabolic pathways [19]. All tests were conducted following standardized protocols and controls to ensure reproducibility and reliability of results [20-22].

Physical and Chemical Analyses of Water Samples

Physical parameters were measured in situ or immediately upon sample receipt, including: Temperature (°C), using a calibrated thermometer, pH**,** using a digital pH meter calibrated at pH 4, 7, and 10, electrical conductivity (µS/cm), using a conductivity meter and total solids and dissolved solids (ppm), measured gravimetrically following filtration.

Chemical analyses were conducted following standard procedures outlined by the American Public Health Association (APHA, 2017)**:** Acidity and alkalinity (expressed as ppm $CaCO_3$), Total hardness (ppm $CaCO_3$), Chloride (Cl^-), nitrate (NO_3^-), phosphate (PO_4^{3-}) concentrations, Dissolved oxygen (DO)**,** biochemical oxygen demand (BOD)**,** and chemical oxygen demand (COD) as indicators of organic pollution, Trace heavy metals including copper (Cu), zinc (Zn), lead (Pb), chromium (Cr), and cadmium (Cd)**,** detected using atomic absorption spectrophotometry. All analyses were performed in triplicate to ensure reproducibility.

Results and Discussion

Table 1, presents the total heterotrophic bacterial counts, hydrocarbon-utilizing bacterial counts, and the percentage of hydrocarbon degraders in the Alpha, Bar, and Eleko water samples.

Table 1: Bacterial Populations

SAMPLE	HETEROTROPHIS BACTERIAL COUNTS (cfu/ml)	TOTAL HYDROCARBON UTILIZERS (cfu/ml)	PERCENTAGE OF HYDROCARBON UTILIZERS (%)
ALPHA	1.90×10^{10}	2.55×10^{7}	0.13%
BAR	7.25×10^{8}	1.00×10^{7}	1.37%
ELEKO	4.00×10^{12}	1.85×10^{7}	0.0005%

Table 2 summarizes the observable colony morphology including colour, form, margin, and elevation of the bacterial isolates obtained from the water samples.

Table 2: Cultural Characteristics of Isolates

ISOLATE	COLOUR	FORM	MARGIN	ELEVATION
A1	White	Circular	Serrated	Subsurface
A3	White	Circular	Undulate	Flat
A4	White	Circular	Serrated	Subsurface
B5	Yellow	Circular	Undulate	Flat
B6	Orange	Circular	Entire	Flat
B9	Peach	Irregular	Entire	Elevated
E11	White	Irregular	Entire	Flat
E12	Peach	Irregular	Entire	Elevated

Table 3 shows the details the biochemical test results for eight hydrocarbon-degrading bacterial isolates, including Gram staining, enzyme activities, salt and temperature tolerance, motility, and metabolic capabilities.

Table 3: Biochemical Characteristics of Degrading Bacterial Isolates

TEST NO	TEST	A1	A3	A4	B5	B6	B9	E11	E12
1	Gram's Stain	+r	-r	+r	-r	+c	-r	+r	-r
2	Spore stain	-	~	-	~	~	~	+	~
3	Catalase	+	+	+	+	+	+	+	+
4	Oxidase	-	-	+	+	+	+	-	+
5	Citrate utilization	-	-	-	~	~	-	-	-
6	Growth at 50 ^{o}C	-	~	-	~	~	-	-	+
7	Growth at 6.5% NaCI	+	~	+	~	~	+	+	+
8	Growth on MSA	~	~	~	+	+	~	~	~
9	Growth on starch agar	+	~	-	~	~	~	+	~
10	KIA	k/a	k/a	~	k/a	~	~	~	k/a
11	Motility	-	~	-	-	~	-	+	-
12	Indole	+	-	-	-	~	-	-	-
13	Urease	-	-	-	-	~	-	-	-
14	Oxidation/ Fermentation of glucose	-/+	+/-	-/+	+/-	+/-	-/-	-/+	-/-

Table 4 outlines key physical parameters such as temperature, pH, conductivity, and total and dissolved solids measured in the three water bodies.

Table 4: Results of Physical Analysis of Water Samples

S/No	PARAMETER	LEVELS DETECTED		
		ALPHA	BAR	ELEKO
1	Temperature at sampling site	29.5 ^{o}C	30 ^{o}C	31 ^{o}C
2	pH at 20 ^{o}C	7.9	7.6	7.8

3	Conductivity (sem)	5.98×10^3	5.66×10^3	5.58×10^3
4	Total solids (ppm)	2990	2830	2790
5	Dissolved solids (ppm)	230	420	300

Table 5 shows the chemical analysis results of the water samples, including nutrient concentrations, oxygen demand indicators, and levels of trace metals and other pollutants.

Table 5: Results of Chemical Analysis of Water Samples

S/No	PARAMETER	LEVELS DETECTED		
		ALPHA	BAR	ELEKO
1	Acidity-P(ppm $CaCO_3$)	10	30	20
2	Alkalinity-M (ppm $CaCO_3$)	150	160	190
3	Total Hardness (ppm $CaCO_3$)	6870	7330	6830
4	Chloride Cl (ppm)	1120	1196	1240
5	Nitrate NO_3 (ppm)	0.28	0.26	0.23
6	Phosphate PO_4^{3-} (ppm)	5.60	4.40	9.20
7	Dissolved oxygen DO (ppm)	4.70	4.90	4.70
8	Biochemical Oxygen Demand BOD (ppm)	63.64	71.82	77
9	Chemical Oxygen Demand COD (ppm)	140	158	170
10	Copper Cu (ppm)	0.06	0.07	0.06
11	Zinc Zn (ppm)	ND	0.33	ND
12	Lead Pb (ppm)	0.69	0.47	0.54
13	Chromium Cr (ppm)	0.59	0.61	0.59
14	Cadmium Cd (ppm)	0.13	0.11	ND

ND- Not Detected

This study assessed the microbial and physicochemical properties of petroleum-contaminated water samples from three locations: Alpha**,** Bar, and Eleko**,** focusing on total heterotrophic bacteria (THB), hydrocarbon-utilizing bacteria (HUB), and their ability to degrade petroleum compounds.

The total heterotrophic bacterial count (THBC) varied significantly among sites: Eleko had the highest THBC (4.00×10^{12} cfu/mL)**,** followed by Alpha (1.90×10^{10} cfu/mL) and Bar (7.25×10^{8} cfu/mL). However, the relative abundance of HUB was inversely proportional**:** Bar exhibited the highest HUB percentage (1.37%)**,** Alpha intermediate, and Eleko the lowest (0.0005%)**,** suggesting that high bacterial loads do not equate to high biodegradation capacity [23]. This pattern aligns with earlier reports by Ghosal et al. [24] where microbial diversity increased under stress, but functionality (e.g., hydrocarbon degradation) was reduced due to competition or toxicity.

Eight bacterial isolates from hydrocarbon-enriched media were identified through biochemical characterization. These included: Pseudomonas aeruginosa, a well-documented hydrocarbon degrader with strong oxidative metabolism. *Bacillus subtilis* is capable of biosurfactant production to increase hydrocarbon bioavailability. *Corynebacterium spp.* is known to degrade short-chain hydrocarbons. *Micrococcus luteus* is able to co-metabolize hydrocarbons under certain conditions. *Alcaligenes spp.***,** *Flavobacterium spp.***,** and *Klebsiella spp*. are facultative hydrocarbon users, often found in polluted environments. *Staphylococcus spp.* although less potent, contributes to organic matter turnover and may tolerate stress [26-30].

These isolates demonstrated varied biochemical traits: catalase-positive, oxidase-variable, motile, salt-tolerant (up to 6.5% NaCl), and able to grow at 50°C. Their diversity suggests ecological resilience, with the potential for consortial action in biodegradation. However, their actual degrading efficiency remains to be quantified via hydrocarbon depletion assays or genomic analysis [31-33].

The physicochemical environment across the sites was moderately conducive to microbial activity, with neutral to slightly alkaline pH (7.6–7.9) and temperatures (29.5–31°C) optimal for mesophilic bacterial growth. However, several stressors were identified: Electrical conductivity (5580–5980 µS/cm) and total hardness (6830–7330 ppm $CaCO_3$) indicated substantial mineralization and ionic load, likely from industrial discharges high chloride levels (1120–1240 ppm) and total solids (2790–2990 ppm) contribute to osmotic stress, possibly suppressing sensitive microbial populations. Elevated BOD (63.64–77 ppm) and COD (140–170 ppm) reflect substantial organic pollution, favoring fast-growing heterotrophs but potentially inhibiting specialized degraders. Heavy metals**:** Lead (Pb: 0.47–0.69 ppm)**,** Chromium (Cr: 0.59–0.61 ppm)**,** Cadmium (Cd: up to 0.13 ppm)

These metals exceed WHO and EPA permissible limits for aquatic environments and are known to inhibit microbial enzymatic pathways, possibly explaining the reduced HUB counts in Eleko despite high THBC [34-37].

The presence of hydrocarbon-degrading bacteria confirms the natural biodegradation potential of these ecosystems. However, their low relative abundance, especially in heavily polluted zones like Eleko, suggests that pollution stress (e.g., metals, salinity, organic overload) is limiting microbial performance. To enhance microbial biodegradation and reduce ecological toxicity, the following interventions are recommended: Immediate identification and mitigation of petroleum discharge sources, installation of oil containment booms and sorbent barriers around vulnerable water bodies [38-40]. Nutrient amendment (e.g., nitrogen and phosphorus) to enhance microbial growth and metabolism. Adjustment of C:N:P ratios to support hydrocarbon metabolism. Introduction of high-performing indigenous or genetically characterized hydrocarbonoclastic bacteria, such as *Pseudomonas putida* or *Alcanivorax borkumensis*. Use of bacterial consortia that can degrade complex hydrocarbons under saline and metal-stressed conditions [41-42]. Planting of native aquatic vegetation (*Typha*, *Eichhornia*, etc.) known to enhance microbial degradation in the rhizosphere. Use of natural chelators (e.g., humic acids) or engineered solutions (e.g., zero-valent iron) to immobilize or reduce metal toxicity. Periodic tracking of HUB populations, microbial diversity indices, and water quality parameters to assess the progress of remediation [43-44].

Conclusion

The study highlights the ecological potential and limitations of microbial communities in petroleum-impacted water bodies. While diverse hydrocarbon-degrading bacteria are present, their effectiveness is constrained by physicochemical stressors, particularly heavy metals and organic pollution. A comprehensive bioremediation strategy combining source control, biostimulation, and ecosystem restoration is essential to restore water quality and support microbial-driven recovery in these environments. This investigation provides a comprehensive simultaneous analysis of microbial heterotrophic and hydrocarbon-utilizing populations alongside detailed physicochemical profiling of three environmentally distinct water bodies in Nigeria. The documented inverse relationship between total bacterial load and hydrocarbon degrader percentage is particularly novel, offering insights into microbial competition and pollutant impacts. Furthermore, detecting heavy metal co-contamination alongside hydrocarbon-utilizing bacteria highlights adaptation dynamics important for future bioremediation strategies in tropical aquatic environments.

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