Physicochemical Properties of Sediment and Isolation of Hydrocarbon-Degrading Bacteria from Crude Oil-Contaminated Iko River Estuary

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Abstract. Crude oil pollution poses a significant threat by contaminating drinking water supplies and soil, posing health hazards to current and future generations and disrupting the ecological balance of affected ecosystems. Thus, the current study evaluated the physicochemical properties of sediment of the Iko River estuary contaminated with crude oil and isolated hydrocarbon-degrading bacteria using appropriate methods. Physicochemical analyses of the sediment showed slight variations in pH, temperature and electrical conductivity along the different sections of the estuary. The pH values were 6.2 (upstream), 5.0 (midstream), and 5.8 (downstream). The temperatures were 29 °C (upstream and downstream) and 30 °C (midstream). Electrical conductivity increased downstream, reaching 173 μScm-1. The isolated organisms were one Gram-negative and four Gram-positive bacterial isolates. The researchers tentatively identified the bacterial isolates as *Bacillus cereus, Bacillus subtilis, Micrococcus spp., Pseudomonas aeruginosa,* and *Bacillus spp*., and found that all isolates except *Micrococcus spp.* were motile. The analysis revealed that of the five isolates, *Pseudomonas aeruginosa* and *Bacillus subtilis* demonstrated strong potential to utilise all crude oil. Researchers could harness these microbial communities to reduce the impact of crude oil pollution and enhance the fertility and productivity of farmlands.

Keywords: Pollution; health hazards; microbial communities; *Bacillus subtilis*; farm lands.

INTRODUCTION

The world demand for fuel has led to the exploration and production of an increasing number of petroleum hydrocarbons. Petroleum hydrocarbons (PHs) are the most abundant pollutants in soil and water environments [1]. It is reported that about eight million tons of petroleum are released into the soil and water environment every year. Leakage of PHs commonly occurs during exploration, production, refining, storage and transportation. Toxic and persistent constituents of PHs, such as olefinic and paraffinic hydrocarbons and monoaromatic hydrocarbons (e.g., benzene, toluene, and xylene), pose a significant threat to human health [2, 3].

Crude oil pollution is a global problem that leads to the uptake and accumulation of toxic chemical pollutants along the food chain and harms the flora and fauna of affected habitats. Hydrocarbons also pose a significant threat, contaminating drinking water supplies and soil, posing health hazards to current and future generations, and disrupting the ecological balance of affected ecosystems. Hence, developing an economical and effective way to remove petroleum hydrocarbons from ecosystems is a significant goal for maintaining ecosystem health and human health [3].

There are several physical, chemical, and biological methods to remove petroleum hydrocarbons from contaminated environments. Biological processes, also known as bioremediation, are considered the most exciting, cost-effective, and environmentally friendly approaches for removing petroleum hydrocarbons compared to chemical or physical processes. However, the mechanisms underlying biological processes degrade slowly, limiting their widespread implementation.

Petroleum hydrocarbons, such as heavy oil, degrade slowly due to their highly hydrophobic properties [2]. Chemical surfactants are nonbiodegradable and thus have a relatively high potential to cause toxicity to living organisms, hydrocarbon-degrading bacteria, and the surrounding environment. They can also inhibit biodegradation through toxic interactions and sequester hydrocarbons into surfactant micelles, thereby decreasing the oxygen uptake rate [4]. The current study evaluated the physicochemical properties of sediment and determined its bacterial diversity in a crude oil-contaminated estuary.

MATERIALS AND METHODS

Study Area. The study area was the mangrove ecosystem of the Iko River Estuary. Iko is located within the petroleum belt of the Niger Delta, Nigeria (70 30' N and 70 45' N, and longitudes 70 30' E and 70 40' E). The Iko River estuary has semi-diurnal tides and a shallow depth of 1 to 7 m at flood and ebb tides. The estuary was more than 20 km long with an average width of about 5 m. Iko River takes its course from Qua Iboe River catchments and drains directly into the Atlantic Ocean at the Bight of Bonny. It has many tributaries, some of which drain into the Imo River estuary, which opens into the Atlantic Ocean. Soft dark mudflats, usually exposed during low tide, mangrove swamps, shoals and sandbars, characterise the shoreline of the Iko River. The area was characterised by a humid tropical climate with rainfall reaching about 3,000 mm per annum.

Sample Collection. The researchers selected three sites in the Iko River estuary to represent a wide range of crude oil contamination. Intertidal sediment samples were aseptically collected using an Eckman sediment grab, placed in 95% ethanol-sanitised plastic containers, and transported to the Microbiology laboratory within 24 hours of collection for further analysis. The researchers kept the samples at ambient temperature.

Physicochemical Analysis of Sediment Samples. Physicochemical parameters of the sediment samples were determined using standard analytical procedures recommended by APHA [5]. The researchers measured the pH of the samples potentiometrically in a 1:2 soil–water suspension, and electrical conductivity (EC) in the same suspension using a conductivity meter. Organic matter content, Organic carbon content, total nitrogen content, phosphate, Nitrate (NO^{-}_{3}), nitrite, sulphate (SO_4^{2-}), chloride (Cl^-), particle size distribution and total hydrocarbon content (THC) were determined using conventional methods [5, 6].

Enumeration and Isolation of Oil Degrading Bacteria (ODB). The density of oil-degrading bacteria (ODB) was estimated using the vapour-phase transfer technique as described by [7]. 1 g of sample was diluted in a series of dilutions. The desired diluents were plated on MSM fortified with sterilised Bonny Light crude oil and were incubated at room temperature for 14 to 21 days, depending on the growth rate on the plates. Colonies of bacteria on plates treated with crude oil were enumerated. The enrichment culture technique was employed. Precisely 1 g of sediment sample was inoculated into three sets of conical flask containing 50 ml of sterile Mineral Salt Medium [K_2HPO_4 – 6 g, NaCl – 12 g, KH_2PO_4 – 6 g, $(NH_4)_2SO_4$ – 6 g, $MgSO_4.7H_2O$ – 2.6 g, $CaCl_2.2H_2O$ – 0.16 g, per liter (pH 7.0 + 0.2)] (MSM) enriched with 1% crude oil as carbon source. The medium was incubated at 28 °C in a shaker incubator (100 rpm) for 7 days. After 7 days of incubation, the samples were serially diluted with sterile water and plated on Nutrient Agar (NA) to obtain viable bacterial cells. The discrete colonies obtained were subcultured using the streak method as described by the author [8] to obtain pure cultures.

Maintenance of Pure Cultures of Oil-degrading Bacterial Isolates. Distinct colonies of the oil-degrading bacteria isolated from estuarine sediment were subcultured into McCartney bottles containing freshly prepared Nutrient Agar slants and incubated at 30 ± 2 °C for 24 hours before storage at 4 °C for characterisation.

Characterisation of Bacterial Isolate. The best crude oil-utilising bacterial isolates were characterised based on their cultural and morphological attributes and responses to standard biochemical tests, as described by the author [8]. Twenty-four-hour-old monocultures of bacteria obtained were subjected to Gram's staining, endospore staining, and several biochemical tests, including the Catalase test, Citrate Utilisation test, Oxidase test, Motility test, Endospore test, Methyl red and Vogues Proskauer test, and Indole test, as well as sugar fermentation tests.

Screening for Crude Oil Utilising Potential of the Bacterial Isolates. The researchers added precisely 15 g of agar-agar to the mineral salt medium, sterilised the medium, and allowed it to solidify. They overlaid the solidified plates with 1% (v/v) sterile crude oil for 15–30 minutes and then streaked the test isolates onto the plate surface. All inoculated plates were incubated at room temperature for 7-14 days with periodic observation. Colonies that eventually developed, showing areas of clearing, were selected and rated. The utilisation was rated based on the diameter and luxurious nature of the developed colonies; i.e., '+', '++' or '+++' indicated the magnitude of the oil-degrading potential.

Deoxyribonucleic Acid (DNA) Extraction. A Zymo-SpinTM IV-HRC Spin Filter was first prepared by snapping off the base, inserting it into a collection tube, and spinning in a microcentrifuge at 8,000 xg for 3 minutes. The researchers extracted genomic DNA from the samples by transferring 0.25 g of each sample into separate ZR BashingBead™ Lysis Tubes. They added 750 µl of lysis solution to each tube and vortexed the mixtures for 5–10 minutes, then transferred each mixture to a bead beater fitted with a 2 ml tube holder assembly and centrifuged it at 10,000 × g for 1 minute. Next, they transferred the supernatant from each tube to a ZymoSpin™ IV Spin Filter in a collection tube, then centrifuged at 7,000 × g for 1 minute. Afterwards, they added 1,200 µL of soil DNA-binding buffer to the filtrate in the collection tube. From the mixture above, 800 µl was transferred to a Zymo-SpinTM IIC Column in a collection tube and centrifuged at 10,000 x g for 1 minute.

The researchers discarded the flow-through from the collection tube, added 200 µl of DNA Prewash buffer to the Zymo-Spin™ IIC, placed the Zymo-Spin™ IIC in a new collection tube, and centrifuged the contents at 10,000 × g for 1 minute. They transferred the Zymo-Spin™ IIC to a new collection tube, added 500 µL of Sample DNA Wash Buffer, and centrifuged at 10,000 × g for 1 minute. Next, they moved the Zymo-Spin™ IIC to a clean 1.5 ml microcentrifuge tube, added 100 µl of DNA Elution Buffer to the column matrix, and centrifuged it at 10,000 × g for 30 seconds. Finally, they transferred the eluted DNA to a prepared Zymo-Spin™ IV-HRC Spin Filter placed in a clean 1.5 ml Eppendorf tube, then centrifuged at 8,000 × g for 1 minute; the resulting filtrate constituted the genomic DNA extract.

Polymerase chain reaction and next-generation sequencing. The researchers subjected the extracted DNA samples to polymerase chain reaction using a 96-well thermal cycler (2ETM, UK). They used labelled Eppendorf tubes bearing sample codes and primer names for amplification and performed the amplifications with the universal primer pair 341F and 785R, which targets the V3 and V4 regions of the 16S rRNA gene. Resulting amplicons were gel-purified, end-repaired, and Illumina-specific adapter sequences were ligated to each amplicon (NEBNext Ultra II DNA library prep kit). Following quantification, the samples were individually indexed (NEBNext Multiplex Oligos for Illumina (Dual Index Primers Set 1), and another AMPure XP bead-based purification step was performed. Amplicons were then sequenced on Illumina's MiSeq platform using a MiSeq V3 (600-cycle) kit according to the manufacturer's protocol. The sequencing platform generated 20 Mb of data per sample (2 × 300 bp paired-end reads), and the researchers performed bioinformatic analyses using the National Centre for Biotechnology Information BLAST (version 2.24) and the CLC bio Genomics Workbench (version 7.5.1).

RESULTS AND DISCUSSION

Physicochemical properties of estuary sediment are presented in Table 1.

Table 1 – Physicochemical properties of estuarine epipelic sediments

Parameter	$Upstream_1$ (Okoro River)	$Midstream_1$ (Okoro River)	$Downstream_2$ (Edonwhii)	WHO Limit
Temperature (°C)	29	30	29	NA
PH	6.2	5.0	5.8	6.5 - 8.5
Total Organic Carbon (%)	3.45	3.00	4.01	NA
Organic Matter (%)	70.37	69.62	71.12	NA
Available Phosphorus	52.44	50.32	51.02	NA
Total Nitrogen	0.39	0.45	0.41	
Electrical Conductivity(µscm-1)	109	128	173	1500
Chloride (mg/kg)	103	116	141	
Nitrate (mg/kg)	14.51	19.43	17.32	40.0

Parameter	Upstream1 (Okoro River)	Midstream1 (Okoro River)	Downstream2 (Edonwhii)	WHO Limit
Nitrite (mg/kg)	0.93	1.30	1.01	40.0
Phosphate (mg/kg)	7.88	9.10	8.42	0.05
Sulphate (mg/kg)	3.24	5.83	5.33	240.0
Particle Size: Silt	5.01	4.77	4.94	
Clay	10.12	9.93	9.82	
Sand	84.87	85.3	85.24	
Total Petroleum Hydrocarbon (mg/kg)	35.35	35.39	35.37	30

Notes: NA – Not applicable.

The temperature of the estuarine sediment ecosystem ranged between 29 °C and 30 °C. The temperature of the upstream (Okoro River) and Downstream (Edonwhii) sediment was 29 °C, whereas that of Midstream1 (Okoro River) was 30 °C. The pH values for the estuarine ecosystem ranged from 5.0 to 6.2, indicating a mildly acidic pH at all stations. The total organic carbon in the estuarine ecosystem ranged from 3.00% to 4.01%. TOC of Midstream1 (Okoro River) was 1.15 and 1.33 times lower than upstream (Okoro River) and Downstream (Edonwhii) sediment. The organic matter content of sediment was 70.37, 69.62, and 71.12% in upstream, lower stream, and downstream sediment, respectively. The electrical conductivity in the estuarine ecosystem ranged from 107 µs/cm to 173 µs/cm, with downstream sediment recording the highest value and upstream sediment recording the lowest.

Chloride concentrations in sediment were 103, 116, and 141 mg/kg in upstream, lower stream, and downstream sediment, respectively, while Nitrate concentrations were 14.51, 19.43, and 17.32 mg/kg in upstream, lower stream, and downstream sediment, respectively. Nitrite in midstream (1.30 mg/g) was 1.29 and 1.40 times higher than upstream and downstream sediment. Phosphate levels were 7.88, 9.10, and 8.42 mg/kg, suggesting that midstream sediment was 1.15 and 1.08 times higher than upstream, lower-stream, and downstream sediment, respectively. In the estuarine sediment ecosystem, sulphate (SO_4^2) concentration ranged from 3.53 mg/kg to 5.83 mg/kg, with midstream sediment having the highest value, followed by upstream and downstream sediment. The estuarine ecosystem recorded a higher total hydrocarbon content of 35.35 mg/kg to 35.39 mg/kg, with midstream recording the highest (35.39 mg/kg) and upstream sediment recording the least HC content. The Particle Size of the sediment was predominantly sand (84.87-85.3 %), followed by silt (4.77-5.01 %) and clay (9.82-10.12 %).

The results presented in Figure 1 revealed a rich bacterial assemblage in the sediment. Culture-dependent techniques showed that counts of hydrocarbon-degrading bacteria in estuarine sediment ranged from 3.40 ± 0.07 to 3.88 ± 0.05 CFU/g. The hydrocarbon-degrading bacteria in the upstream, midstream, and downstream were 3.56 ± 0.005, 3.40 ± 0.07, and 3.88 ± 0.05 CFU/g, respectively.

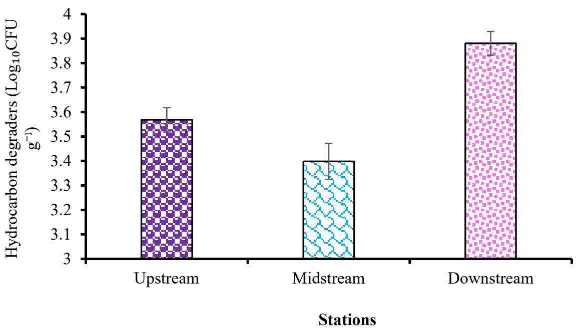

Figure 1 – Hydrocarbon-degrading bacterial counts in sediment samples

Hydrocarbon-degrading bacterial isolates were characterised and identified based on their morphological, microscopic, and biochemical characteristics (Table 2). The result revealed 1 Gram-negative and 4 Gram-positive bacterial isolates. The researchers tentatively identified the bacterial isolates as *Bacillus cereus, Bacillus subtilis, Micrococcus spp., Pseudomonas aeruginosa,* and *Bacillus spp*., and all isolates except *Micrococcus sp*. exhibited motility. The results for indole production, hydrogen sulphide production, the methyl red test, the Voges-Proskauer test, and sugar fermentation are presented in Table 2. The analysis revealed that of the five isolates, *Pseudomo-*

nas aeruginosa and *Bacillus subtilis* demonstrated strong potential to utilise all crude oil.

Table 2 – Cultural, microscopy and biochemical characteristics of cellulose-degrading bacteria isolated from Estuarine sediment

Gram Reactions	Shape	Catalase	Coagulase	Motility	Starch hydrolysis	Citrate	Urease	MR	VP	Spore formation	H2S	Oxidase	Indole	Glucose	Maltose	Xylose	Lactose	Fructose	Sucrose	Mannitol	Galactose	Probable organisms
+	rod	+	-	+	+	-	-	-	+	+	-	-	-	AG	A	-	-	A	A	-	A	*Bacillus cereus*
+	rod	+	-	+	+	+	-	-	+	+	-	-	-	AG	A	A	-	A	-	-	A	*Bacillus sublilis*
+	Cocci in pairs	+	-	-	+	+	+	+	-	-	-	+	-	-	A	A	-	A	-	A	A	*Micrococcus spp.*
-	rod	+	-	+	-	+	-	-	+	-	+	-	+	A	-	-	AG	AG	AG	AG	AG	*Pseudomonas aeruginosa*
+	Rod in a short chain	+	-	+	+	+	+	+	-	-	-	-	-	AG	A	A	-	A	A	-	A	*Bacillus spp.*

Notes: + = Positive, - = Negative; A = Acid production; AG = Acid and Gas production; G = Gas production.

The researchers demonstrated this trend by observing a remarkable increase in isolate biomass when they exposed the organisms to crude oil as the sole carbon source (Table 3).

Table 3 – Crude oil utilisation potentials of the bacterial isolate

Bacterial isolate	Growth of crude oil after 7 days	Growth of crude oil after 14 days
Bacillus cereus	+	-
Pseudomonas aeruginosa	++++	++++
Bacillus subtilis	+++	++++
Micrococcus spp.	+++	+++
Bacillus spp.	++	++

Notes: - = no growth; + = weak (1-5 mm); ++ = moderate (6 -10) mm; +++ = strong (11-15); ++++ = very strong (16 – 20 mm).

Table 4 shows the sequencing results of the hydrocarbon-degrading bacterial isolates in the sample. The bacterial isolates were identified mainly *as Bacillus subtilis subsp. Subtilis str*, 168 and *Pseudomonas aeruginosa* PAOI.

Table 4 – Sequencing Result showing Corresponding Accession Numbers, Percentage Match and Identity of Bacteria in the Gene Bank

Isolate Code	Sample type	DNA Type	Gene Bank Accession Number	%-age match	Organism in the Gene Bank
IsoA	DNA	Genomic	NC_000964	98.93	Bacillus subtilis subsp. Subtilis str. 168
IsoB	DNA	Genomic	NC_002516.2	97.95	Pseudomonas aeruginosa PAOI

Estuaries are highly sensitive ecosystems that lie at the convergence of freshwater and sea, with distinct physical, chemical, and biological characteristics. Estuaries function as transitional zones where river water mixes with seawater, creating dynamic, complex aquatic environments [9–11]. This combination exposes estuaries to significant volumes of human waste while simultaneously creating unusual, nutrient-rich habitats [12]. These processes significantly influence water quality, making estuaries vulnerable to environmental degradation. However, this ecosystem serves as a breeding and nidification area for numerous commercially valuable fish and crustacean species [13]. Regular monitoring procedures are necessary to determine the temporal and spatial fluctuations in water quality. Regular monitoring procedures are required to determine temporal and spatial fluctuations in water quality. To date, many studies have examined the physicochemical parameters of various estuaries in Nigeria [6, 10, 11, 14]. In this study, the researchers observed wide variations in the measured parameters at all stations. Temperature is a key regulator of biogeochemical processes in ecosystems, influencing the growth, activity, and survival of organisms [15]. Sediment temperatures were similar (mesophilic) at all the sites. The sediment samples exhibited mesophilic temperatures, which support the growth and proliferation of a diverse range of microorganisms in aquatic ecosystems. This temperature range, neither too hot nor too cold, promotes optimal growth and activity of bacteria, fungi, and other microbes, contributing to the overall biodiversity and function of aquatic ecosystems.

The electrochemical property of pH in estuaries is influenced by numerous factors, including vegetation, tidal flooding, benthic primary productivity, the dominant respiratory pathway or pathways, and sediment composition [16]. Despite sediment samples exhibiting a slightly acidic pH across all sites, pH values varied among sites, indicating a diversity of environmental conditions within the ecosystem. This variation in pH, even if slight, can have significant impacts on the microbial communities inhabiting the sediment and, in turn, affect the overall ecology of the area [17]. The slightly acidic values recorded in sediment could be attributed to increased input of humic materials from the associated swamps and creeks, dilution of ionic concentrations by rainwater and poor buffering capacity of flood waters [6], thereby causing a general drop in pH throughout the system as the sampling was done in the rainy season. The pH of rainfall can be as low as 5.6, primarily due to dissolved carbon dioxide (CO_2) [6]. Furthermore, the pH values recorded in the sediments were within the normal range of values observed for estuarine sediments (5-7) [15].

Total organic carbon (TOC) and organic Matter (OM) are important environmental factors that regulate the structure and function of sedimentary microbial communities. Total organic carbon values indicate that the Midstream sediment had lower TOC content than the other two locations, with implications for the microbial communities present in each area. The lower TOC in the Midstream sediment limited bacterial species growth and diversity. In comparison, the slightly higher TOC in the upstream and downstream sediments supported a more diverse and abundant bacterial community (Table 1). This variation in TOC levels across the estuary may be influenced by factors such as input of organic material from land, sediment transport dynamics, and local hydrological conditions [18]. Electrical conductivity is an essential parameter to estimate, as it indicates the overall mineralisation of water. The electrical conductivity (EC) of sediments from the studied estuary ranged from 109 to 173 μS/cm. The mean EC values obtained were within the [19] safe limit of 1500.0 μS/cm. This range of electrical conductivity values (107 to 173 μs/cm) across the estuarine ecosystem suggests that different locations have varying levels of dissolved ions in the sediments, which may influence microbial communities and biogeochemical processes [20]. The low ion concentrations in the water channels contribute to the low EC values in the studied sediments.

Nutrients are considered among the most essential features of the estuarine environment. It influences the growth, reproduction and metabolic activities of the living beings. Nutrients, including nitrates and phosphates, are indeed influenced by seasonal variations, tidal conditions, and freshwater inputs from land sources. These factors can affect nutrient availability and movement within the estuary, potentially leading to eutrophication. Phosphorus, along with Nitrogen, causes explosive algal growth [21]. A high concentration of phosphate observed in estuary sediment might be due to the intrusion of seawater, as well as heavy rainfall, and the mixing of land runoff from agricultural fields contaminated with superphosphates and alkyl phosphates from soap and detergents used by the public for bathing and washing clothes [22]. During this study, the estuarine midstream sediment had the highest nitrate and nitrite concentrations, but remained within the standard required for unpolluted water of 40.0 mg/kg [19]. The values of nitrate and nitrite recorded were higher than 0.090 mg/l, 0.083 mg/l, and 0.085 mg/l for nitrite, and 8.03 mg/l, 8.11 mg/l, and 8.13 mg/l for nitrate for upstream, midstream, and downstream, respectively, of the same ecosystem reported by [23]. These differences could be attributed to seasonal variation. Nitrate and nitrite concentrations can change with the seasons, as the biological and chemical processes that regulate these nutrients in the environment are affected by temperature, rainfall, and sunlight [24]. The sulphate content of the studied sediments ranged from 3.24 to 5.83 mg/g, which is notably higher than the values reported by authors [1] (83.72-89.56 mg/kg). However, the sulphate concentrations obtained in the studied sediments were still below the 240.0 mg kg-1 limit for unpolluted sediment recommended by the sources [19]. Based on these findings, the results suggest that sulphate pollution has not significantly impacted the studied sediments.

The total hydrocarbon content (THC) in sediments can provide insight into the presence and potential sources of organic pollutants in the estuary. The total hydrocarbon content was slightly higher in midstream sediment (35.39 mg/kg) than in upstream (35.35 mg/kg) and downstream (35.37 mg/kg) samples. The slight difference in THC values between the midstream, upstream, and downstream sediments suggests that there may be minor variations in the amount of hydrocarbon contamination in the sediments across the estuary. This variation could be due to a range of factors, such as differences in the sources of hydrocarbon contamination (e.g., urban runoff, industrial effluents, or natural oil seepage), sediment deposition patterns, and biological degradation processes. The values for the different stations exceeded the WHO-recommended 30 mg/kg total hydrocarbon concentration for unpolluted sediment [19]. The high levels of total hydrocarbon content (THC) in the sediments may result from anthropogenic activities associated with oil and gas operations in the area. The presence of elevated total hydrocarbon content (THC) in sediments may cause a significant drop in dissolved oxygen (DO) levels in the studied river estuaries, potentially harming the health and survival of aquatic life [1, 25]. This result contrasts with the work of authors [26], who reported higher total hydrocarbon contents (>200mg/kg) in sediment from the Iko River. Researchers found variable particle-size percentages in sediments across all locations. Sand dominated the sediments (87.87–88.3%), followed by clay (9.82–10.12%) and silt (1.77–2.01%). The predominance of sand in the sediments is in agreement with the observation by authors [23] that in the sediments of the Iko River, sand and clay are predominant. The distribution of these fractions is influenced by strong tides and longshore drift, which govern the movement of sediments in the estuary. The high clay mineral content in the Iko River sediments is a notable feature, given that clay particles have a well-established affinity for adsorbing both metallic and organic pollutants. This abundance of clay minerals suggests that the Iko River sediments may serve as an important reservoir for metallic pollutants, effectively reducing their bioavailability and mitigating their impact on the aquatic ecosystem by binding and retaining these pollutants.

Variation in hydrocarbon-degrading bacterial population densities across regions may be influenced by environmental factors such as temperature, pH, salinity, and nutrient availability. The type and concentration of hydrocarbons present in the different areas may have also affected the population densities of hydrocarbon-degrading bacteria, as other bacteria have different metabolic preferences [27, 28]. The hydrocarbon-degrading bacterial isolates identified were *Bacillus cereus, Bacillus subtilis, Micrococcus spp., Pseudomonas aeruginosa*, and *Bacillus spp*. Gram-positive and Gram-negative bacteria play a role in contaminant degradation, with Gram-negative bacteria generally dominating this process; however, sediment samples were dominated by Gram-negative hydrocarbonoclastic bacteria. These bacterial species have previously been implicated in crude oil biodegradation and exhibit varying degrees of crude oil-degrading capability, as reported in previous studies [28, 29]. Bacillus is a genus encompassing a variety of species, some of which have been reported to degrade hydrocarbons [17].

Many microorganisms possess the metabolic ability to degrade hydrocarbon compounds as their sole carbon and energy source, despite their high insolubility in water. The hydrocarbonoclastic activity of these microorganisms is mainly dependent on their ability to produce the necessary enzymes to break down the resistant components of hydrocarbons, rather than on their nutrient requirements. This study investigated the hydrocarbonoclastic potential of estuarine sediment using the hydrocarbon overlay method, revealing that *Pseudomonas* aeruginosa and *Bacillus subtilis* exhibited vigorous hydrocarbonoclastic activity (11-15 mm). These findings support the role of *Pseudomonas spp.* in the utilisation of crude oil and its products, as previously reported by authors [30, 31]. The results of this study support the previous research by authors [17], which demonstrated that *Bacillus subtilis* can utilise crude oil. This potential may be due to the species' inherent haemolytic and emulsification activities, which could facilitate the degradation of hydrocarbons, thereby enabling the utilisation of carbon atoms for cellular growth and energy production.

CONCLUSIONS

Various factors, including human activities, influence the composition of estuary water. This study found that sediment in the Iko River Estuary was rich in organic Matter and supported a diverse heterotrophic microbial community. The total hydrocarbon content in the sediment exceeded recommended standards, indicating potential anthropogenic contamination. Despite this, the majority of environmental attributes were within suitable levels for microbial processes and ecological health.

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