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Bacterial and physicochemical properties of a crude oil polluted freshwater wetland at Apoi Creek, Bayelsa State, Nigeria

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Abstract

Illegal crude oil bunkering and artisanal refining are becoming the leading cause of oil spill in the Niger Delta. In this study, we used standard physicochemical and microbiological methods to study a freshwater wetland in Apoi Creek, Bayelsa State that was affected by oil spill and an adjacent unpolluted site. The results show that both study areas are slightly acidic with low salinity and macronutrients particularly nitrate, phosphate and potassium. Heavy metals (zinc, lead and nickel) occurred in traces in unpolluted soil, which were generally below 0.1 mg/kg in both strata, but significantly higher levels ($P<0.05$) of zinc and lead occurred in the polluted soil. In the topsoil strata, TOC, TOM and ETPH were 0.60±0.01%, 1.03±0.64% and 85±7.07 mg/kg in the unpolluted soil but 5.12±0.02%, 8.80±0.03% and 1,404±1.41 mg/kg respectively in the polluted soil ($P<0.05$). In the subsoil strata, the trend was similar, but with higher concentrations. In the topsoil, THB was $1.21±2.12×10^8$ cfu/g in the unpolluted site and $9.40±4.95×10^8$ cfu/g in the polluted site ($P<0.05$). In the subsoil, they were also in the order of 10^8 ($P>0.05$). In the topsoil strata, HUB density was $5.65±9.19×10^7$ cfu/g in the unpolluted site and $8.95±0.71×10^8$ cfu/g in the polluted soil ($P>0.05$). In the subsoil, the population density of HUB in the unpolluted site was $4.25±0.71×10^7$ cfu/g, which was significantly lower ($P<0.05$) than the $8.20±5.66×10^8$ recorded in the polluted site. The dominant bacteria identified using molecular method based on 16S rDNA gene sequencing include *Bacillus altitudinis*, *Fictibacillus macauensis*, *Micrococcus luteus* and *Enterobacter chengduensis*. We conclude that the oil spill impacted the wetland via increased hydrocarbons and heavy metal content.

Key words: *Heterotrophic bacteria, Hydrocarbon utilizers, Illegal bunkering, Niger Delta, Oil exploration, Oil spill*

1. Introduction

Nigeria is a major crude oil producing and exporting country, but dependent on foreign countries for the supply of refined petroleum products to meet her domestic demand. This is due to outdated technology of

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existing government refineries, which are poorly maintained. Commercial oil and gas exploration in Nigeria, which started in the 1950s, is mostly concentrated in the Niger Delta region of the country (Ohimain, 2022). The Niger Delta, which is about 76,000 sq km is the largest wetland in Africa and the third largest in the world (Ayanlade and Proske, 2015; Edo and Albrecht, 2021; Ohimain *et al.*, 2024). The Niger Delta ecosystems consists of several landscapes including lowland rainforests, coastal mangroves and beach ridges, of which the freshwater rainforests are the most productive (Ohimain, 2022).

Apoi Creek, which is located in Bayelsa State, Central Niger Delta is a tidal freshwater, lowland swamp-forest, which comprised largely of marshes, fresh water swamps and mangrove forests with numerous meandering creeks (Edo and Albrecht, 2021). Apoi Creek is estimated to have an area of about 29,213 ha, which is a designated forest reserve, and a wetland with Ramsar Site No. 1751 (Ramsar, 2008; Edo and Albrecht, 2021). Apoi Creek has other relevance especially for host communities. It hosts several important plant and animal species including the Niger Delta Red Colobus monkey (*Piliocolobus epieni*), which has been ranked as endangered/critically endangered species in the IUCN list of threatened species, which is endemic to the area. The area is a breeding and spawning ground for fisheries. The Apoi Creek support local economic activities through the provision of water, fisheries, farmland, timber and non-timber forest products (Ramsar, 2008). Exploitation of these resources especially through fishing, hunting, harvesting, wood logging, farming and oil and gas exploration has mounted pressure on the ecosystem (Ayanlade and Proske, 2015; Akeem and Lewiska, 2021; Edo and Albrecht, 2021).

Oil and gas exploration activities in the Apoi Creek has benefitted the Nigerian state, but accompanying environmental impacts, also mount pressure on the ecosystem. Important oilfields in the area include Ogbainbiri, Clough Creek, Apoi among others. These oilfields have installed infrastructure that are spread over the area including flowstations, well heads, jetties and many linear structures such as flowlines, pipelines and dredged canals. Oil spills arising from their infrastructure is a major course of concern (Edo and Albrecht, 2021). Recently, the major cause of spillage in the Niger Delta is linked to illegal oil bunkering and artisanal refining activities especially from pipelines (Luke *et al.*, 2021), which sometimes results in explosions and fires leading to air emissions and impacting soil and water quality, and biota especially vegetation, fauna and microbes.

Studies on the effects of oil spill on the physicochemical and microbiological properties of soils and sediments in the Niger Delta are quite common in literature especially covering prolific oilfields such as Ogoni land (Joel and Amajuoyi, 2009; Ogbonna *et al.*, 2021), Bonny River (Ibietela and Rogers, 2022), Brass River (Luke *et al.*, 2021), Santa Barbara River especially in the Nembe Creek (Ighariemu *et al.*, 2023; Isukul *et al.*, 2023; Yaabari *et al.*, 2024), Eket (Udotong *et al.*, 2008) among others. But such studies appear not to cover Apoi Creek area. Besides, molecular studies of microbial guilds present in oil spill environment is not common in the Niger Delta, but are now only recently been studied in few places such as Emuoha (Chikere *et al.*, 2020), Ogoni land (Chikere *et al.*, 2019; Iturbe-Espinoza *et al.*, 2022) and Santa Barbra River (Allen-Adebayo *et al.*, 2024; Ohimain *et al.*, 2024). Hence, this study is focused on assessing the effect on wetland soil physicochemical and microbial properties by an oil spill from a pipeline traversing Apoi Creek that was allegedly caused by illegal bunkering activity. The study went further to use molecular techniques to characterize some of the dominant bacteria isolated from the spill site.

2. Materials and methods

2.1. Field sampling

Soil samples from a crude oil spill allegedly due to illegal bunkering, were sampled between December 2023 and January 2024. The site is dominated by oil palm trees. The samples were collected from two strata, i.e., surface soil (0-15 cm) and subsurface soil (15-30 cm) using a soil auger. Samples were collected in triplicates from nine samples both from polluted (4.7785^{o}N, 5.9800^{o}E) and nearby unpolluted sites (4.8227^{o}N, 5.9626^{o}E) soils, which served as the control. The samples were collected into sterile glass jars and preserved at 4°C (39.2°F) in a ice chest and transported to the Microbiology Laboratory, Niger Delta University, for analysis.

2.2. Laboratory analysis

2.2.1. Physicochemical analysis

The soil samples were air-dried under ambient temperature and analyzed in duplicates using standard

Figure 1: Sampling crude oil polluted palm forest at Apoi Creek

techniques described in Page *et al.* (1982). pH was analysed electrometrically using a standardized pH-211 meter (Hanna instruments). Electrical conductivity and salinity were measured electrochemically using Hach HQ 1140 conductivity/TDS meter (Figure 1). Moisture content was determined gravimetrically using analytical weighing balance, ZSa 210 (SCIENTECH).

Nitrates was determined spectrophotometrically at wavelength of 220 nm (JENWAY 650 UV/VIS spectrophotometer) after Kjeldahl digestion. Phosphate was determined spectrophotometrically at wavelength 690 nm (JENWAY 650 UV/VIS spectrophotometer). Total Organic Carbon (TOC) and Total Organic Matter (TOM) were determined by the Walkley-Black chromic acid wet oxidation method. Extractable Total Petroleum Hydrocarbons (ETPH) was determined gravimetrically after n-hexane extraction. Potassium was determined by flame photometry (Perkin-Elmer). Heavy metals including Lead (Pb), Nickel (Ni) and Zinc (Zn) were determined by atomic absorption spectrophotometry (Perkin-Elmer AA5100 PC, Boston, USA).

2.2.2. Microbiological analysis

The population of Total Heterotrophic Bacteria (THB) and Hydrocarbon Utilizing Bacteria (HUB) in the soil were determined by the pour plate method using nnutrient agar and Bushnell Haas media with 1% crude oil added respectively (Pepper and Gerba, 2005). Identification of bacterial colonies was carried out using cultural, morphological and biochemical characteristics (Benson, 2002; Cheesbrough, 2004), while the probable species were identified using the identification scheme described in Bergey's manual (Bergey, 1994). The dominant bacteria isolates were further characterized using molecular method based on 16S rDNA gene sequencing (Weisburg *et al.*, 1991; Muyzer *et al.*, 1993).

2.3. Molecular identification

2.3.1. Bacterial genomic DNA extraction

Bacterial DNA extraction was carried out in among the dominant isolates using a mini-preparation kit designed for ZR fungus/bacteria DNA extraction. Samples were collected from the pure culture of probable isolates and was suspended in 200 ul of isotope buffer in a ZR-Bashing-Bead Lysis tube and added 750 ul of lysis solution to the tube. The tubes were fastened in a bead beater installed with a 2 ml tube holder assembly, which was

operated at peak speed for 5 minutes. The ZR Bead lysis tube was centrifuged for 1 minute at 10,000 xg using EBA 12R centrifuge. Exactly 400 ul of supernatants were pipetted to a Zymo-Spin IV spin filter (top orange) in a collection tube and was spun for 1 minute at 7000 xg using EBA 12R centrifuge. Exactly 1.2 ml of fungal/bacterial DNA binding buffers were added to the filtrate in the collection tube, which brought the final volume up to 1600 ul, of which half the volume was pipetted into a Zymo-Spin IIC column in the collection tube and spun for 1 minute at 10,000xg using EBA 12R centrifuge. The flow-through was wasted from the collection tube. The residual volume was pipetted to the same Zymo spin and spun. Exactly 200 ul of DNA Pre-Wash buffer were added to the Zymo-Spin IIC in a fresh receiving tube and operated for 1 minute at 10,000 g using EBA 12R centrifuge, which was followed by pipetting into it, 500 ul of fungal/bacterial DNA wash buffer and spun at 10,000 g using EBA 12R centrifuge for 1 minute. The Zymo-Spin IIC column was transferred to a fresh 1.5-mL centrifuge tube, where 100 ul of DNA elution buffer were pipetted into the column matrix, and spun for 30 seconds at 10,000xg using EBA 12R centrifuge to extract the DNA. The ultra-pure DNA was then preserved at -20 ^{o}C. The extracted genomic DNA was quantified by the Nanodrop 1000 spectrometer (Thermo scientific spectrometer 1000).

2.3.2. 16S rRNA amplification

The amplification of the extracted genome of the bacteria was carried out using the 16s RNA region of the rRNA genes of the presumptive species. They were amplified using a thermal cycler (ABI 9700 Applied Biosystems) at a final volume of 50 microlitres for 35 cycles using the primer; 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3'. The PCR mix included: the X2 Dream Taq Master mix (DNTPs, Taq polymerase, MgCl), the primers prepared to 0.4M concentration and the extracted DNA, which was used as template. The ramping conditions were as follows: Initial denaturation, 95 ^{o}C for 5 minutes; denaturation, 95 ^{o}C for 30 seconds; annealing, 52 ^{o}C for 30 seconds; extension, 72 ^{o}C for 30 seconds for 35 cycles and final extension, 72 ^{o}C for 5 minutes. The amplified genes were determined on a 1% agarose gel at 120V for 15 minutes and visualized on a UV transilluminator (IndiaMart Lumina UV transillumator).

2.3.3. Sequencing

The amplified genes were sequenced by Inqaba Biotechnological, Pretoria South Africa using the BigDye Terminator kit on a 3510 ABI sequencer. The apparatuses of the sequencing comprised 0.25 ul BigDye® terminator v1.1/v3.1, 2.25 ul of 5 x BigDye sequencing buffer, 10 uM PCR primer, and 2-10 ng PCR template per 100 bp. The sequencing was carried out at a final volume of 10 ul under the following conditions; 32 cycles of 96 °C for 10 s, 55 °C for 5 s and 60 °C for 4 min.

2.4. Phylogenetic Analysis

The sequences obtained were edited using the bioinformatics algorithm Trace edit. Comparable sequences were obtained from the National Center for Biotechnology Information (NCBI) data base using BLASTN, which were aligned using ClustalX. The evolutionary history of the isolates were extrapolated using the Neighbor-Joining method in MEGA 6.0 (Saitou and Nei, 1987). The bootstrap consensus tree that was inferred from 500 replicates (Felsenstein, 1985) that was collected, was used to represent the evolutionary history of the taxa studied. The evolutionary distances were calculated using the Jukes-Cantor technique (Jukes and Cantor, 1969).

2.5. Statistical analysis

The results were compiled in Microsoft Excel and analyzed using statistical software IBM SPSS version 26.0. T test was carried out between the polluted and unpolluted soil with alpha set at 0.05.

3. Results and discussion

The results of the physicochemical analysis of unpolluted and crude oil polluted soil is presented in Table 1. The moisture content of the soil was significantly higher ($P<0.05$) in the polluted soil than unpolluted soil at both strata. The pH at the top and subsoil were slightly acidic in the polluted and unpolluted sites ($P>0.05$). Soils of the freshwater landscape in the Niger Delta has been well documented to be slightly acidic (Chikere *et al.*, 2020). Electrical conductivity in the top soil and subsoil in the unpolluted site was 27±0.00 µS/cm in top

and subsoil strata, which was significantly lower (P<0.05) than the 41±0.00 µS/cm and 43±1.41 µS/cm recorded at the polluted site respectively. Salinity was similarly significantly lower in the unpolluted site (P<0.05). Hence, the data shows that the study area is freshwater in nature. Soil macronutrients particularly nitrate, phosphate and potassium were low, i.e., generally below 1 mg/kg in both strata of polluted and unpolluted soil. These nutrients are known to be liming in most soils.

Table 1: Physicochemical Analysis of the unpolluted and Crude oil-polluted top (0-15 cm) and sub (15-30 cm) soil from Apoi Creek

	Strata	Unpolluted	Polluted	T-test	P-value
pH	Topsoil	6.41±0.01	6.36±0.00	9.00	0.07
	Subsoil	6.40±0.00	6.38±0.01	5.00	0.13
ETPH (mg/kg)	Topsoil	85±7.07	1404±1.41	-329.75	0.00
	Subsoil	75±7.07	1523±0.71	-321.67	0.00
Zn (mg/kg)	Topsoil	0.05±0.00	4.40±0.00	-4.35	0.14
	Subsoil	0.01±0.01	4.61±0.01	-461	0.00
Pb (mg/kg)	Topsoil	0.04±0.00	15.30±0.00	-15.26	0.04
	Subsoil	0.06±0.01	15.36±0.01	-153	0.00
Ni (mg/kg)	Topsoil	0.00±0.00	0.05±0.00	-0.05	0.97
	Subsoil	0.01±0.01	0.06±0.01	-0.5	0.71
K (mg/kg)	Topsoil	0.12±0.01	0.10±0.01	2	0.30
	Subsoil	0.13±0.01	0.08±0.01	0.5	0.71
P (mg/kg)	Topsoil	0.43±0.01	0.40±0.01	-0.3	0.82
	Subsoil	0.45±0.01	0.37±0.02	8	0.08
Nitrate (mg/kg)	Topsoil	0.69±0.01	0.86±0.01	-11.67	0.05
	Subsoil	0.78±0.01	0.89±0.01	-1.1	0.47
Salinity (mg/kg)	Topsoil	12.50±0.70	20±0.00	-15	0.04
	Subsoil	13.00±0.00	25.5±0.71	-25	0.03
M.C (%)	Topsoil	22.3±0.00	45.1±0.00	-22.8	0.03
	Subsoil	24.2±0.01	53.25±0.07	-726.25	0.00
T.O.C (%)	Topsoil	0.60±0.01	5.12±0.02	-452	0.01
	Subsoil	0.64±0.04	7.52±0.01	-459	0.01
T.O.M (%)	Topsoil	1.03±0.64	8.80±0.03	-17.15	0.04
	Subsoil	1.03±0.01	9.87±0.01	-589	0.00
E.C (µS/cm)	Topsoil	27±0.00	41±0.00	-14	0.05
	Subsoil	27±0.00	43±1.41	-16	0.04

Note: Data is expressed as mean ± standard deviation (S.D). pH (potential of hydrogen), ETPH (Extractable Total Petroleum Hydrocarbons), Zn (Zinc), Pb (Lead), Ni (Nickel), P (Phosphorus), M.C (Moisture Content), T.O.C (Total Organic Carbon), T.O.M (Total Organic Matter), E.C (Electrical Conductivity).

Heavy metals that were tested including zinc, lead and nickel, all occurred in traces in unpolluted soil, generally below 0.1 mg/kg in both strata. But significantly higher levels ($P<0.05$) of zinc and lead occurred in the polluted soil. In the polluted soil, zinc was 4.40±0.00 and 4.61±0.01 mg/kg, while lead was 15.30±0.00 mg/kg and 15.36±0.01 mg/kg in the top and subsoil respectively. Several studies elsewhere in the Niger Delta have shown that heavy metal pollution often accompanied oil spills (Ighariemu *et al.*, 2023; Isukul *et al.*, 2023; Allen-Adebayo *et al.*, 2024). Hydrocarbon parameters determined including Total Organic Carbon (TOC), Total Organic Matter (TOM) and Extracted Total Petroleum Hydrocarbons (ETPH) were all significantly higher ($P<0.05$) in the polluted soil. In the topsoil strata, TOC, TOM and ETPH were 0.60±0.01%, 1.03±0.64% and 85±7.07 mg/kg in the unpolluted soil but where 5.12±0.02%, 8.80±0.03% and 1,404±1.41 mg/kg respectively in the polluted soil ($P<0.05$). In the subsoil strata, the trend was similar, but higher values were recorded, which indicated that the spilled oil is probably seeping through the soil profile.

The population of bacteria in the unpolluted and polluted soil are presented in Table 2. In the topsoil, THB was $1.21±2.12×10^8$ cfu/g, which was significantly higher ($P<0.05$) than the $9.40±4.95×10^8$ cfu/g that was recorded in the polluted site. In the subsoil, they were also in the order off 10^8 ($P>0.05$), but were not significantly different in both strata. In the topsoil stratum, HUB density was $5.65±9.19×10^7$ cfu/g, which was not significantly different ($P>0.05$) from that $8.95±0.71×10^8$ cfu/g was recorded in the polluted soil. In the subsoil, the population density of HUB in the unpolluted site was $4.25±0.71×10^7$ cfu/g, which was significantly lower ($P<0.05$) than the $8.20±5.66×10^8$ cfu/g recorded in the polluted site.

Table 2: Population of culturable Total Heterotrophic Bacteria (THB) and Hydrocarbon Utilizing Bacteria (HUB) in unpolluted and crude oil-polluted top (0-15 cm) and sub (15-30 cm) soil from Apoi Creek

	Strata	Unpolluted	Polluted	T-test	P-value
THB	Topsoil	$1.21±2.12×10^8$	$9.40±4.95×10^8$	13.50	0.05
	Subsoil	$9.20±6.36×10^8$	$6.55±7.77×10^8$	2.60	0.23
HUB	Topsoil	$5.65±9.19×10^7$	$8.95±0.71×10^8$	-4.71	0.13
	Subsoil	$4.25±0.71×10^7$	$8.20±5.66×10^8$	-11.286	0.05

Note: Data in are expressed as mean $CFUg^{-1}$ ± standard deviation.

Table 3 presented the cultural characteristics of the bacterial isolates from both soils that was used for their presumptive identification. However, the dominant species that were further characterized confirmed the dominance of the Bacteriaceae family among others (Figure 2 and 3). *Bacillus altitudinis* was confirmed in the unpolluted subsoil, while *Fictibacillus macauensis* was detected in unpolluted topsoil and polluted subsoil (Table 4). *Micrococcus luteus* and *Enterobacter chengduensis* were detected in the topsoil strata of the oil-polluted soil.

Luke *et al.* (2021) studied three oil-spill sites impacted by artisanal refining activities in three Niger Delta communities, namely, Bolo, Twon Brass and Ekpemu, which are respectively located in Rivers, Bayelsa and Delta States, Nigeria. They reported the population of THB and HUB in the soil to be in the order of 10^5 cfu/g and presumptively identified the following bacteria; *Pseudomonas aeruginosa, Pseudomonas putida,* and *Bacillus subtilis*. Similarly, Udotong *et al.* (2008) studied wetland impacted by oil spill in Eket, Akwa Ibom State. They reported acidic pH and the population range of THB was in the range of 10^6-10^7 cfu/g and presumptively identified *Bacillus* sp. among several other species including *Arthrobacter*, *Acinetobacter, Beijerinckja, Alcaligenes, Serratia, Pseudomonas, Micrococcus, Achromobacter, Flavobacteria, Enterobacter,* and *Enterococcus.*

Joel and Amajuoyi (2009) carried out physicochemical and microbiological studies of an oil polluted site in Gokana, Rivers State. They reported population of THB in the range of 10^3-10^6 cfu/g and oil and grease values in the range of 620-32,040 mg/kg in topsoil that was linked to vegetation death. In Emuoha, which is a freshwater wetland environment in Rivers State, Chikere *et al.* (2020) studied an oil spill site and reported a pH of 6.92, ETPH of 6231 mg/kg, zinc of 60.4 mg/kg, lead of 22.4 mg/kg and nickel of 35.5 mg/kg in the topsoil,

Table 3: Cultural, morphological and biochemical characteristics of isolates

Isolate	Cultural characteristics	Microscopic characteristics	Gram stain	Biochemical test				Presumptive Organism
				Oxidase test	Catalase test	Indole production test	H_2S test	
PS 1	White slimy irregular convex colonies with lobate margin	Gram positive rods	+	-	+	-	+	*Bacillus sp*
PS 2	Green, large non slimy, having irregular margins	Gram negative rods	-	-	+	-	-	*Bacillus sp*
PS 3	White slimy irregular convex colonies with lobate margin	Gram positive rods	+	-	+	-	+	*Bacillus sp*
PSS 1	Small, blue green, round smooth muccoid, flat and opaque colonies	Gram negative rods	-	+	+	-	-	*Pseudomonas sp*
PSS 2	Creamy, slimy, having rough ends	Gram positive rods	+	-	+	-	-	*Bacillus sp*
PSS 3	Greenish yellow, small round and smooth apaque colonies	Gram positive cocci	+	-	+	-	+	*Micrococcus sp*
CS 1	Creamy, smooth, muccoid, convex colonies with entire margin	Gram negative rods	-	-	w+	+	+	*Enterobacter sp*
CS 2	Greenish yellow, small round and smooth apaque colonies	Gram positive cocci	+	-	+	-	+	*Micrococcus sp*
CS 3	Small, blue green, round smooth muccoid, flat and opaque colonies	Gram negative rods	-	+	+	-	-	*Pseudomonas sp*
CSS 1	Small yellowish, round, smooth irregular, flat, convex and translucent colonies	Gram positive cocci	+	-	+	-	+	*Staphylococcus sp*
CSS 2	Yellow white, slimy irregular convex colonies with lobate margin	Gram positive rods	+	-	+	-	+	*Bacillus sp*
CSS 3	Creamy, slimy, having rough ends	Gram positive rods	+	-	+	-	-	*Bacillus sp*

Note: PS (Pristine surface), PSS (Pristine subsurface), CS (crude polluted surface), CSS (crude polluted subsurface), sc (sub-culture), + (positive), - (negative) and w+ (weak Positive).

whereas in the subsoil, they were 6.56, 9112 mg/kg, 89.1 mg/kg, 14.8 mg/kg and 36.2 mg/kg respectively. They also used metagenomic approach to identify the predominant bacteria during remediation and listed *Bacillus* among other species such as *Methylobacterium, Mycobacterium*, *Rhodoplanes*, and *Burkholderia*. Our

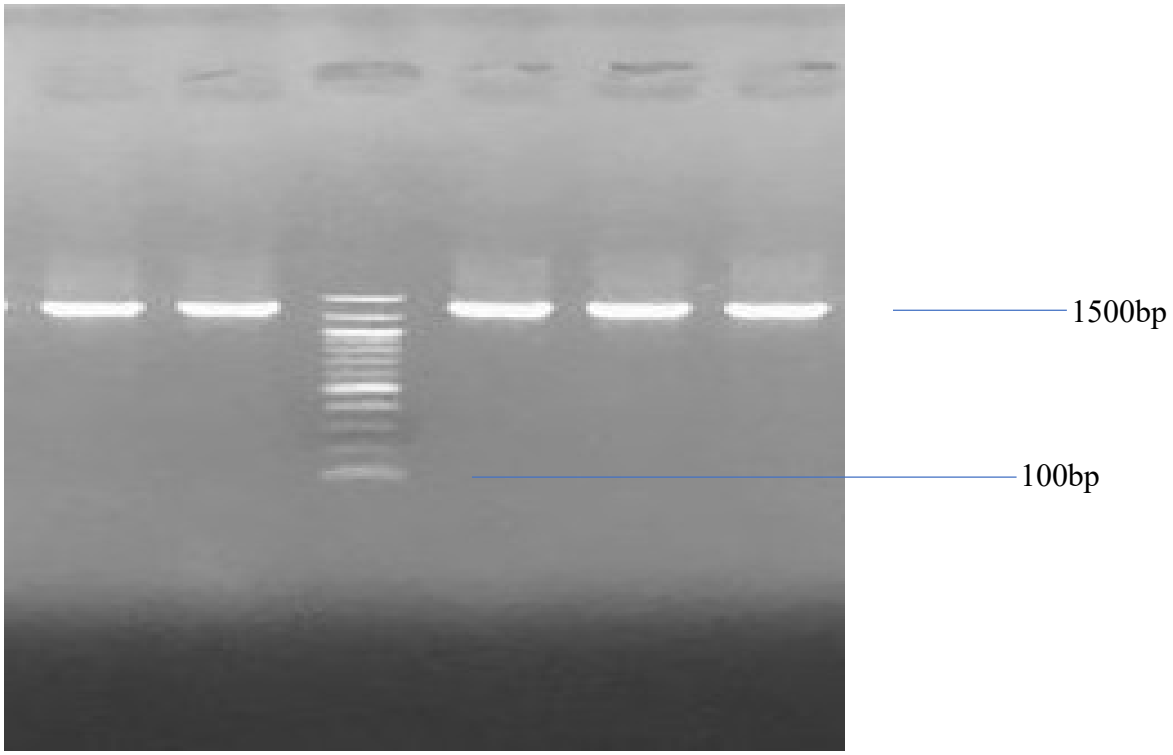

Figure 2: Agarose gel electrophoresis of the dominant bacterial isolates

Note: Lane 1-5 represents 16SrRNA gene bands (1500 bp). Lane C represents the 100 bp Molecular ladder. C1 = unpolluted topsoil 1 (UT1), C2 = unpolluted subsoil 2 (US2), C3 = polluted topsoil 2 (PT2), C4 = polluted topsoil 1 (PT1), C5 = polluted subsoil 2 (PS2).

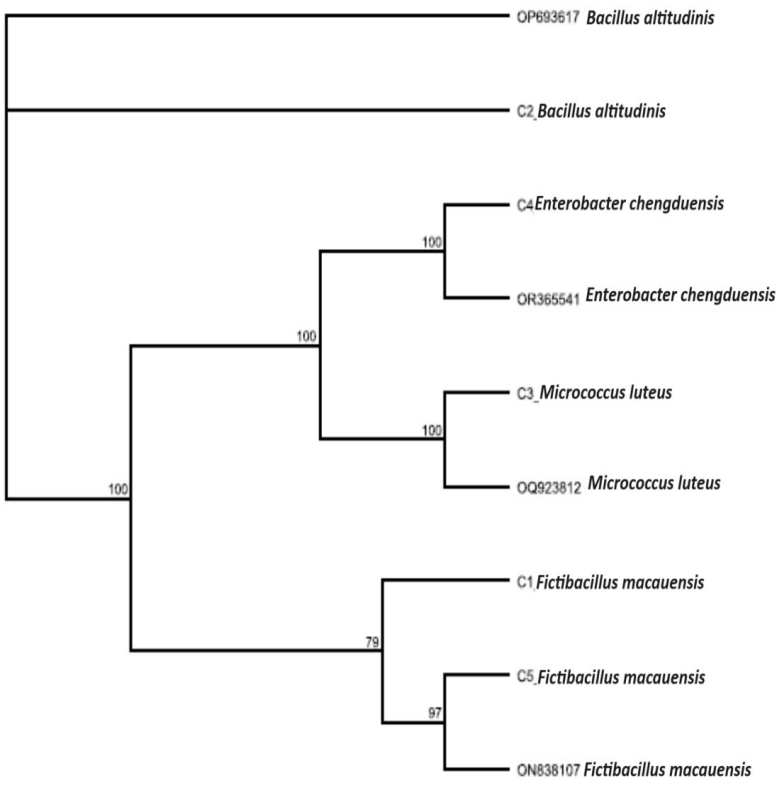

Figure 3: Phylogenetic tree showing the evolutionary relationship between identified bacterial isolates

Table 4: Distribution of the isolates identified via molecular study

Code of isolate	Pollution status	Strata	Phylogenetically homology BLAST search
UT1	Unpolluted	Topsoil	*C1 Fictibacillus macauensis*
US2	Unpolluted	Subsoil	*C2 Bacillus altitudinis*
PT2	Polluted	Topsoil	*C3 Micrococcus luteus*
PT1	Polluted	Topsoil	*C4 Enterobacter chengduensis*
PS2	Polluted	Subsoil	*C5 Fictibacillus macauensis*

study confirmed other reports (Ramsar, 2008; Edo and Albrecht, 2021) that mentioned oil spill as a threat to the Apoi Creek freshwater wetlands.

4. Conclusion

The study was carried out to assess the effects of oil spill caused by illegal bunkering on the freshwater swamp in Apoi Creek. Samples were collected from two strata, i.e., topsoil and subsoil, from the oil impacted wetland and nearby unpolluted areas for comparison. The results of analysis showed a significant increase in ETPH, zinc, lead and HUB in the polluted site over the unpolluted site, with the subsoil values generally higher, which tend to suggest that the spill is seeping through the soil strata. We conclude that the oil spill impacted the wetland through increase in hydrocarbons and heavy metals.

Author contributions

This work is based on the M.Sc. research work of the first author supervised by the second author. The first author conceived and carried out the laboratory and data analyses, the second author wrote the initial manuscript, while all authors approved the final version.

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