



Microbiology and Heavy Metal Content of Wetlands Impacted by Crude Oil Pollution in Rivers State, Southern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author MPC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors DNO and JOW managed the analyses of the study. Author JOW managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Wetland soils constitute vast, under-exploited and sometimes undiscovered ecologies in many countries of the World, including Nigeria. A total of 54 wetland soil samples including surface and subsurface soil at depths of 0-15 cm and 15-30 cm were collected using a sterile hand auger for a period of three months between August and October and subjected to standard and analytical microbiological procedures. The wetland soil samples were further subjected to atomic absorption spectroscopy (AAS) to check for presence and concentration of heavy metals. Results obtained showed that apart from heterotrophic bacterial and fungal counts, hydrocarbon utilizing bacteria (HUB) counts were higher in the surface soil ranging from $12.06 \pm 3.43^b \times 10^7$ cfu/g at Iwofe to $6.19 \pm 2.67^a \times 10^7$ cfu/g at Chokocho while subsurface soil had HUB ranging from $8.91 \pm 6.67^a \times 10^3$ cfu/g at Eagle Island to $4.93 \pm 3.95^a \times 10^3$ cfu/g at Chokocho. Heavy metals such as Fe, Pb, Cd and Ni were recorded in concentrations above FEPA permissible limit in the surface and subsurface soil across the three wetlands. The heavy metal concentration in each wetland however, decreased with an increase in soil depth. According to literatures, elevated levels of heavy metals in soils decrease

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microbial population, diversity and activities. However, the microbial population in this study increased with increasing heavy metal concentration which indicates that the microbes can tolerate or utilize heavy metals in their systems; as such can be used for bioremediation of heavy metal polluted soils.

Keywords: Heavy metals; wetlands; crude oil pollution; soil microorganisms; FEPA.

1. INTRODUCTION

Wetlands are among the ecosystems most vulnerable or susceptible to anthropogenic activities. Dredging of canals, placement of pipelines, flow lines and oil pollution are major threats to wetlands. Due to storm water pollution on wetlands, heavy metals and hydrocarbons accumulate in wetland sediments. Therefore, depletion of wetlands may result to reduction of mangroves and swamps, loss of biodiversity, reduction in ecological services, reduction of flood retention and reduced aquifer recharge, loss of breeding grounds for aquatic lives and livelihoods [1]. Oil spillage is a major cause of loss of wetlands, indiscriminate oil activities, including the exploration, exploitation and transportation of crude oils in water fronts can also lead to spillages on wetland soils. In some cases, pollution of wetlands is mostly due to equipment failure and bursting of pipelines, this affects the ecology of wetland soils and destroys the values of the beauty of wetlands. The wetland soils in Rivers State, Southern Nigeria are polluted regularly by diverse petroleum products due to the local refining processes otherwise known as “kpo fire” which are carried out along most water fronts in the state. Very often, wetlands are drained, then houses and other buildings are built on the land that used to house diverse species of microorganisms, birds, fish and amphibians. Studies have revealed that contaminants can transfer significant levels of toxic and persistent metals into the soil environment and eventually these metals are taken up by plants which are transferred into the food chain. The threats have induced changes that eroded the ecological and socio-economic values as well as services derived from the wetland [2]. Most of these activities are done out of ignorance, lack of awareness, understanding and poor advocacy on waste management issues relating to wetland values. Bacteria are present in high amounts in wetland environments and the largest group of wetland bacteria is *Proteobacteria* which are capable of a number of important functions ranging from nitrogen fixation, denitrification, iron and sulfate reducers. Other examples of wetlands microorganisms

include *Pseudomonas* which is capable of degrading contaminants such as naphthalene, toluene, etc., *Desulfovibrio* which are sulfate reducers, *Geobacter* which are iron reducers, *Streptomyces* which degrade resistant substrates such as Xenobiotics, *Arthrobacter* which degrade toxic compounds, *Bacillus* and *Clostridium species* which are facultative aerobes. The choice of this study is based on the fact that wetlands in Rivers State serve as major areas where various activities involving indiscriminate dumping of waste, bunkering activities, transportation of petroleum products, sand dredging and other anthropogenic activities endangering wetlands resources. The aim of this study therefore is to investigate the microbiology and heavy metal concentration in wetland soils impacted by crude oil pollution which may result in pollution of water resources due to seepage or erosion or runoff of leachates into the aquifers due to the porous nature of Niger Delta soils.

2. MATERIALS AND METHODS

2.1 Description of Study Area

This study was carried out in three wetlands in Rivers State; Iwofe (4°48'46.551" N, 6°56'12.0906"E), Eagle Island (4°47'47.302"N, 6°58'24.5496"E) and Chokocho (4°59'53.75688" N, 7°3'39.93084"E). These three wetlands are located in the South-South geopolitical zone of Nigeria. These three (3) wetland locations were selected because spills have occurred on them and they serve as major areas where various activities involving indiscriminate dumping of waste, bunkering activities, transportation of petroleum products, sand dredging, and other anthropogenic activities endangering wetlands are carried out. The vegetation in each wetland comprised of grasses, ferns, oil and raffia palms. Iwofe wetland has visible clumps of crude oil on the soil surface of the wetland and serves as a major dumpsite for both household waste and sewages. Eagle Island wetland is used by boat makers for wood works and boat making, while Chokocho wetland serves as an area where major anthropogenic activities such as sand

dredging and indiscriminate dumping of waste is carried out.

2.2 Sample Collection

Wetland soil samples were collected with the aid of a hand auger using the method by Pepper et al. (2015). In each station, samples were collected from three different points at different depths of 0-15 cm and 15-30 cm and made up into composite samples. But each of the sampling was done 50 m away from each sampling station. The hand auger used was cleaned after each collection to reduce contamination between samples. A total of 54 samples were collected for a period of three months (August to October) from the three wetlands. The soil samples were put in sterile polyethylene bags and conveyed to the Microbiology Research Laboratory of the Department of Microbiology, Rivers State University, Port Harcourt for analyses within 2 hours of sample collection.

2.3 Materials used

The materials including glass wares used for this study were sterilized for about one hour in the hot air oven at about 170°C. Preparation of the culture media such as Nutrient and Sabouraud Dextrose Agar was in accordance with the manufacturers instruction, while Mineral Salt Agar (MSA) was compounded as described by Nrior and Jirigwa [3]. Sterilization was done using the autoclave at 15psi (121°C) for 15 minutes. MSA was composed of 0.29 g of KCl, 10 g of NaCl, 0.42 g of MgSO₄.7H₂O.

2.4 Microbiological Analysis

2.4.1 Serial dilution

The dilution method adopted was the ten-fold serial dilution technique in which 1g of the soil sample was added into 9ml test tubes containing sterile diluent. This was done consecutively until appropriate dilutions of 10² to 10⁶ were reached [4].

2.4.2 Inoculation and incubation of bacterial and fungal cultures

Inoculation of heterotrophic and hydrocarbon utilizing bacteria was done by aseptically transferring aliquots (0.1 ml) of the dilution of 10⁻⁵ and 10⁻⁶ and 10⁻² and 10⁻³ onto properly dried nutrients agar and mineral salt agar plates

(containing fungosol 100 g (µ/ml)) in duplicates using the spread plate method and they were incubated at 35-37°C for 24 hours and 37°C for 3-5 days respectively [5]. Sub culturing of bacterial isolates was done to obtain pure cultures. Bacterial colonies were picked with sterile inoculating loop and streaked on freshly prepared well dried nutrient agar (NA) plates [6]. Colonial morphology such as shape, edge, color, elevation, surface, opacity and their consistency were recorded. Biochemical assay was based on Gram staining reaction, Motility, Catalase, Oxidase, Indole, Methyl red, Citrate, Voges Proskauer, Starch hydrolysis and Sugar fermentation test [3].

Inoculation of heterotrophic fungi and hydrocarbon utilizing fungi was also done by aseptically transferring aliquots (0.1 ml) of the dilution of 10⁻² and 10⁻³ onto Sabouraud dextrose agar (SDA) plates and Mineral Salt Agar (MSA) plates (containing chloramphenicol to suppress bacterial growth) in duplicates using spread plate method. The inoculated plates were then incubated at 28 ± 2°C for 5-7 days [7]. Fungal isolates from the heterotrophic and hydrocarbon utilizing bacteria populations were sub-cultured onto freshly prepared SDA plates. Fungal identification was based on macroscopic and microscopic characteristics.

2.4.3 Cultural characterization of bacterial and fungal isolates

The cultural characteristics of the bacterial isolates were based on appearance on the media, shape, color, moisture, size, elevation, opacity etc. The fungal isolates were identified based on cultural characteristics such as colony growth pattern and pigmentation.

2.4.4 Morphological characterization bacterial and fungal isolates

Pure cultures of bacterial isolates were identified based on biochemical tests which include; gram staining, motility, catalase, oxidase, citrate utilization, indole production, methyl red test, sugar fermentations, starch hydrolysis and microscopic techniques [8,9]. The identification of bacterial isolates was confirmed by comparing them with Bergey's Manual of Determinative Bacteriology after microscopic examination.

The fungal isolates were identified morphologically based on conidial morphology and pigmentation. The technique described by

[8] was also adopted for the identification of the isolated fungi using cotton blue in lacto phenol stain. This was done by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungal cultures was removed and placed in a drop of lacto-phenol. The mycelium was spread on the slide with a needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope using $\times 10$ and $\times 40$ objective lenses. The morphological characteristics and appearance of the fungal isolates seen were identified in accordance with standard scheme for identification of fungi as adopted by Williams and Dimbu [10].

2.4.5 Characterization and identification of fungal isolates

Discrete fungal isolates from the 5-7 days incubated plates were selected based on the differences in their morphologies and purified by sub-culturing on freshly prepared Sabouraud Dextrose Agar plates using spread plate technique. The sub-cultured plates were marked, labelled properly and incubated at $28 \pm 2^\circ\text{C}$ for 5-7 days. Pure cultures obtained after 5-7 days of incubation were subjected to characterization

and identification based on cultural and morphological characteristics such as colony growth pattern, conidial morphology, and pigmentation.

2.4.6 Purification and preservation of bacterial cultures

Ten percent (10%) glycerol solution was prepared, dispensed in McCartney bottles and autoclaved at 121°C for 15 minutes, and allowed to cool, discrete colonies were purified by repeated sub-cultures unto Nutrient Agar. Pure cultures were inoculated in duplicates then stored in nutrient agar slants kept in the refrigerator at 4°C for further tests [11].

2.4.7 Heavy metal concentration

Heavy metals (Micro nutrients) of the soil was determined using the Atomic Absorption Spectrophotometer (UNICAM AA 919 model) [12].

2.4.8 Molecular identification

Selected hydrocarbon utilizing bacterial isolates were subjected to molecular identification via the 16srRNA sequencing, plasmid profiling and phylogenetic tree.

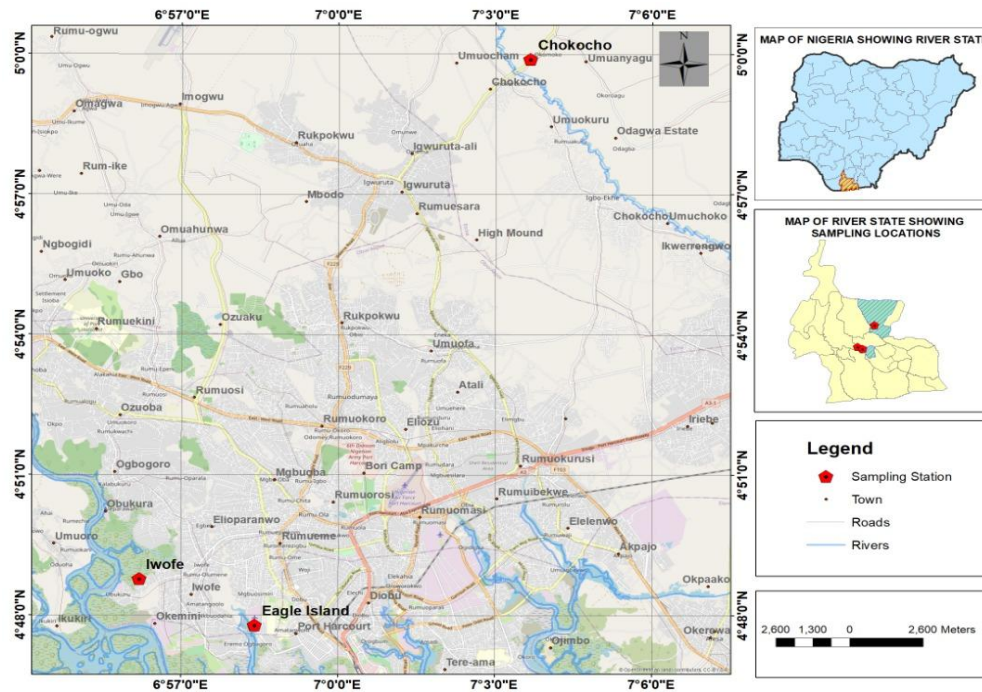


Fig. 1. Map showing the three wetland locations sampled in this study



Fig. 2. Plate showing one of the sampling locations (Iwofe) impacted by crude oil pollution/spillage

2.4.9 Statistical analysis

All statistical analysis (ANOVA) was performed using SPSS version 22 to test for significance. Duncan Multiple Range Test (a mean separation tool) was used to separate means where differences existed in the data obtained.

3. RESULTS

3.1 Microbial Analyses

3.1.1 Microbial population

Result of the microbial population of the surface soil (0-15 cm) in the three wetlands is presented in Table 1. Heterotrophic bacterial count was highest in Iwofe ($17.25 \pm 16.44 \times 10^7$ cfu/g) and lowest in Chokocho ($6.57 \pm 3.41 \times 10^7$ cfu/g). There was no significant difference ($p \geq 0.05$) in the total heterotrophic bacterial (THB) count in the surface soil (0-15 cm) across the three wetlands. Eagle Island had the highest total heterotrophic fungi count ($3.99 \pm 3.10 \times 10^4$ cfu/g) and Chokocho had the lowest ($1.64 \pm 1.19 \times 10^4$ cfu/g). There was a significant difference ($p \leq 0.05$) in the total

heterotrophic fungal (THF) count in the surface soil (0-15 cm) across the three wetlands sampled. Hydrocarbon Utilizing Bacterial (HUB) count was $6.19 \pm 2.67 \times 10^3$ cfu/g (least) and $12.06 \pm 3.43 \times 10^3$ cfu/g (highest) for Chokocho and Iwofe. There was a significant difference ($p \leq 0.05$) in the HUB count in the surface soil (0-15 cm) across the three wetlands. Eagle Island also had the highest Hydrocarbon Utilizing Fungal (HUF) count ($3.31 \pm 1.99 \times 10^3$ cfu/g) and the least HUF was recorded in Chokocho ($1.63 \pm 1.52 \times 10^3$ cfu/g). There was no significant difference ($p \geq 0.05$) in the HUF count in the surface soil across the three wetlands sampled.

The microbial population of the wetlands at a depth of 15-30 cm (subsurface soil) is presented in Table 2. The Heterotrophic bacterial count was least in Chokocho ($5.39 \pm 2.22 \times 10^7$ cfu/g) and highest in Eagle Island ($10.28 \pm 6.18 \times 10^7$ cfu/g). There was no significant difference ($p \geq 0.05$) in the THB count in the subsurface soil across the three wetlands. Chokocho had the least heterotrophic fungal count of ($1.02 \pm 0.29 \times 10^4$ cfu/g) while Iwofe had the highest ($1.14 \pm 0.39 \times 10^4$ cfu/g). There was no significant

difference ($p \geq 0.05$) in the THF count in the subsurface soil across the three wetlands. The Hydrocarbon Utilizing Bacterial (HUB) count was lowest in Chokocho ($4.93 \pm 3.95 \times 10^3$ cfu/g) and highest in Eagle Island ($8.91 \pm 6.67 \times 10^3$ cfu/g). There was no significant difference ($p \geq 0.05$) in the HUB count in the subsurface soil (15-30 cm) across the three wetlands sampled. The Hydrocarbon Utilizing Fungal (HUF) count was lowest in Chokocho ($1.13 \pm 1.98 \times 10^3$ cfu/g) and highest in Iwofe ($5.58 \pm 0.15 \times 10^3$ cfu/g). There was a significant difference ($p \leq 0.05$) in the HUF count in the subsurface soil across the three wetlands sampled. Generally, hydrocarbon utilizing bacterial and fungal counts were higher in the surface soil (0-15 cm) than in the subsurface soil (15-30 cm) for the three wetland locations as shown in Tables 1 and 2.

3.1.2 Microbial isolates

About 6 genera of bacteria were genetically identified in this study from the three wetlands of which *Bacillus* was the most occurring. The organisms isolated and identified in this study include *Bacillus*, *Staphylococcus*, *Pseudomonas*, *Micrococcus*, *Enterobacter* and *Proteus*. The fungal isolates were mostly of the genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. These isolates have significant ability to utilize crude oil as a sole source of carbon and energy, and the dominance of these organisms have been reported by different researchers as crude oil degraders [13,14].

3.1.3 Heavy metal content

The heavy metal content of the surface soil across the three wetlands is presented in Table 3. There was a significant difference ($p \leq 0.05$) in all heavy metals present in the surface soil across the three wetlands sampled. The quantity of Iron present in the surface soil of the wetlands was lowest in Iwofe (6358.00 ± 82.02 mg/kg) and highest in Chokocho (12768.00 ± 309.71 mg/kg). Lead present in the surface soil of the wetlands ranged between 42.50 ± 0.00 mg/kg (least) to 119.2 ± 5.66 mg/kg (highest) for Chokocho and Iwofe respectively. Cadmium present in the surface soil of the wetlands was lowest (1.90 ± 0.00 mg/kg) in Iwofe and highest (7.10 ± 0.14 mg/kg) in Chokocho. The quantity of Chromium present in the surface soil of the wetlands was 0.003 ± 0.00 mg/kg (least) in Iwofe and 21.70 ± 1.41 mg/kg (highest) in Chokocho. The quantity of Copper present in the surface soil of the wetlands was lowest (0.001 ± 0.00 mg/kg) in Iwofe and highest (17.7 ± 2.83 mg/kg) in Chokocho. Manganese present in the surface soil of the wetlands was 20.40 ± 2.83 mg/kg (least) in Iwofe and 141.80 ± 2.83 mg/kg (highest) in Chokocho. The quantity of Nickel present in the surface soil of the wetlands was lowest (16.70 ± 4.24 mg/kg) in Iwofe and highest (42.30 ± 2.83 mg/kg) in Chokocho. Zinc present in the surface soil of the wetlands was lowest (46.90 ± 0.00 mg/kg) in Eagle Island and highest (96.10 ± 4.24 mg/kg) in Chokocho.

Table 1. Microbial population of the surface soil at a depth of 0-15 cm

Wetlands	THB (10^7 CFU/g)	THF (10^4 CFU/g)	HUB (10^3 CFU/g)	HUF (10^3 CFU/g)
Chokocho	6.57 ± 3.41^a	1.64 ± 1.19^a	6.19 ± 2.67^a	1.63 ± 1.52^a
Eagle Island	8.97 ± 7.55^a	3.99 ± 3.10^b	10.46 ± 6.23^{ab}	3.31 ± 1.99^a
Iwofe	17.25 ± 16.44^a	1.99 ± 0.74^a	12.06 ± 3.43^b	1.06 ± 8.01^a

Key: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi)

*Means with the same superscript along the columns is not significantly different ($p \geq 0.05$)

Table 2. Microbial population of the sub surface soil at a depth of 15-30 cm

Wetlands	THB (10^7 CFU/g)	THF (10^4 CFU/g)	HUB (10^3 CFU/g)	HUF (10^3 CFU/g)
Chokocho	5.39 ± 2.22^a	1.02 ± 0.29^a	4.93 ± 3.95^a	1.13 ± 1.98^a
Eagle Island	10.28 ± 6.18^a	1.12 ± 0.50^a	8.91 ± 6.67^a	1.54 ± 1.94^a
Iwofe	5.58 ± 0.08^a	1.14 ± 0.39^a	5.78 ± 8.43^a	5.58 ± 0.15^b

Key: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi)

*Means with the same superscript along the columns is not significantly different ($p \geq 0.05$)

Results of the heavy metal content of the subsurface soil (15-30 cm) across the wetlands is presented in Table 4. There was a significant difference ($p \leq 0.05$) in the quantity of Iron, Lead, Cadmium, Chromium, Copper, Manganese and Zinc in the subsurface soil of the three wetlands studied. The quantity of Iron present in the subsurface soil of the wetlands was 243.50 ± 5.66 mg/kg (least) in Chokocho and 11842.00 ± 8.49 mg/kg (highest) in Iwofe. The quantity of Lead present in the subsurface soil of the wetlands was lowest (26.00 ± 1.41 mg/kg) in Chokocho and highest (111.10 ± 0.14 mg/kg) in Iwofe. Cadmium present in the subsurface soil of the wetlands was lowest (3.50 ± 0.42 mg/kg) in Iwofe and highest (9.30 ± 0.283 mg/kg) in Eagle island. Chromium present in the subsurface soil of the wetlands was 0.003 ± 0.00 mg/kg (least) in Iwofe and 5.90 ± 2.83 mg/kg (highest) in Chokocho. Copper present in the subsurface soil of the wetlands was lowest (0.001 ± 0.00 mg/kg) in Iwofe and highest (7.50 ± 1.414 mg/kg) in Chokocho. The quantity of Manganese present in the subsurface soil of the wetlands was lowest (28.00 ± 2.83 mg/kg) in Iwofe and highest (48.70 ± 7.07 mg/kg) in Chokocho. Zinc present in the subsurface soil of the wetlands was

28.80 ± 1.41 mg/kg (least) in Eagle Island and 38.00 ± 5.66 mg/kg (highest) in Chokocho. There was no significant difference ($p \geq 0.05$) in the amount of Nickel present in the subsurface soil across the three wetlands sampled. The quantity of Nickel present in the subsurface soil of the wetlands was lowest (20.90 ± 0.00 mg/kg) in Iwofe and highest (25.70 ± 4.24 mg/kg) in Eagle island.

4. DISCUSSION

The study on the Microbiology and Physicochemistry of Wetlands in Rivers State was designed to provide baseline data on the potentials of this vast unexploited environment. Generally, the result of the bacterial population from the soils followed similar trends as reported by previous works done by [12,15]. In the surface soil (0-15 cm) Iwofe wetland had the highest population of heterotrophic bacteria $17.25 \pm 16.44 \times 10^7$ cfu/g followed by Eagle Island wetland $8.97 \pm 7.55 \times 10^7$ cfu/g then Chokocho wetland $6.57 \pm 3.41 \times 10^7$ cfu/g and there was no significant difference ($p \geq 0.05$) in the heterotrophic bacterial count in the three wetlands.

Table 3. Heavy metals concentration of the surface soil at a depth of 0-15 cm

Parameter	Chokocho	Eagle Island	Iwofe	FEPA Limits
Fe (Mg/Kg)	12768.00 ± 309.71^b	6358.00 ± 82.02^a	6064.00 ± 1.41^a	300-400
Pb (Mg/Kg)	42.50 ± 0.00^a	104.2 ± 1.41^b	119.2 ± 5.66^c	1.6
Cd (Mg/kg)	4.60 ± 1.41^b	7.10 ± 0.14^b	1.90 ± 0.00^a	3.0
Cr (Mg/Kg)	21.70 ± 1.41^b	0.003 ± 0.00^a	0.003 ± 0.00^a	150
Cu (Mg/Kg)	17.7 ± 2.83^b	0.001 ± 0.00^a	0.001 ± 0.00^a	70-80
Mn (Mg/Kg)	141.80 ± 2.83^c	42.60 ± 1.41^b	20.40 ± 2.83^a	Not Fixed
Ni (Mg/Kg)	42.30 ± 2.83^b	42.30 ± 2.83^b	16.70 ± 4.24^a	0.1
Zn (Mg/Kg)	96.10 ± 4.24^c	46.90 ± 0.00^a	68.20 ± 8.49^a	300-400

*Key: Fe: Iron, Pb: Lead, Cd: Cadmium, Cr: Chromium, Cu: Copper, Mn: Manganese, Ni: Nickel, Zn: Zinc

*Means with same superscript across the columns indicate no significant difference ($p \geq 0.05$)

Table 4. Heavy metals concentration of the subsurface soil at a depth of 15-30 cm

Parameter	Chokocho	Eagle Island	Iwofe	FEPA Limits
Fe (Mg/Kg)	243.50 ± 5.66^a	5430.00 ± 70.71^b	11842.00 ± 8.49^c	300-400
Pb (Mg/Kg)	26.00 ± 1.41^a	72.10 ± 1.41^b	111.10 ± 0.14^c	1.6
Cd (Mg/kg)	5.30 ± 0.00^b	9.30 ± 283^c	3.50 ± 0.42^a	3.0
Cr (Mg/Kg)	5.90 ± 2.83^b	0.003 ± 0.00^a	0.003 ± 0.00^a	150
Cu (Mg/Kg)	7.50 ± 1.414^b	0.001 ± 0.00^a	0.001 ± 0.00^a	70-80
Mn (Mg/Kg)	48.70 ± 7.07^c	36.30 ± 4.24^{ab}	28.00 ± 2.83^a	Not Fixed
Ni (Mg/Kg)	25.70 ± 0.00^a	25.70 ± 4.24^a	20.90 ± 0.00^a	0.1
Zn (Mg/Kg)	38.00 ± 5.66^c	28.80 ± 1.41^a	46.00 ± 5.66^a	300-400

*Keys: Fe: Iron, Pb: Lead, Cd: Cadmium, Cr: Chromium, Cu: Copper, Mn: Manganese, Ni: Nickel, Zn: Zinc

*Means with same superscript across the columns indicate no significant difference ($p \geq 0.05$)

The high occurrence of bacteria in the wetlands can be attributed to the nature of the environment, the age of the spill, the total petroleum hydrocarbon content and the high amounts utilizable organic matter present in the wetland soil [16]. Iwofe wetland is used as a site for dumping of refuse, including household waste and sewage thus enriching the soil with various forms of utilizable organic matter. Compared to Eagle Island and Chokocho wetlands, Iwofe has the oldest oil spill and the lowest total petroleum hydrocarbon content. When crude oil or other petroleum products are spilled into an environment, a series of events leading to its degradation takes place; the first few days after the spill, between 20-40% of the oil mass turns into gases, the volatile gases evaporate leaving the heavier components. Natural attenuation which is a variety of physical, chemical, or biological processes that, under favorable conditions, act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil also sets in [17]. After a few months to a year, microorganisms which are not able to utilize the hydrocarbons present in the soil either mutate or to completely die off due to environmental stress. The organisms in the environment fully adapt and reproduce; most of the crude oil components are also completely broken down. Due to these factors, sites with older spills have lesser total petroleum hydrocarbon (TPH) content; lesser TPH content implies higher chances for the proliferation of microorganisms. The result from this study is in agreement with works done by [18-20]. Hydrocarbon-degrading microorganisms are ubiquitously distributed in crude oil polluted wetland soil environments. According to Odukuma and Dickson [18], populations of hydrocarbon degraders normally constitute less than 1% of the total microbial communities, but when oil pollutants are present in an environment, the hydrocarbon-degrading populations increase, typically to 10% of the community. This study revealed that not all the members of the heterotrophic population could utilize the crude oil and petroleum products spilled in the wetlands studied, hence a decrease in the count of hydrocarbon utilizing organisms compared to the heterotrophic microbial count which is in agreement with Udotong et al. [12]. As with the heterotrophic bacteria count, Iwofe also had the highest population of hydrocarbon utilizing bacteria in the surface soil $12.06 \pm 3.43 \times 10^3$ cfu/g and Chokocho the least HUB count $6.19 \pm 2.67 \times 10^3$ cfu/g across the wetlands. Generally, the surface soil had a

higher population of hydrocarbon utilizing organisms than the subsurface soil across the wetlands because it has better growth supporting conditions such as sufficient amounts of oxygen, nutrients, surfaces that microbes can adhere to and high moisture content due to run off from the surrounding water bodies. Eagle Island wetland had the highest heterotrophic fungi counts at both depths across the three wetlands, with values $3.99 \pm 3.10 \times 10^4$ cfu/g and $1.12 \pm 0.50 \times 10^4$ cfu/g. The high occurrence of fungi mostly *Penicillium species* in the Eagle Island wetland soil can be attributed to the nature of the environment, and the pH of the soil. *Penicillium species* can be commonly found in temperate regions growing on wood and decaying vegetative matter and Eagle Island wetland is mostly occupied by carpenters who carry out wood work including carving of boats in the water front [21]. Furthermore, *Penicillium species* has an optimum pH between 7.0 and 9.0 and the wetland soil in Eagle Island has a pH of 7.20 [22,23].

Heavy metals are non-biodegradable and accumulate in the environment with no known homeostasis mechanism for their removal. High level of heavy metals may affect human health by hindering normal functioning of organs/systems such as liver, kidney, central nervous system, bones, among others or acting as cofactors in other diseases. A few metals are fundamental to life and assume key parts as wellsprings of vitamins, and minerals in the working of body organs. Studies have revealed that contaminants can transfer significant level of toxic and persistent metals into the soil environment and eventually these metals are taken up by plants which are transferred into the food chain. The surface soil is considered to represent the plough layer and average root zone for nutrient and heavy metal uptake by plant roots. Iron (Fe) concentration in the wetlands decreased with an increase in soil depth. In the surface soil, the concentrations of Fe recorded in the three wetlands were above the maximum permissible limit of 300-400mg/kg set for iron by FEPA [24]. However, in the subsurface soil, Eagle Island and Chokocho had Fe concentrations higher than the permissible limit set by FEPA [24] with values 5430.00 ± 70.71 mg/kg and 11842.00 ± 8.49 mg/kg respectively but Iwofe had a concentration below the permissible limit with a concentration of 243.50 ± 5.66 mg/kg. The high concentration of Fe in the wetlands can be attributed to mainly anthropogenic activities such as indiscriminate dumping of sewages,

dumping of municipal and industrial wastes including oil spills in the wetlands. Lead (Pb) in high amounts is very toxic to humans and is additionally a dangerous and cancer-causing metal. Pb may cause perpetual health disorders, including migraine, crabbiness, stomach torment, nerve harms, kidney harm, circulatory strain, lung tumor, stomach growth, and gliomas. In the surface soil, the concentration of Pb recorded in the three wetlands were above the permissible limit of 1.6mg/kg for Pb set by FEPA while in the subsurface soil only Iwofe wetland had Pb concentration of 111.10 ± 0.14 mg/kg above FEPA [24] permissible limit. The high concentration of Pb in the wetlands can be attributed to vehicular discharges and oil spill during the transportation of artisanal crude oil (kpo fire), metal plating and greasing up oils. It can also be due to the wearing of tires and run-offs from the roadsides. Lead contamination can also be ascribed to ignition of fuel of the loading trucks that contains tetraethyl lead as anti-knock agent, the indiscriminate dumping of sewages and household waste in the wetland environment cannot also be ruled out [25]. When Pb is ingested by humans it affects the Central Nervous System and can also cause Lungs, Kidney and Liver damage [26]. Cadmium (Cd) is classified as human carcinogen because Cd causes kidney failure and fragile bones in human [26]. Nickel-cadmium batteries, cadmium-pigment, glasses, paints and enamels, ceramics, cadmium coated ferrous and non-ferrous products, cadmium alloys, cadmium electronics or electronic compounds are among the anthropogenic sources of cadmium in the soil environment. There was a significant difference ($p \leq 0.05$) in the concentration of Cd in the three wetlands at both depths sampled. Eagle Island and Chokocho wetlands had concentrations of 9.30 ± 283 mg/kg and 5.30 ± 0.00 mg/kg which is above FEPA [24] permissible limit of 3.0 mg/kg at both depths. A measure of Chromium (Cr) is required for typical body capacities; while its high fixations may cause poisonous quality, including liver and kidney issues and genotoxic cancer-causing agent. The concentration of Cr recorded in the three wetlands at both depths were below the permissible limit set by FEPA [24]. Copper (Cu) in high amounts can be toxic for humans especially children. There was a significant difference ($p \leq 0.05$) in the concentration of Cu in the three wetlands at both depths. The concentration of Cu in the surface soil of the wetlands ranged from 0.001 mg/kg least recorded in Iwofe to 17.7 mg/kg highest recorded in Chokocho. The concentration of Cu recorded

in the three wetland soils studied were below the permissible limit set by FEPA [24]. Manganese (Mn) in high amounts can be toxic for humans especially children. The presence of Manganese (Mn) in the three wetland soils sampled can be associated with oil refinery activities including oil spillage and "kpo fire" [25]. Nickel (Ni) is the 24th most abundant element in the environment. It can be found in the air, soil, sediment and water. Even though Ni is an essential element for plants, excessive amounts can adversely impact the quality of the environment for flora and fauna. There was a significant difference ($p \leq 0.05$) in the concentration of Ni in the surface soil of the three wetlands while in the subsurface soil, there was no significant difference ($p \geq 0.05$). The concentration of Ni recorded in the Iwofe wetland surface soil 16.70 ± 4.24 mg/kg was above the permissible limit of 0.1 mg/kg set by FEPA [24]. Zinc (Zn) occurs naturally in the environment. Although, most Zn finds its way into the environment because of human activities such as mining, melting metals, steel production as well as burning coal and certain wastes can release Zn into the environment. There was a significant difference ($p \leq 0.05$) in the concentration of Zn in the three wetlands at both depths. The concentration of Zn in the surface soil ranged from 46.90 mg/kg least recorded in Eagle Island to 96.10 mg/kg highest recorded in Chokocho while in the subsurface soil Zn concentration ranged from 28.80 mg/kg least recorded in Eagle Island to 46.00 mg/kg highest recorded in Iwofe. The concentration of Zn recorded in the three wetlands at both depths were below the permissible limit of 300-400 mg/kg set by FEPA [24]. According to literature, microbial population decreases significantly with increasing heavy metal concentration [27,28]. However, the microbes in this study increased with increase in heavy metal concentration which indicates that they tolerate and use heavy metals in their systems; as such these microbes can be used for bioremediation of heavy metal polluted soils.

5. CONCLUSION

Conclusively, the study revealed a decrease in microbial counts of the isolates with increase in soil depth in the three wetland locations studied which is in accordance with [12]. The major reason for this may not necessarily be tied to the tolerance of the different microorganisms on oil pollutants alone but also the physicochemical parameters such as the moisture content of the soil. The low number of indigenous hydrocarbon utilizers in the soil and the toxicity of crude oil on

the natural flora could be to an extent the cause of persistence of hydrocarbon pollutants in the environment [29]. According to literature, elevated levels of heavy metals in soils decrease microbial population, diversity and activities. However, the microbes in this study increased with increase in heavy metal concentration which indicates that they tolerate and use heavy metals in their systems; as such these microbes can be used for bioremediation of heavy metal polluted soils.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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