Seasonal Influence on the Microbiology and Physicochemistry of Crude Oil Polluted Wetlands

ABSTRACT

Pollutants such as crude oil spills in an environment, affect the physical, chemical and biological properties of that environment. This study was conducted to determine the effect of seasonal variations on the microbiological and physicochemical characteristics of three crude oil-polluted wetlands in Rivers State, Nigeria. Soil samples were collected at 3 depths (0-15cm, 15-30cm and 30-45cm) with the aid of a hand auger, sampling was done for a period of twelve months covering the rainy (March to August) and dry seasons (September to February). Samples were analyzed for physicochemical characteristics, using standard analytical and microbiological methods. The data obtained was analyzed using the statistical package for social science (SPSS) version 22 and Duncan's multiple range test was used to separate means where differences occurred. Microbiological analyses showed that significantly higher microbial counts were observed in the dry season than in the rainy season in the wetland samples which could be due to favourable environmental conditions during the period of study. Wetland soil total heterotrophic bacterial (THB) count in the wet season ranged from $1.09x10^7$cfu/g to $10.45x10^7$cfu/g and from $2.43x10^7$cfu/g to $18.56x10^7$cfu/g in the dry season. Total heterotrophic bacteria had the highest population in all soil depths (0-15cm, 15-30cm and 30-45cm) in both seasons. Hydrocarbon-utilizing bacteria such as *Bacillus subtilllis, B. rigui, B. flexus, Lysinibacillus macrolides,Staphylococcus aureus, Proteus penneri* and fungi such as *Aspergillus species, Fusarium species, Mucor species* and *Rhizopus species* were genetically identified in this study. Temperature and other physicochemical parameters in the wetlands were altered due to the variation in season. Higher temperature ranges were observed in the dry season than in the rainy season as seen in Iwofe, temperature ranged from 26.50^0C (0-15cm) to 28.50^0C (30-45cm) in the rainy season while in the dry season it ranged from 31.50^0C (0-15cm) to 33.50^0C (30-45cm) which was above FEPA permissible limit of 30^0C for soil temperature. The study showed that season of study as well as crude oil spillage have significant effects on the microbiology and physicochemical characteristics of wetlands and therefore, contribute to microbiological degradation in wetlands.

Keywords: *Pollutant, Wetland, Physicochemical, Permissible, Anthropogenic.*

1. INTRODUCTION

A wetland is best described as an intermediate land located between marine and terrestrial ecosystem which is often characterized by a shallow water table [1]. Globally, the importance of wetlands is rapidly gaining momentum and progressively receiving appropriate attention because they in several ways add to a loveable and healthy environment [2, 3]. Wetlands get their water either from ground water usually seeping up from an aquifer or a nearby water body such as a river or lake or from precipitation after rainfall [4]. Wetlands have the features of both terrestrial and marine ecosystems with temporal or seasonal influence by both systems [5]. Wetlands are responsible for providing a variety of valuable ecosystem services, including trapping nutrients from land prior to their introduction to aquatic ecosystems, abating flood events, enhancing biodiversity and improving water quality [6]. During the rainy season, wetlands tend to retain water which they release back into surrounding environments in the dry season, thereby, stabilizing the water table. Besides, in any flood scenario, wetlands play a significant role in mitigating floods by entrapping suspended solids and accompanying nutrients. As such, the water released by wetlands into rivers and lakes often contains less suspended solids and more nutrients than water flowing directly into these environments as fish species and planktons feed on those nutrients [7, 8]. Wetlands are vital resources to the natural ecosystem because they serve as breeding grounds and feeding regions for wildlife and they protect and create shelter for sea creatures.

The Niger delta is a wetland of about 76,000sq/km and has the largest mangrove forest in Africa (11,134 sq/km) and the third largest in the world [9]. Spillage of crude oil and its refined products is a problem facing the entire nations of the World including the under-developed and developed nations. It has caused a threat to our environment by imposing a health hazard to humans, causing a

decrease in agricultural productivity on soil and economic loss. Despite the fact that the oil sector has contributed immensely to the development of the nation, it has also caused a lot of catastrophic damage to the environment. Oil spillage has a major impact on the ecosystem into which it is released. Immense tracts of the mangrove forests, which are especially susceptible to oil spills have been destroyed. An estimated 5-10% of Nigeria's mangrove ecosystem has been wiped out due to oil exploration [10]. Marshes, which are an example of wetlands constitute an important component of river, estuarine, and coastal ecosystems are extremely sensitive to oil pollution [11] and can be severely damaged by spills, which block carbon fixation by stifling plant transpiration and through this mechanism and others can kill marsh vegetation [12]. Pollution caused by petroleum and its derivatives is the most prevalent problem in oil-producing regions of the world. Oil spillage is an accidental discharge of crude oil into the environment. These spills endanger public health, impair drinking water, devastate natural resources such as wetlands and disrupt land farming and other agricultural systems. The Niger Delta region of Nigeria supports a lot of petroleum exploration and exploitation activities, which has subsequently led to a wide scale of pollution of its rivers, swamps and farmlands with petroleum hydrocarbon. A serious issue of concern is the local refineries that have sprung up in several areas in the Niger Delta (Port Harcourt inclusive) giving rise to what is now known as the 'Kpo fire' business (artisanal refineries). It is usually done in coastal areas where water bodies are present for transporting products as well as water needed for certain processes. The wastewater from refining as well as spills during refining and transportation ends up in the water bodies and surrounding wetlands. These activities are largely or if not totally unregulated, therefore, research tending towards understanding the effect of these pollutants on the microbiology and physicochemistry of the environment is of primary importance.

2. MATERIALS AND METHODS

2.1. Description of Study Area

This study was carried out in three crude oil-polluted wetlands in Rivers State. The sample stations were Rumuolumeni/Iwofe in Obio Akpor Local Government Area, Eagle Island in Port Harcourt City Local Government Area and Borokiri in Port Harcourt City Local Government Area all in Rivers State, Nigeria. These wetlands 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. Eastern Bypass/Ogbunuabili in Port Harcourt City Local Government Area served as the control station. The wetlands are located in the South-South geopolitical zone of Nigeria and the coordinates of the stations are shown in Fig 1**.**

2.2. Experimental Design (Sampling Period)

Samples were collected randomly (CRD) from the wetlands in Rivers State. Samples were taken once a month, for a period of twelve (12) months.

2.3. Sample Collection

Soil samples were collected with the aid of a hand auger using the method described by Pepper and Gerba (2015). Soil samples were collected at 3 depths (0-15cm, 15-30cm and 30-45cm) and three positions; one meter apart in each wetland and bulked in order to obtain composite samples. The hand auger used was cleaned after each collection to reduce contamination between samples. A total of two hundred and eighty eight (288) soil samples were collected for a period of twelve (12) months from the wetland stations in Rivers State. The soil samples were put into sterile polyethylene bags and conveyed to the Post Graduate Microbiology Research Laboratory of the Department of Microbiology, Rivers State University, Port Harcourt for analyses within 24 hours. Sampling was done in the mornings between 10am to 12pm each day for a period of twelve (12) months covering both wet (March to August, 2022) and dry seasons (September to February, 2022) at an interval of once a month. Samples were collected in triplicates from each sample location.

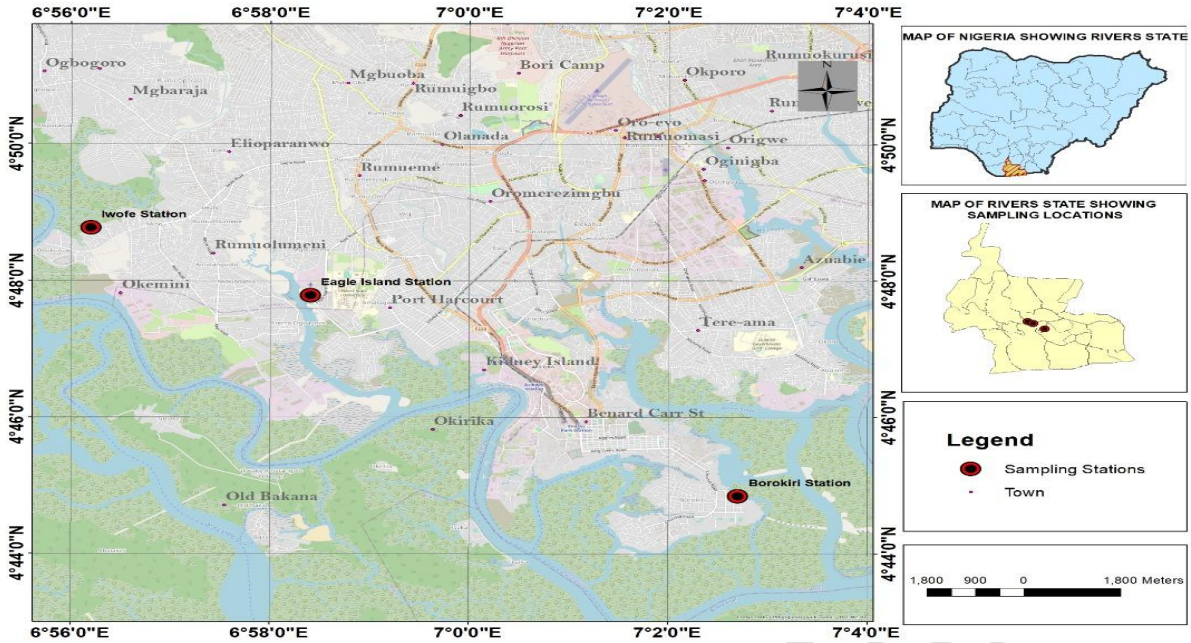

Fig. 1. Map of Rivers State Showing the Sampling Locations

2.4. Microbiological Analyses

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 10^2 to 10^6 were reached [13].

2.4.2. Inoculation and Enumeration of Heterotrophic Bacterial

Aliquots (0.1 ml) of dilutions $x10^5$ and $x10^6$ were inoculated unto surface dried nutrient agar media plates (containing fungosol 100 g (µ/ml) in triplicates and spreading was done with a flamed bent glass spreader. The plates were incubated at 37^0 C for 24 hours. Total heterotrophic bacterial count was enumerated as described by Prescott *et al.* (2005). Bacterial colonies that appeared on the nutrient agar plates were counted and the mean was expressed as cfu/g [14].

2.4.3. Inoculation and Enumeration of Heterotrophic Fungal

This was determined using the sabouraud dextrose agar. Chloramphenicol was used to suppress bacterial growth [15]. The spread plate technique as described by [16] was adopted. An aliquot (0.1ml) of the appropriately serially diluted samples were inoculated in duplicates onto sterile pre-dried sabouraud dextrose agar plates and then spread evenly with a sterile glass spreader. The inoculated plates were then incubated at 28 ± 2^0C for 3-7 days [17].

2.4.4. Inoculation and Enumeration of Hydrocarbon Utilizing Bacteria (HUB) and Fungi (HUF)

The vapour phase transfer method of [18] was adopted to determine the population of hydrocarbon utilizing bacteria (HUB). Aliquot (0.1 ml) of the serially diluted soil sample was inoculated on Bushnell Haas Agar medium (Containing either Chloramphenicol or Fungosol) using the spread plate technique as described by [19]. Sterile filter paper discs soaked in filter-sterilized crude oil which served as the only carbon source in the mineral salt agar was placed aseptically in the cover of the inoculated agar plates in duplicates. The plates were incubated for 3-5 days at ambient temperature (37^0C) for hydrocarbon utilizing bacteria and at 28^0C for 3-7 days fr hydrocarbon utilizing fungi. After the incubation period, the number of colonies was counted and the colonies were determined in cfu/g.

2.4.5. Cultural characterization of bacterial and fungal isolates.

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

2.4.6. Morphological Characterization of Bacteria

Pure cultures of bacterial isolates were identified based on gram staining, motility and biochemical tests which include; catalase, oxidase, citrate utilization, indole production, methyl red test, sugar fermentations, starch hydrolysis and microscopic techniques [20, 21]. The identification of bacterial isolates was confirmed by comparing them with Bergey's Manual of Determinative Bacteriology after microscopic examination.

2.4.7. Morphological Characterization of Fungal Isolates

The fungal isolates were identified morphologically based on conidial morphology and pigmentation. The technique described by [20] was also adopted for the identification of isolated fungi using cotton blue in lacto phenol stain. This was done by placing a drop of the stain on a 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 ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal isolates seen were identified in accordance with the standard scheme for the identification of fungi as adopted by Williams and Dimbu [22].

2.4.8. 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 the spread plate technique. The sub-cultured plates were marked, labeled properly and incubated at 28 ± 2°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.

2.4.9. 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 plates. Pure cultures were inoculated in duplicates and then stored in nutrient agar slants kept in the refrigerator at 4°C for further tests [23].

2.5. Molecular Identification

Selected hydrocarbon utilizing bacterial isolates were subjected to molecular identification via the16srRNA sequencing and phylogenetic analysis.

2.6 Physicochemical Analyses

The instruments and methods used for the measurement of the physicochemical characteristics of importance in this study are listed in Table.1.

Table 1 Soil physicochemistry methodology

Sample	Parameter/Instrument	Methodology
Soil	Temperature (Hanna Multipurpose Meter)	[24]
	Electrical Conductivity (Conductivity cell)	[25]
	pH (pH-911 Pen type)	[26]
	Moisture Content	[27]
	Chlorine (Hanna Multipurpose Meter)	[28]
	Bromine (Hanna Multipurpose Meter)	[28]
	Nitrogen (Hanna Multipurpose Meter)	[28]
	Phosphorous (Hanna Multipurpose Meter)	[28]
	Potassium (Hanna Multipurpose Meter)	[28]

2.7. Data Analyses

The data obtained was analysed using analysis of variance (ANOVA) to test for significance and where differences occur Duncan multiple range test was used to separate the means using the Statistical Package for Social Science (SPSS) version 22 [29].

3. RESULTS

3.1. Microbial Population of the Wetlands in Wet season

Results of the microbial population of the crude oil polluted wetlands as well as the control at three depths (surface 0-15cm, subsurface 15-30cm and deep soil 30-45cm) in the wet season is shown in Table 2 In the top soil (0-15cm), total heterotrophic bacterial count (THBC) ranged from $3.20x10^7$cfu/g (Borokiri) to $10.45x10^7$cfu/g (Control). In the sub soil, THBC ranged from $1.27x10^7$cfu/g (Borokiri) to $7.92x10^7$cfu/g (Control) and from $1.27x10^7$cfu/g (Borokiri) to $7.02x10^7$cfu/g. There was a significant difference ($p \leq 0.05$) in the total heterotrophic bacterial count across the soil depths in the wetlands. The microbial population of the wetland soil was observed to decrease with increasing soil depths. The control location had the highest population of total heterotrophic bacteria at all depths while Borokiri had the least population. Fungal count (FC) in the top soil (0-15cm) ranged from$1.08 x10^5$cfu/g (Iwofe) to $3.42x10^5$cfu/g (control) and from $1.06x10^5$cfu/g (Eagle Island) to $2.56x10^5$cfu/g (Control) in the subsurface soil. FC in the deep soil ranged from $1.06x10^5$cfu/g (Eagle Island) to $2.03x10^5$cfu/g (Control). There was a significant difference ($p \leq 0.05$) in the total heterotrophic fungal count across the soil depths in the wetlands. The control location also had the highest population of fungi at all depths compared to the crude oil polluted wetlands. The control location had the least population of hydrocarbon utilizing bacteria (HUB) ranging from $1.01x10^5$cfu/g (deep soil) to $1.22x10^5$cfu/g (top soil) and fungi $1.07x10^5$cfu/g (deep soil) to $1.09x10^5$cfu/g (top soil) across the wetlands. Iwofe had the highest HUB count ranging from $5.25x10^5$cfu/g (top soil) to $1.87x10^5$cfu/g (deep soil) and HUF counts $1.80 x10^5$cfu/g (top soil) to $1.05x10^5$cfu/g (deep soil) across the wetlands. There was a significant difference ($p \leq 0.05$) in the hydrocarbon utilizing bacterial and fungal counts across the soil depths in the wetlands.

3.2. Microbial Population of the Wetlands in Dry Season

The microbial population of the crude oil polluted wetland soils and control at three depths (surface 0-15cm, subsurface 15-30cm and deep soil 30-45cm depths) in the dry season is shown in Table 3. Total heterotrophic bacterial count (THBC) in the top soil ranged from $7.88x10^7$cfu/g (Borokiri) to $18.56x10^7$cfu/g (Control) and from $3.55x10^7$cfu/g (Borokiri) to $16.43x10^7$cfu/g (Control) in the subsurface soil. The deep soil had a THBC range of $2.43x10^7$cfu/g (Borokiri) to $10.46x10^7$cfu/g (Control). There was a significant difference ($p \leq 0.05$) in the total heterotrophic bacteria across the soil depths in the wetlands. The microbial population in the dry season was observed to decrease with an increase in soil depth across the wetlands. The control location also had the highest population of total heterotrophic bacteria and fungi at all depths compared to the crude oil polluted wetlands. The control location also had the least population of THFC ranging from $2.56x10^7$cfu/g (deep soil) to $5.65x10^7$cfu/g (top soil) and Borokiri the least population ranging from $1.03 x10^7$cfu/g (deep soil) to $1.87x10^7$cfu/g (top soil). The HUBC and HUFC followed the trend of a decrease in counts with an increase in soil depth; as surface soil had more counts than the deep soil levels across the wetlands. The highest hydrocarbon utilizing bacterial (HUB) count in the surface soil was observed in Eagle Island ($5.90x10^5$cfu/g) and the least was observed in the control location ($1.32x10^5$cfu/g). There was a significant difference ($p \leq 0.05$) in the hydrocarbon utilizing bacterial and fungal counts across the wetlands. In both seasons, the microbial population was higher in the surface soil (0-15cm) than in the subsurface soil and deep soil (15-30cm and 30-45cm) across the three wetlands as shown in Figs 2 to 5.

3.3. Physicochemical Analyses of the Wetland Soil in Wet and Dry Season

Results of the physicochemical characteristics of the wetlands soils at three depths (0-15cm, 15-30cm and 30-45cm) in wet and dry seasons are presented in Tables 4 and 5. In the wet season, the temperature ranged from 26.00^0C in Iwofe (15-30cm) to 29.50^0C in Borokiri (30-45cm) which was within the FEPA permissible limit of $20\text{-}30^0$C for soil temperature (Table 4.). In the dry season, the temperature ranged from 26.65^0C in Borokiri (0-15cm) to 33.50^0C in Iwofe (30-45cm), which was slightly above FEPA permissible limit of $20\text{-}30^0$C for soil temperature. The temperature ranges observed in this study showed an increase in temperature with increasing soil depth in both seasons. Soil temperature was significantly different ($p \leq 0.05$) at the various depths of the soil across the wetlands in both seasons. Electrical conductivity (EC) of the various wetland soils showed varying

differences (p≤0.05) in both seasons. The EC level in Borokiri ranged from 58.35µS/cm to 77.07µS/cm in the rainy season and 56.48µS/cm to 72.74µS/cm in the dry season which was below FEPA limit of 1000 µS/cm. Apart from the control station, other stations (Eagle Island, Iwofe and Borokiri) had EC levels above FEPA permissible limit in both wet and dry seasons. The soil pH across the wetland was slightly acidic in both seasons. pH ranged from 6.35 to 7.55 in the rainy season and 6.25 to 7.65 in the rainy season at Iwofe surface soil (0-15cm) and deep soil (30-45cm). pH ranges were within the FEPA limit of 6.50 to 8.50 in both seasons across the wetlands. There was a significant difference (p≤0.05) in the pH of the various depths of the soil across the wetlands in both seasons. There was no significant difference (p≥0.05) in the amount of Chlorine and Bromine present in the crude oil polluted wetlands at all soil depths in both seasons. Eagle Island, Iwofe and Borokiri had chlorine and bromine levels of 0.01Mg/Kg at all depths in both seasons, however, chlorine levels in the control location ranged from 90.0Mg/Kg to 102.0Mg/Kg in the rainy season and 84.03Mg/Kg to 97.03Mg/Kg in the dry season. Nitrogen, phosphorous and potassium which are essential trace elements in soil were present in relatively high quantities across the wetlands in both seasons. Phosphorus content ranged from 4.50Mg/Kg (Borokiri 30-45cm) to 208.92Mg/Kg (Control 0-15cm) in the rainy season and from 3.30Mg/Kg to 215.32Mg/Kg (control 0-15cm). The phosphorous level in the wetlands was slightly above FEPA permissible limit of 5.0Mg/Kg across all the depths in both seasons except in Borokiri deep soil (30-45cm). The amount of nitrogen, phosphorous and potassium in the soil varied significantly (p≤0.05) across all the wetlands in both seasons.

Table 2 Mean Microbial Population of the Wetland Soils in Wet Season

Wetland	Depth (cm)	THB ($x10^7$CFU/g)	THF ($x10^5$CFU/g)	HUB ($x10^5$CFU/g)	HUF ($x10^4$CFU/g)
Eagle Island	0-15	7.41 ± 2.16^{c}	1.08 ± 0.69^{b}	3.13 ± 1.18^{c}	1.52 ± 0.69^{c}
	15-30	3.40 ± 1.66^{b}	1.06 ± 0.42^{ab}	1.98 ± 0.91^{b}	1.08 ± 0.32^{b}
	30-45	1.90 ± 0.97^{a}	1.04 ± 0.28^{a}	1.00 ± 0.39^{a}	1.04 ± 0.34^{a}
Iwofe	0-15	9.28 ± 7.59^{a}	1.73 ± 0.79^{c}	5.25 ± 2.97^{c}	1.80 ± 0.81^{c}
	15-30	5.33 ± 1.91^{a}	1.09 ± 0.31^{b}	2.82 ± 1.06^{b}	1.09 ± 0.51^{b}
	30-45	3.07 ± 1.94^{a}	1.08 ± 0.49^{a}	1.87 ± 0.92^{a}	1.05 ± 0.26^{a}
Borokiri	0-15	7.80 ± 2.65^{c}	1.10 ± 0.66^{a}	2.22 ± 0.99^{a}	1.50 ± 0.40^{a}
	15-30	3.20 ± 1.23^{b}	1.06 ± 0.37^{a}	1.57 ± 0.96^{a}	1.05 ± 0.17^{b}
	30-45	1.27 ± 0.49^{a}	1.06 ± 0.28^{a}	1.18 ± 0.78^{a}	1.03 ± 0.20^{c}
Control	0-15	10.45 ± 3.42^{a}	3.42 ± 1.86^{b}	1.22 ± 0.23^{a}	1.09 ± 0.42^{a}
	15-30	7.92 ± 2.34^{b}	2.56 ± 1.23^{a}	1.34 ± 0.33^{a}	1.02 ± 0.21^{a}
	30-45	7.02 ± 1.32^{c}	2.03 ± 0.12^{c}	1.01 ± 0.95^{a}	1.07 ± 0.01^{a}

***Mean with the same superscript along the columns is not significantly different (p≥0.05)**

Keys: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi), 0-15cm (Surface Soil), 15-30cm (Subsurface Soil), 30-45cm (Deep Soil).

Table 3.Mean Microbial Population of the Wetland Soils in Dry Season

Wetland	Depth (cm)	THB ($x10^7$CFU/g)	THF ($x10^5$CFU/g)	HUB ($x10^5$CFU/g)	HUF ($x10^4$CFU/g)
Eagle Island	0-15	11.80±2.97^a	1.93±0.76^a	5.90±1.54^a	1.93±0.23^b
	15-30	10.40±12.79^a	1.70±1.13^a	4.43±4.74^a	1.60±0.47^{ab}
	30-45	7.43±6.47^a	1.88±0.70^a	3.13±1.95^a	1.33±0.26^a
Iwofe	0-15	17.85±6.08^c	1.75±0.17^a	5.33±3.93^a	1.90±0.26^a
	15-30	8.97±2.02^b	1.43±0.85^a	4.83±1.80^a	1.60±0.26^a
	30-45	5.62±2.59^a	1.20±0.87^a	2.87±1.88^a	1.33±0.06^a
Borokiri	0-15	7.88±2.58^a	1.87±0.78^a	2.23±1.71^a	1.90±0.39^a
	15-30	3.55±2.79^a	1.43±0.78^a	2.83±1.62^a	1.70±0.31^a
	30-45	2.43±1.05^b	1.03±0.46^a	1.47±0.85^a	1.48±0.39^a
Control	0-15	18.56±8.79^a	5.67±1.23^b	1.32±0.45^a	1.54±1.10^a
	15-30	16.43±5.34^b	3.34±1.43^c	1.23±0.34^c	1.41±0.45^b
	30-45	10.46±4.45^c	2.56±1.40^a	1.12±0.43^b	1.32±0.34^c

***Mean with the same superscript along the columns is not significantly different (p≥0.05)**

Keys: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi), 0-15cm (Surface Soil), 15-30cm (Subsurface Soil), 30-45cm (Deep Soil).

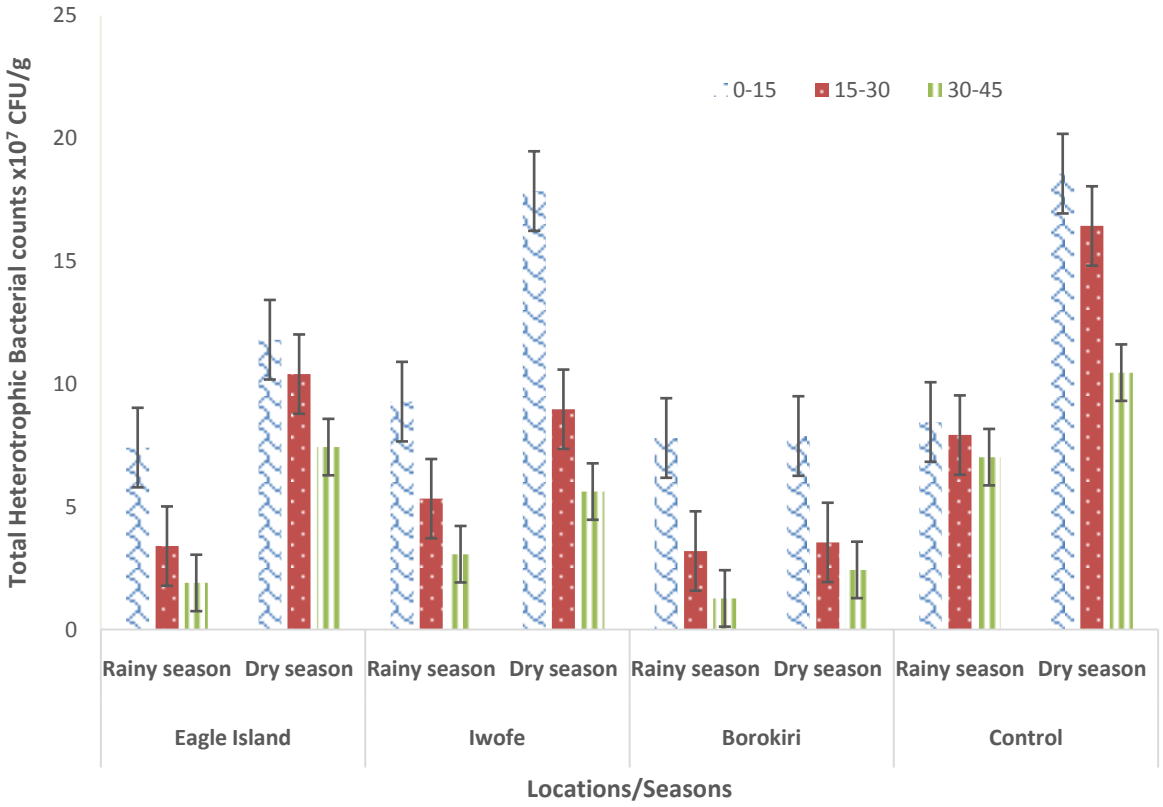

Fig. 2: Total heterotrophic bacterial count of soil across the wetlands in wet and dry seasons

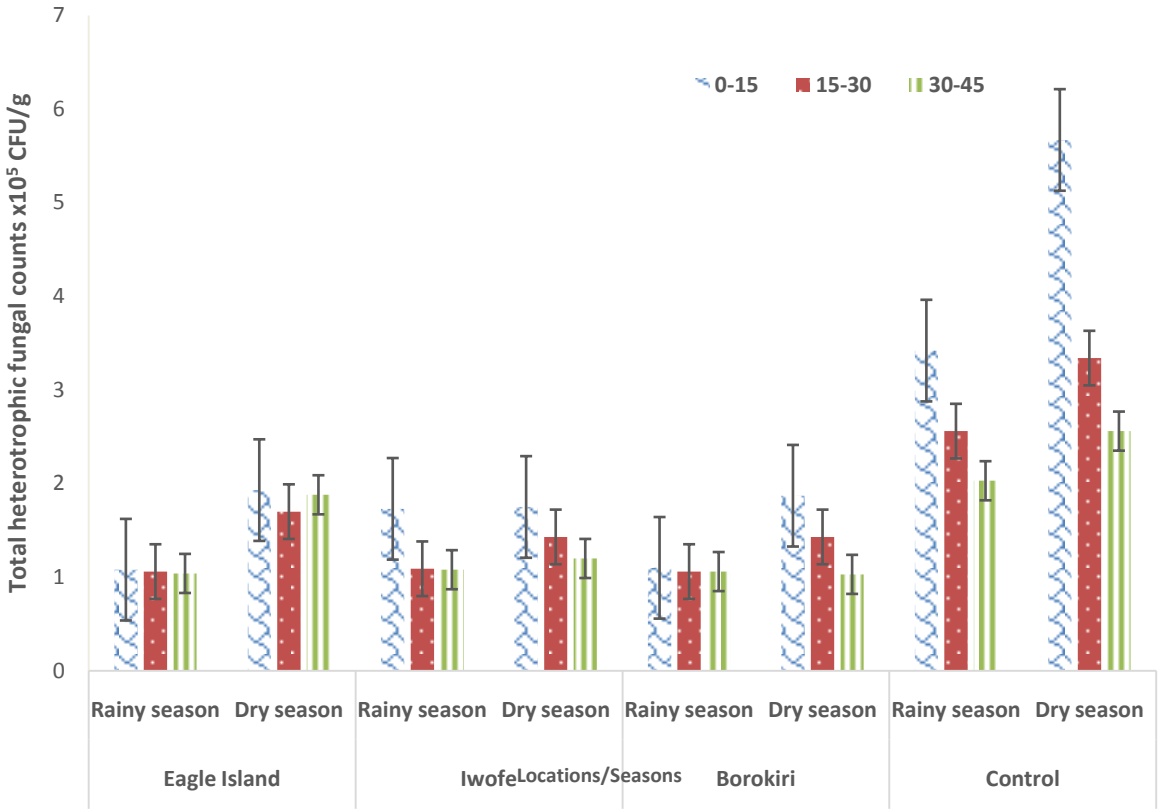

Fig. 3.: Fungal counts of soil across the wetlands in wet and dry seasons

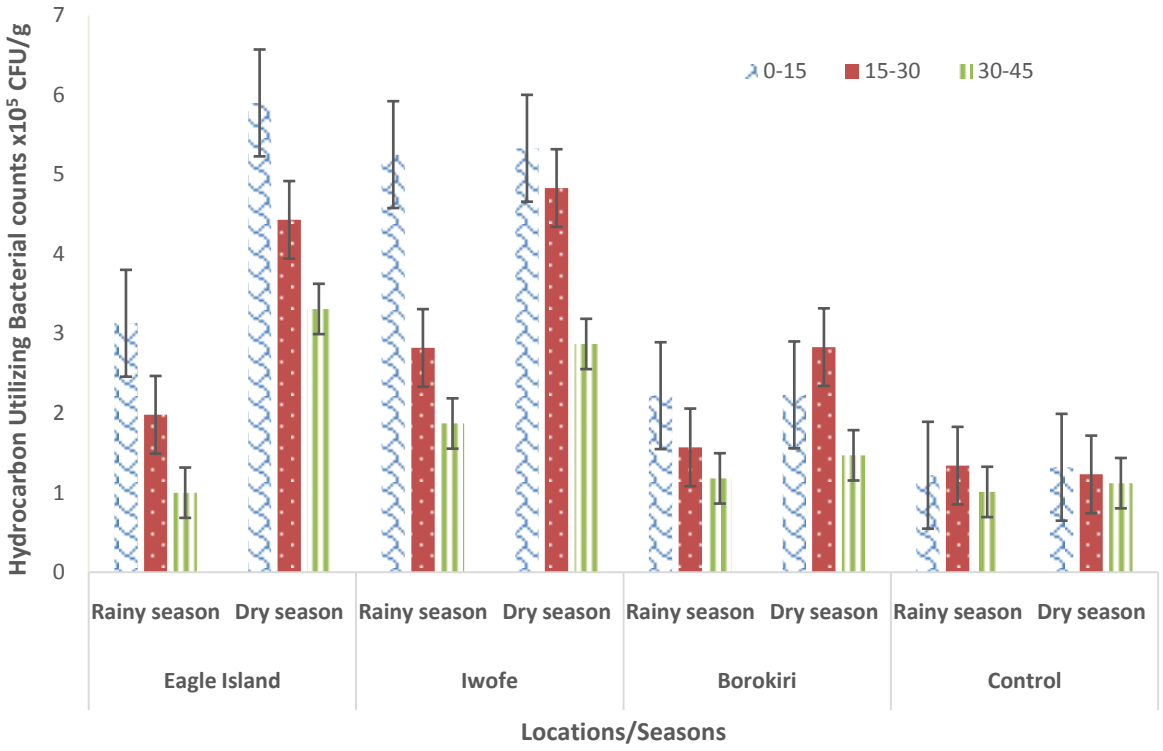

Fig. 4 : Hydrocarbon utilizing bacterial counts of soil across the different wetlands in wet and dry seasons

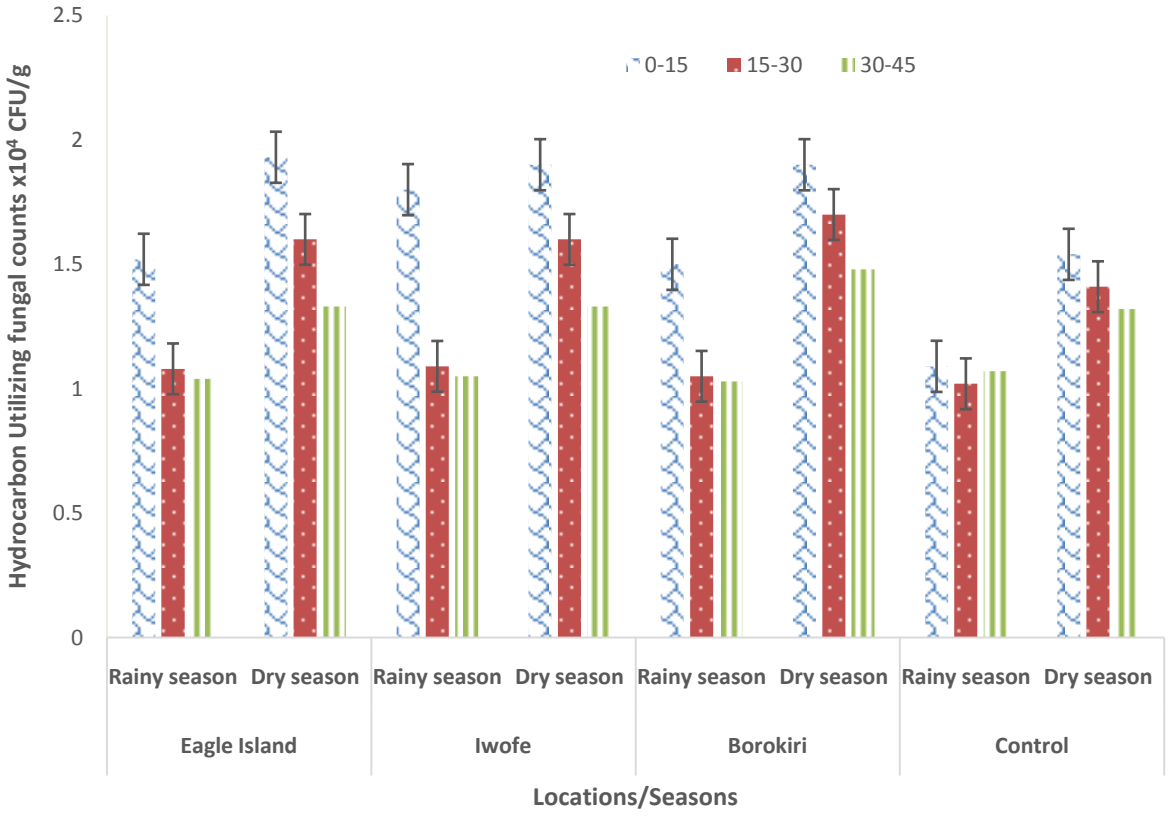

Fig. 5: Hydrocarbon utilizing fungal counts of soil across the wetlands in wet and dry seasons

Table 4 Physicochemical Characteristics of the Wetland Soil in the Rainy Season

Location	Depth (cm)	Temp. ^{o}C	EC (µS/cm)	pH	M.C (%)	Cl (mg/Kg)	Br (mg/Kg)	N (mg/Kg)	P (mg/Kg)	K (mg/Kg)
Borokiri	0-15	27.00±0.00^{a}	77.07±0.00^{c}	6.46±0.01^{a}	22.55±0.07^{a}	0.01±0.00^{a}	0.01±0.00^{a}	113.00±1.4^{a}	9.85±0.07^{b}	10.50±0.71^{a}
	15-30	29.00±0.00^{a}	67.500±0.70^{b}	6.83±0.01^{ab}	18.75±0.07^{b}	0.01±0.00^{a}	0.01±0.00^{a}	83.50±0.71^{b}	7.50±0.71^{a}	73.00±1.41^{c}
	30-45	29.50±0.71^{a}	58.35±0.07^{a}	6.92±0.01^{ab}	16.75±0.07^{c}	0.01±0.00^{a}	0.01±0.00^{a}	59.50±0.71^{c}	4.50±0.57^{c}	87.45±0.07^{b}
Eagle Island	0-15	26.50±0.71^{a}	5455.50±0.71^{d}	7.30±0.14^{cd}	13.95±0.00^{d}	0.01±0.00^{a}	0.01±0.00^{a}	940.50±0.77^{g}	9.55±0.07^{d}	810.50±0.71^{d}
	15-30	27.50±0.71^{b}	3436.50±0.71^{h}	7.50±0.71^{ab}	14.38±0.01^{e}	0.01±0.00^{a}	0.01±0.00^{a}	561.00±1.41^{h}	25.50±0.71^{e}	500.50±0.71^{e}
	30-45	28.50±0.71^{a}	2509.50±0.71i	6.85±0.07^{cd}	20.00±1.41^{f}	0.01±0.00^{a}	0.01±0.00^{a}	436.00±0.00^{i}	29.50±0.71^{f}	297.50±0.71^{f}
Iwofe	0-15	26.50±0.71^{b}	1073.50±0.71^{e}	6.35±0.07^{c}	14.34±0.02^{g}	0.01±0.00^{a}	0.01±0.00^{a}	990.50±0.71^{f}	70.50±0.7071^{g}	490.50±0.71^{g}
	15-30	26.00±0.00^{a}	2582.50±0.71^{a}	7.40±0.14^{cd}	13.50±0.71^{h}	0.01±0.00^{a}	0.01±0.00^{a}	490.50±0.71^{e}	160.50±0.71^{h}	390.50±0.71^{h}
	30-45	28.50±0.71^{a}	2946.50±0.71^{g}	7.55±0.07^{d}	15.35±0.07i	0.01±0.00^{a}	0.01±0.00^{a}	311.00±1.4^{d}	196.00±1.41^{i}	332.50±0.71^{i}
Control	0-15	27.87±0.72^{a}	802.09±0.72^{h}	7.06±0.08^{d}	25.34±0.08^{h}	90.00±0.02^{a}	0.01±0.00^{a}	1208.00±2.11^{e}	208.92±1.24^{g}	1482.00±0.71^{e}
	15-30	28.30±0.71^{b}	562.00±0.71^{e}	7.20±0.07^{c}	22.87±0.07^{a}	96.50±0.04^{b}	0.01±0.00^{a}	874.00±0.71^{d}	151.90±0.72^{h}	987.50±0.73^{h}
	30-45	28.90±0.72^{a}	309.20±0.72^{f}	7.54±0.71^{d}	20.01±0.07^{b}	102.00±0.03^{a}	0.01±0.00^{a}	646.00±0.72^{e}	138.50±0.35^{i}	704.90±0.71^{i}

FEPA LIMIT (1991)	20-30	1000	6.50-8.00	Not fixed	250	Not Fixed	Not Fixed	5.0	Not Fixed

Table 5: Physicochemical Characteristics of the Wetland Soil in Dry Season

Location	Depth (cm)	Temp. ^{0}C	EC (µS/cm)	pH	M.C (%)	Cl (mg/Kg)	Br (mg/Kg)	N (mg/Kg)	P (mg/Kg)	K (mg/Kg)
Borokiri	0-15	26.65±0.07^a	72.74±0.01^a	6.43±0.03^a	20.85±0.07^a	0.01±0.00^a	0.01±0.00^a	102.50±0.71^a	9.50±0.71^a	5.50±0.71^e
	15-30	27.50±0.71^b	60.50±0.71^b	6.83±0.01^a	15.70±0.14^b	0.01±0.00^a	0.01±0.00^a	53.00±1.41^a	6.00±1.41^a	62.00±1.4^a
	30-45	30.50±0.71^b	56.48±0.01^c	7.20±0.14^a	10.35±0.07^c	0.01±0.00^a	0.01±0.00^a	34.30±0.14^b	3.30±0.14^b	90.60±0.14^f
Eagle Island	0-15	31.50±0.71^a	14340.50±0.71^d	7.25±0.071^a	13.85±0.07^d	0.01±0.00^a	0.01±0.00^a	520.50±0.71^c	7.60±0.14^c	471.00±1.41^a
	15-30	32.50±0.71^a	8390.50±0.71^e	7.50±0.71^a	19.50±0.71^e	0.01±0.00^a	0.01±0.00^a	530.50±0.71^d	14.55±0.071^d	510.50±0.71^g
	30-45	32.50±0.71^a	3578.00±0.00^f	6.82±0.01^a	23.25±0.21^f	0.01±0.00^a	0.01±0.00^a	538.50±0.7^e	17.40±0.14^e	601.00±1.41^b
Iwofe	0-15	31.50±0.71^a	15380.50±0.71^g	6.25±0.07^b	0.87±0.014^g	0.01±0.00^a	0.01±0.00^a	525.50±0.71^f	13.55±0.07^f	486.00±1.41^h
	15-30	32.00±0.00^a	4910.50±0.71^h	7.40±0.14^a	8.85±0.071^h	0.01±0.00^a	0.01±0.00^a	990.50±0.71^g	15.50±0.71^g	491.00±1.41^c
	30-45	33.50±0.71^a	2780.50±0.71^i	7.65±0.21^a	14.50±0.71^i	0.01±0.00^a	0.01±0.00^a	1010.50±0.71^h	18.65±0.07^h	498.50±0.71^d
Control	0-15	28.31±0.72^a	809.08±0.72^h	7.10±0.23^a	20.60±0.73^h	84.03±0.02^b	0.01±0.00^a	1350.34±0.82^i	215.32±0.08^f	1503.00±0.72^d
	15-30	29.21±0.62^a	560.08±0.76^h	7.20±0.21^b	18.92±0.23^g	90.32±0.03^b	0.01±0.00^a	897.87±0.07^a	162.73±0.03^g	991.00±0.07^c
	30-45	30.00±0.34^a	310.00±0.63^a	7.50±0.22^b	18.00±0.07^f	97.73±0.01^b	0.01±0.00^a	679.85±0.09^e	140.92±0.07^e	724.00±0.08^b

FEPA LIMIT (1991)	20-30	1000	6.50-8.00	Not Fixed

***Means with same alphabet across the column shows no significant difference (p≥0.05)**

Keys: Temp: Temperature, EC: Electrical Conductivity, pH: Hydrogen Ion Concentration, MC: Moisture Content, Cl: Chlorine, Br: Bromine, N: Nitrogen, P: Phosphorous, K: Potassium.

4. DISCUSSION

Soil microbial communities are major components of microbial food webs, biogeochemical cycles and energy flow. Bacteria and fungi are the predominant microorganisms in these habitats [30]. Their biodiversity is structured and determined by the temporal and spatial variability of physicochemical and biotic parameters and thus, can reflect local environmental conditions [31]. The assessment of the effect of seasonal variation on the microbial population of the wetland in this study revealed that the season of study contributed greatly to microbial proliferation. Significantly higher counts were observed during the dry than the rainy seasons in the wetland soils and this conformed to a similar study carried out by Unimke *et al.* [32]. The increase in the microbial population of the wetland soil during the dry season can be linked to the slight increase in temperature during the research period in the wetlands. The slightly lower population of microorganisms in the wet/rainy season can be linked to changes in temperature and other physicochemical characteristics of the soil such as nutrient content, moisture content, oxygen concentration and many other parameters of importance in this study. The wetland soils under study revealed total heterotrophic bacteria as having the highest population in all soil depths (0-15cm, 15-30cm and 30-45cm) in both seasons. The occurrence of total heterotrophic bacteria as the highest occurring organisms could be attributed to the tolerance of these microorganisms to a wide variation of physicochemical properties (temperature, pH, nutrient content, moisture content, oxygen concentration) which prevailed in the wetland in both seasons. Fungi constituted the second highest number of microbes that inhabit the wetland while the hydrocarbon utilizing microorganisms were the least among the groups of microorganisms isolated from the wetland soil in both seasons. The high fungal counts could be attributed to the slightly acidic nature of this environment since fungal growth is enhanced by the acidic nature of an environment [33]. The result from this study followed the same trend for wetland microbial populations as reported by Udotong *et al.* [33], Bassey *et al*. [34], Youssef *et al.* [30] and John and Eduok [35]. The results also showed that the microbial population in the wetland soil decreased with an increase in soil depth. The microbial population of the wetlands followed the order 0-15cm > 15-30cm > 30-45cm; microbial counts were higher in surface soils (0-15 cm) than in the sub-surface soils (15-30cm) and deep soil (0-45cm) across all the wetlands. This could be attributed to the higher availability of favourable growth factors such as nutrients, moisture content and oxygen being more available at the surface soil (0-15cm) than at the deeper portions (15-30cm and 30-45cm). These results corroborate with other studies in which a similar trend for wetland soils has been reported [35, 33, 32]. Ten (10) bacteria were identified in this study using 16srRNA from the three wetlands of which *Bacillus* was the most occurring. The organisms isolated and identified in this study include *Bacillus subtillis, B. rigui, B. flexus, Lysinibacillus macroides, Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterobacter aerogenes, and Proteus penneri.* The fungal isolates *were* mostly of the genera *Aspergillus species, Fusarium species, Mucor species, Penicillium species and Rhizopus species.* These isolates have the ability to utilize crude oil as their sole source of carbon and energy and the dominance of these organisms has been reported by different researchers as crude oil degraders [36, 37]. Generally, the temperature values obtained from the wetland soils and control in both seasons fell within the mesophilic range (20-45^{0}C). This indicated that the temperature of the wetlands at the three depths supported the growth of mostly mesophilic microorganisms. Furthermore, soil temperature was higher in the dry season than in the rainy season and this could be attributed to an increase in the amount of sunlight received by the soil in the dry season. The study also revealed an increase in temperature with an increase in the depth of the soil which is in agreement with works done by Udotong *et al.* [33]. Soil pH is a good indicator of the possible nutrient status of the land. Soil having pH < 5.6 is usually considered to be acidic in nature, pH from 5.6 to 6.0 ranges is moderately acidic while < 5.5 are strongly acidic in nature [38]. Similarly, soil pH is the most important factor that affects the heavy metal solubility and their availability to the plants [39, 40]. The results in this study also indicate that heavy metal concentrations were higher in impacted soils compared to the control soils in the wet and dry seasons. The trend of heavy metal concentrations in the soil was in the order: Fe > Zn > Pb > Cu > Cd >Ni > Hg > Ar > Ba. The varying concentrations of heavy metals for the impacted and non-impacted wetlands suggest the influence of soil properties, metal properties and environmental factors such as run-off that washed the crude oil exploration product wastes into the wetlands. Heavy metal contamination of the soil from petroleum hydrocarbons has also been reported in several studies [41, 42].

5. CONCLUSION

Wetlands are destroyed by crude oil spillage because of the high porosity of the soil and the sediment properties oil is quickly absorbed, retained and re-liberated once the wet season starts, thus, aggravating pollution. Most of the wetlands in Rivers State are either encroached into by urbanization

or polluted by crude oil spillage. This study provides information on the effect of seasonal variation on the microbiology and physicochemistry (including nutrient content and heavy metal content) of three coastal wetlands (Iwofe, Eagle Island and Borokiri, Eastern bypass/ Ogbunuabali served as control) in Rivers State polluted by oil spills due to the transportation of artisanal crude oil and other anthropogenic activities. The study revealed that seasonal variation had an effect on the microbial population in the wetlands. The study revealed that the wetland with the highest total petroleum hydrocarbon content (Borokiri) had the highest concentrations of heavy metals. However, heavy metals were present in all three crude oil polluted wetlands either in minute or high levels, which is poisonous to humans. According to the literature, microbial population decreases significantly with increasing heavy metal concentration. However, the microbes in this study increased with an increase in some heavy metal concentrations which indicate that they tolerate and use heavy metals in their systems; as such these microbes can be used for bioremediation of heavy metal-polluted soils.

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