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Effect of crude oil pollution on the microbiology of Coastal Wetlands in river state

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Abstract
This study was conducted to determine the effect of crude oil pollution on the microbial population of Coastal Wetlands in Rivers State. A total of 288 soil samples were collected for a period of twelve (12) month from three crude oil polluted wetlands and subjected to microbiological analyses. In the wet season, total heterotrophic bacterial count (THBC) in the wetland soil, sediment and surface water ranged from $1.90x10^7$ cfu/g (Eagle Island) to $10.45x10^7$ cfu/g (Control); $2.89x10^7$cfu/g (Borokiri) to $9.86x10^7$ cfu/g (control) and $2.57x10^7$ cfu/ml (Borokiri) to $8.67x10^7$ cfu/ml (Control) respectively. Dry season THBC in soil, sediment and surface water ranged from $2.43x10^7$ cfu/g (Borokiri) to 18.5610^7 cfu/g (control); $4.28x10^7$ cfu/g (Borokiri) to $15.45x10^7$ cfu/g (Control) and $2.10x10^7$ cfu/ml (Borokiri) to $7.34x10^7$ cfu/ml (Control). There was a significant difference ($p \leq 0.05$) in the microbial population across the wetlands in both seasons. The Total petroleum hydrocarbon and Polycyclic aromatic hydrocarbon concentrations was higher in the crude oil polluted wetland than the control sample in both seasons. The study revealed a decrease in microbial population with increase in TPH and PAH content in the wetlands.

Keywords: Wetlands, petroleum, hydrocarbon, population, microbial

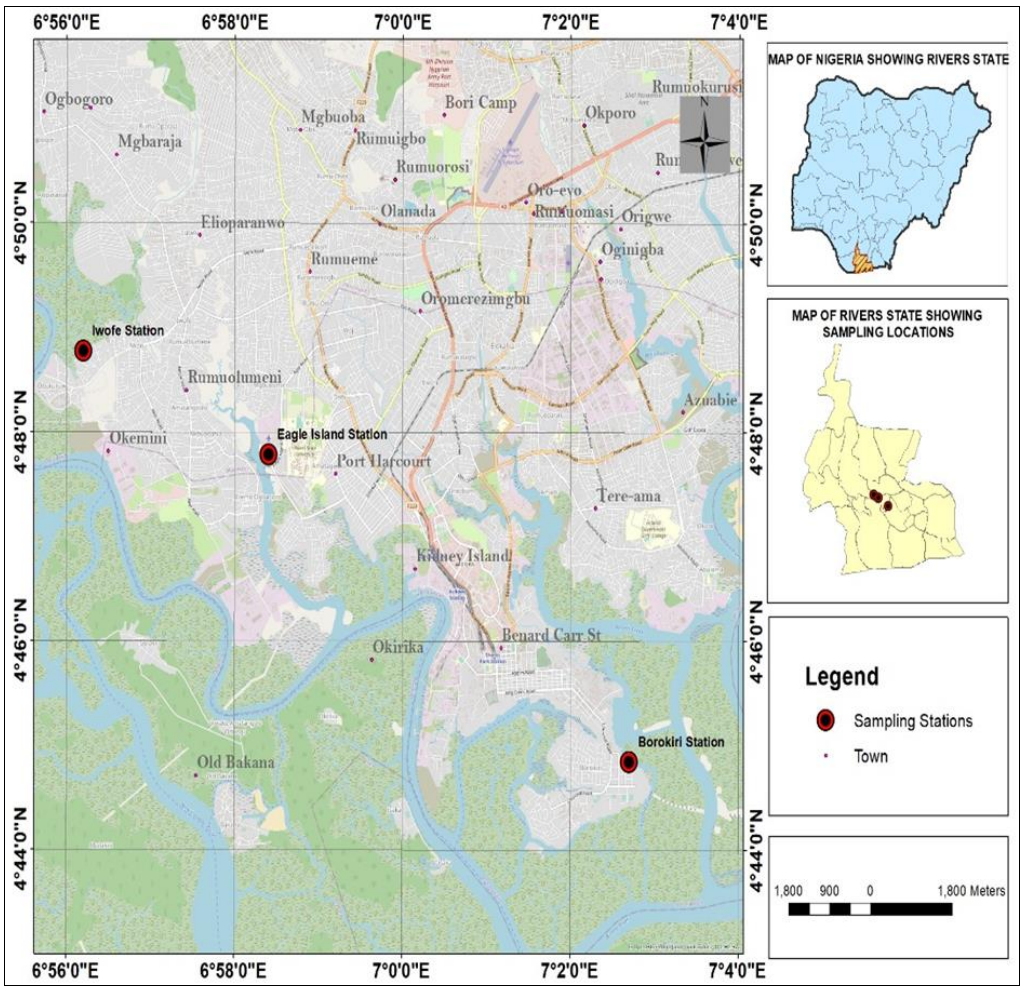
1. Introduction
A wetland is a specific habitat that experiences seasonal or persistent flooding of water and supports oxygen-free processes [1]. The peculiar flora of aquatic plants adapted to their particular hydric soil is the main property that sets wetlands apart from other land types or bodies of water. Water purification, storage, processing of nitrogen and other nutrients, shoreline stabilisation, support of plants, animals, and microbial life are just a few of the functions that wetlands do. Wetlands are also considered the most biologically diverse of all ecosystems, serving as home to a wide range of plants, animals and microbial life. Whether any individual wetland performs all these functions, the degree to which it performs them depends on characteristics of that wetland and the lands and waters near it [2]. As obtainable in every natural habitat, wetlands are very significant in the preservation of biodiversity, serve as tourist and recreation attraction that contributes to the economy of wherever they are found. Consequently, the act of eliminating such viable wetland systems due to industrial development, urbanization and other related factors mean a gradual extinction of micro and macro biota and eventual deterioration of water quality [3, 4]. Although the oil industry has made significant contributions to the country's prosperity, it has also seriously harmed the environment. The ecosystem that an oil spill is released into is significantly impacted. The mangrove forests, which are particularly vulnerable to oil spills, have been devastated in large-scale. According to estimates from oil drilling, 5–10% of Nigeria's mangrove ecology has been destroyed [5]. Marshes, an example of a wetland, play a significant role in river, estuarine, and coastal ecosystems. They are extremely sensitive to oil pollution [6] and can sustain significant damage from spills, which prevent carbon fixation by inhibiting plant transpiration and, through this mechanism and others, can kill marsh vegetation [7]. Numerous stressors brought on by humans have an impact on the marine environment, and the degradation is visible not just in coastal areas but also in remote locations like the deep seas and Polar Regions. Recent instances, however, demonstrate that marine oil spills are unpredictable occurrences that may cause serious harm to coastal communities as a whole as well as to the marine ecosystems and species. The soil, fresh water, lakes, streams, estuaries, and overall ecology of the oil-rich Niger Delta region have all been noticeably degraded as a result [5]. The way petroleum hydrocarbons behave when they enter marine ecosystems is closely related to the type of contaminant, the entry method, and the physical properties of

the receiving fresh water system [5, 8]. When bulk oil is spilled into water that is moving quickly, it will behave differently from when it is thrown into still water. By creating a physical barrier, non-volatile components may prevent volatile ones from evaporating. This may then prefer disintegration as the route of loss, encouraging more entry into the water than the atmosphere [5, 8]. The plants in marshes may completely die as a result of heavy crude oil pollution [9]. Additionally, compared to clean areas, soils contaminated with crude oil are hydrophobic [10]. Oil spills have a variety of detrimental short- and long-term effects that cause economic and financial losses. By entering or obstructing the pores of the leaves, oil hinders photosynthesis and transpiration in plants [11]. Additionally, water-soluble poisons that might harm aquatic organisms may be discharged. Fuel oil from spills has been observed to linger in marsh soils for at least five (5) years before being released into surface waters. High hydrocarbon levels in shellfish living in contaminated marshes or exposed to hydrocarbons there from oil spills in the environment [12] are indicators of this persistence. The majority of these substances are either potential or known carcinogens, making polycyclic aromatic hydrocarbons (PAH), which are generally stable petroleum ingredients, one of the most crucial environmental analytes. Unfortunately, due to their low aqueous solubility, low volatility, and resistance to degradation, PAHs can build up to levels where they could have hazardous effects on the environment. As a result, they need to be constantly monitored. High hydrocarbon levels in shellfish that live in polluted marshes or that have been exposed to hydrocarbons there from environmental oil spills [12] are signs of this persistence. Polycyclic aromatic hydrocarbons (PAH), which are typically stable petroleum constituents, are one of the most important environmental analytes since the majority of these compounds are either recognised or probable carcinogens. Unfortunately, PAHs can accumulate to levels where they could have harmful impacts on the environment because of their low aqueous solubility, low volatility, and resistance to degradation. They must therefore be constantly watched over as a result.

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 Iwofe/ Rumuolumeni 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 2.1.



Map 1: Map of Rivers State Showing the Sampling Locations

2.2 Sample Collection

Samples were collected and conveyed to the Post Graduate Microbiology Research Laboratory of the Department of Microbiology, Rivers State University, and Port Harcourt for analyses within 24 hours.

2.2.1 Wetland Soil

Soil samples were collected with the aid of a hand auger using the method described by Pepper and Gerba [13]. Soil samples were collected at 3 depths (0-15 cm, 15-30 cm 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 wetlands.

2.2.2 Sediment Samples

Using a grab sampler, sediment samples were taken from the river's intertidal surface. Before and after use, the grab sampler was thoroughly cleaned with water from the same watercourse to get rid of any apparent silt. The sampler was lowered to the water's surface at each sampling location, and the topmost layer of material was heaved out. At each sampling phase, replicates of the sediment sample were taken out of the grab's cup and placed in sterile sample containers. For a total of twelve (12) months, sediment samples from the wetland stations totaled forty eight (48).

2.2.3 Surface water

The technique developed by Ugochukwu *et al*. [14] was used to gather samples of surface water. The surface water was aseptically collected using sterile 1.5-liter screw-capped bottles. Two distinct locations (approximately 50m apart) along the direction of the river flow were chosen for the collection of the water samples at each station. With one hand holding the base of the sterilised sample container, it was lowered about 30 cm below the water's surface, with the sample container's mouth facing away from the direction of the water flow [15]. To create a composite sample, 500ml of the sample that was taken at each moment was combined. The samples were immediately taken to the lab for analysis after being collected and put in a cooler with ice blocks. A total of forty eight (48) surface water samples was collected for a period of twelve (12) from the wetland stations.

2.3 Microbiological Analyses

2.3.1. Serial Dilution

The dilution method adopted was the ten-fold serial dilution technique in which 1g/ml of the sample (soil, sediment or water) was added into 9ml test tubes containing sterile diluent. This was done consecutively until appropriate dilutions of 10^2 to 10^6 were reached [16].

2.3.2. Inoculation and Enumeration of Heterotrophic Bacterial

Using a flamed bent glass spreader, aliquots (0.1 ml) of the dilutions $x10^5$ and $x10^6$ were inoculated onto surface-dried nutritional agar medium plates that contained 100g/ml of fungosol. The plates were incubated for 24 hours at 370 C. According to Prescott *et al.* [17] description, the total number of heterotrophic bacteria was counted [17]. On the nutrient agar plates, bacterial colonies formed. The number of colonies was measured, and the mean was represented as cfu/g for soil and sediment and cfu/ml for water [18].

2.3.3. Inoculation and Enumeration of Heterotrophic Fungal

This was determined using the sabouraud dextrose agar. Chloramphenicol was used to suppress bacterial growth [19]. The spread plate technique as described by Prescott *et al*, [17] was adopted. An aliquot (0.1 ml) 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 °C for 3-7 days [20].

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

The vapour phase transfer method of Mills and Cowell [35] 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 Chikere and Ekwuabu [22]. 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 °C) for hydrocarbon utilizing bacteria and at 28 °C for 3-7 days for hydrocarbon utilizing fungi. After the incubation period, the number of colonies was counted and the colonies were determined in cfu/g or cfu/ml.

2.3.5. Cultural characterization of bacterial and fungal isolates

The appearance on the media, shape, colour, wetness, size, elevation, opacity, and other factors were used to determine the cultural features of the bacterial isolates. Based on cultural traits such as colony development pattern and colour, the fungal isolates were identified.

2.3.6. 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 [23, 24]. The identification of bacterial isolates was confirmed by comparing them with Bergey's Manual of Determinative Bacteriology after microscopic examination.

2.3.7. Morphological Characterization of Fungal Isolates

Conidial morphology and pigmentation were used to morphologically identify the different fungal isolates. Cotton blue in lacto phenol stain was used to identify the isolated fungi following the method given by Cheese brough [23]. This was accomplished by using a mounting needle to apply a drop of the stain to a clean slide, followed by the placement of a small piece of the representative fungal cultures' aerial mycelia in a drop of lactophenol. A needle was used to disseminate the mycelium across the slide. To remove air bubbles, a cover slip was put delicately and lightly. After mounting, the slide was examined using a light microscope and 10 and 40 objective lenses. The physical traits and outward appearance of the observed fungal isolates were identified using the Williams and Dimbu [20] standard scheme for fungi identification.

2.3.8. Characterization and identification of fungal isolates

Based on the differences in their morphologies, distinct fungal isolates from the 5-7 day incubated plates were chosen, and they were purified using the spread plate technique by sub-culturing on recently produced Sabouraud Dextrose Agar plates. The sub-cultured plates were appropriately marked and labelled before being incubated for 5-7 days at 28±2 °C. Pure cultures were obtained after 5-7 days of incubation and were subjected to morphological and cultural characteristics such as colony development pattern, conidial morphology, and pigmentation for characterisation and identification.

2.3.9. Purification and preservation of bacterial cultures

Ten percent (10%) glycerol solution was prepared, dispensed in McCarteny 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 then stored in nutrient agar slants kept in the refrigerator at 4°C for further tests [25].

2.4 Total Petroleum Hydrocarbon

Total Petroleum Hydrocarbon was measured ex situ using Gas chromatograph- Flame ionization detector (GC-FID) Agilent 7890A according to the methods of ASTDM 3921 and US EPA 8015 analytical protocol (TPI, 2007) as reported by Chikere *et al.* [26] and in accordance with Nigerian requirements of Department of Petroleum Resources (DPR), National Oil Spill Detection Response Agency (NOSDRA) and Federal Ministry of Environment (FMEnv).

2.5 Polycyclic Aromatic Hydrocarbon

As sample extracts are forced through an immobile, inert stationary phase (1,3 dimethyl siloxane), components with low solubility take a longer time to elute, resulting in the differential mobilities of the fractional components of the polycyclic aromatic hydrocarbon (PAHs), the polycyclic aromatic hydrocarbon (PAH) content of the samples was determined by flame ionisation detection. The FID detector, whose response was based on the composition of the individual constituent fractions, automatically detects samples as they exit the column (at a constant flow rate).

2.5.1 Determination of polycyclic aromatic hydrocarbons (PAH) in Wetland Soil

2.5.1.1 Extraction process

The soil samples were air dried, homogenized, sieved and extracted using hot extraction (sohxlet extractor). A gravimetric method was used to determine oil and grease [27]. Extracts were subjected to silica gel clean-up to remove polar organic substances leaving non- polar hydrocarbon in the solvent.

2.5.1.2 Measurement of polycyclic aromatic hydrocarbons

As sample extracts are being forced through an immobile, vminert stationary phase (1,3 dimethyl siloxane), components of low solubility take a longer elution time, leading to the differential mobilities of the fractional components of the polycyclic aromatic hydrocarbon (PAHs), the polycyclic aromatic hydrocarbon content of the soil sample was determined using the principle of Gas Chromatography by flame ionisation detection. The FID detector, whose response was based on the composition of the individual constituent fractions, automatically detects samples as they exit the column (at a constant flow rate).

2.5.1.3 Determination of PAHs concentration in the Extract

The analysis was done using gas chromatography with flame ionisation detection. While an Agilet HP5 gas chromatographic capillary column with 100% 1,3 dimethyl siloxane stationary phase material was used to separate the vapour components of various hydrocarbon fractions, helium gas, which flows at a rate of 14.81 psi, served as the mobile phase. Serving as igniting gases were hydrogen and air at a flow rate of 30 psi [28].

2.6. 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 wetland soils 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 3.1. In the top soil (0-15cm), total heterotrophic bacterial count (THBC) ranged from 3.20x107cfu/g (Borokiri) to 10.45x107cfu/g (Control). In the sub soil, THBC ranged from 1.27x107cfu/g (Borokiri) to 7.92x107cfu/g (Control) and from 1.27x107cfu/g (Borokiri) to 7.02x107cfu/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 from1.08 x105cfu/g (Iwofe) to 3.42x105cfu/g (control) and from 1.06x105cfu/g (Eagle Island) to 2.56x105cfu/g (Control) in the subsurface soil. FC in the deep soil ranged from 1.06x105cfu/g (Eagle Island) to 2.03x105cfu/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 1.01x105 cfu/g (deep soil) to 1.22x105cfu/g (top soil) and fungi 1.07x105 cfu/g (deep soil) to 1.09x105cfu/g (top soil) across the wetlands. Iwofe had the highest HUB count ranging from 5.25x105cfu/g (top soil) to 1.87x105cfu/g (deep soil) and HUF count 1.80x105cfu/g (Top soil) to 1.05x105 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 30-45 cm depths) in the dry season is shown in Table 3.2. Total heterotrophic bacterial count (THBC) in the top soil ranged from 7.88x107cfu/g (Borokiri) to 18.56x107 cfu/g (Control) and from 3.55x107 cfu/g (Borokiri) to 16.43x107 cfu/g (Control) in the subsurface soil. The deep soil had a THBC range of 2.43x107 cfu/g (Borokiri) to 10.46x107 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 2.56x107 cfu/g (deep soil) to 5.65x107 cfu/g (top soil) and Borokiri the least population ranging from 1.03 x107 cfu/g (deep soil) to 1.87x107 cfu/g (top soil). The HUBC and HUFC followed the trend of decrease in counts with 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 (5.90x105cfu/g) and the least was observed in the control location (1.32x105 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 (0-15 cm) than in the subsurface soil and deep soil (15-30 cm and 30-45 cm) across the three wetland as shown in Figs 3.1 to 3.4.

3.3 Microbial Population of the Wetland Sediment in Wet Season

Result of the mean microbial population of the wetland sediment in wet season is presented in Table 3.3. The control location had the highest population of total heterotrophic bacteria ($9.84x10^7$ cfu/g) and fungi ($3.57x10^5$ cfu/g) while Borokiri and Iwofe had the least populations of total heterotrophic bacteria ($2.89x10^7$cfu/g) and fungi ($1.02x10^5$ cfu/g) respectively. There was a significant difference ($p \leq 0.05$) in the heterotrophic bacterial and fungal count of the sediment across the wetlands. Hydrocarbon utilizing bacterial and fungal counts were highest in Iwofe ($4.03x10^5$cfu/g and $1.83x10^4$ cfu/g) and lowest in the control sediment ($1.02x10^5$ cfu/g and $1.20x10^4$ cfu/g). There was no significant difference ($p \geq 0.05$) in the hydrocarbon utilizing bacterial and fungal count in the sediment across the wetlands.

3.4 Microbial Population of the Wetland Sediment in Dry season

Table 3.4 shows the results of the mean microbial population of the wetland sediments in dry season. The control location had the highest population of total heterotrophic bacteria ($15.45x10^7$ cfu/g) and fungi ($2.78x10^7$cfu/g) while Borokiri had the least total heterotrophic bacterial count ($4.28x10^7$ cfu/g) and Eagle Island the least heterotrophic fungal count ($1.78x10^5$cfu/g). There was a significant difference ($p \leq 0.05$) in the total heterotrophic bacterial and fungal count of the sediment across the wetlands in the dry season. Iwofe had the highest population of hydrocarbon utilizing bacteria (HUB) in the dry season ($5.37x10^5$ cfu/g) while the control location had the least population ($1.76x10^5$ cfu/g). There was no significant difference ($p \geq 0.05$) in the hydrocarbon utilizing bacterial count of the sediment across the wetlands in the dry season. Hydrocarbon utilizing fungal (HUF) count was highest in Borokiri sediment ($1.97x10^4$ cfu/g) and least recorded in the control sediment ($1.07x10^4$ cfu/g). There was no significant difference ($p \geq 0.05$) in the hydrocarbon utilizing fungal count of the sediment across the wetlands in the dry season. Generally, the microbial population in the wetland sediment was higher in the dry season than in the rainy season as shown in Figs 3.5 to 3.8.

3.5 Mean Microbial Population of the Wetland Surface Water in Wet Season

Result of the mean microbial population of the wetland surface water in wet season is presented in Table 3.5. The control had the highest population of total heterotrophic bacteria ($8.67x10^7$ cfu/ml) and fungi ($3.42x10^4$ cfu/ml) while Borokiri had the least total bacterial population ($2.57x10^7$ cfu/ml) and Iwofe the least fungal count ($1.72x10^4$ cfu/ml). There was a significant difference ($p \leq 0.05$) in the total heterotrophic bacterial count in the surface water across the wetlands. The same trend followed for total coliform count (TCC), ranging from $1.90x10^4$cfu/ml in Iwofe to $4.23x10^4$cfu/ml in the Control. There was a significant difference ($p \leq 0.05$) in the total coliform count in the surface water across the wetlands. The highest fecal coliform count was recorded in the control location ($2.11x10^4$ cfu/ml) and the least in Iwofe ($1.83x10^4$ cfu/ml). There was a significant difference ($p \leq 0.05$) in the faecal coliform count in the surface water across the wetlands. Hydrocarbon utilizing bacterial count ranged from $1.45x10^4$ cfu/ml in the control location to$1.78x10^4$cfu/ml in Eagle Island. There was no significant difference ($p \geq 0.05$) in the hydrocarbon utilizing bacterial count in the surface water across the wetlands. Borokiri surface water had the highest population of hydrocarbon utilizing fungi $6.38x10^4$ cfu/ml while the control had the least ($3.67x10^4$ cfu/ml). There was no significant difference (p≥0.05) in the hydrocarbon utilizing fungal count in the surface water across the wetlands.

3.6 Mean Microbial Population of the Wetland Surface Water in Dry Season

Table 3.6 presents the results of the mean microbial population of the surface water across the wetlands in the dry season. Total heterotrophic bacterial population ranged from $2.10x10^7$cfu/ml (Borokiri) to $7.34x10^7$ cfu/ml (control**)** while fungal population ranged from $1.27x10^4$ cfu/ml (Borokiri) to $4.21x10^4$ cfu/ml (control). There was a significant difference ($p \leq 0.05$) in the total heterotrophic bacterial and fungal counts in the surface water across the wetlands. The Control also had the highest population of total coliform ($4.02x10^4$ cfu/ml) while Iwofe had the least population ($1.67x10^4$ cfu/ml). There was a significant difference ($p \leq 0.05$) in the total coliform count in the surface water across the wetlands. The highest population of fecal coliform was recorded in the Control location ($2.32x10^4$cfu/ml) and the least population was recorded in Borokiri ($1.47x10^4$ cfu/ml). There was no significant difference ($p \geq 0.05$) in the fecal coliform count in the surface water across the wetlands. Eagle Island surface water had the highest population of hydrocarbon utilizing bacteria ($1.70x10^4$ cfu/ml) and fungi ($1.93x10^4$ cfu/ml) while the control location had the least population of bacteria ($1.01x10^4$ cfu/ml) and fungi ($1.22x10^4$ cfu/ml) recorded.

There was a significant difference ($p \leq 0.05$) in the hydrocarbon utilizing bacterial count in the surface water across the wetlands. Figs 4.9 to 4.12 show the effect of seasonal variation on the microbial population in the surface water across the wetlands.

Table 1: 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 ($p \geq 0.05$)

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

Table 2: 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 ($p \geq 0.05$)

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 Sediments in Rainy/Wet Season

Wetland	THB (x10^{7}CFU/g)	THF (x10^{5}CFU/g)	HUB (x10^{5}CFU/g)	HUF (x10^{4}CFU/g)
Control	9.86±4.45^b	3.57±0.2^a	1.02±0.12^a	1.20±0.11^a
Eagle Island	4.03±1.79^a	1.62±0.09^b	1.92±0.93^a	1.62±0.32^a
Iwofe	8.38±1.73^c	1.02±0.35^c	4.03±2.46^a	1.83±0.26^a
Borokiri	2.89±1.60^b	1.60±0.24^a	1.67±0.96^a	1.65±0.26^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).

Table 4: Mean Microbial Population of the Wetland Sediments in Dry Season

Wetland	THB (X10^{7}cfu/g)	THF (X10^{5}cfu/g)	HUB (X10^{5}cfu/g)	HUF (10^{4}cfu/g)
Control	15.45±4.22^a	2.78±0.56^b	1.76±0.81^a	1.07±0.45^a
Eagle Island	6.21±4.16^a	1.78±0.93^b	2.67±0.59^a	1.67±0.29^a
Iwofe	14.22±10.70^c	1.94±1.32^c	5.37±3.21^a	1.90±0.36^a
Borokiri	4.28±2.13^b	1.90±0.30^a	1.98±0.71^a	1.97±0.23^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).

Table 5: Mean Microbial Population of the Wetland Surface Water in Wet Season

Wetland	THB (X10^{7}cfu/ml)	THF (x10^4 cfu/ml)	TCC (×10^4 cfu/ml)	FCC (×10^4 cfu/ml)	HUB (x10^4 cfu/ml)	HUF (x10^4 cfu/ml)
Control	8.67±1.68^b	3.42±1.65^a	4.23±1.32^a	2.11±0.21^b	1.45±0.34^a	3.67±1.53^a
Eagle Island	7.07±1.56^b	2.18±1.08^a	3.18±0.92^b	1.95±0.77^b	1.78±0.45^a	5.40±2.25^a
Iwofe	2.77±0.70^a	1.72±0.46^a	1.90±0.55^a	1.83±0.59^b	1.75±0.40^a	4.43±2.80^a
Borokiri	2.57±0.59^a	2.03±0.71^a	2.23±0.91^{ab}	1.90±0.37^a	1.72±0.15^a	6.38±4.89^a

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

Keys: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), TCC (Total Coliform Count), FCC (Fecal Coliform Count), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi).

Table 6: Mean Microbial Population of the Wetland Surface Water in Dry Season

Location	THB (x10^7 cfu/ml)	THF (x10^4 cfu/ml)	TCC (×10^4 cfu/ml)	FCC (×10^4 cfu/ml)	HUB (x10^4 cfu/ml)	HUF (x10^4 cfu/ml)
Control	7.34±0.98^b	4.21±0.45^b	4.02±0.56^b	2.32±0.23^a	1.01±0.21^b	1.22±0.02^a
Eagle Island	6.97±0.86^b	3.17±0.35^b	3.00±0.10^b	1.67±0.41^a	1.70±0.36^b	1.93±0.58^a
Iwofe	2.70±0.30^a	1.63±0.11^a	1.67±0.15^a	1.97±0.25^a	1.30±0.40^a	1.63±0.15^a
Borokiri	2.10±0.26^a	1.27±0.23^a	2.20±0.79^a	1.47±0.51^a	1.00±0.20^a	1.47±0.05^a

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

Keys: THB (Total Heterotrophic Bacteria), THF (Total Heterotrophic Fungi), TCC (Total Coliform Count), FCC (Fecal Coliform Count), HUB (Hydrocarbon Utilizing Bacteria), HUF (Hydrocarbon Utilizing Fungi).

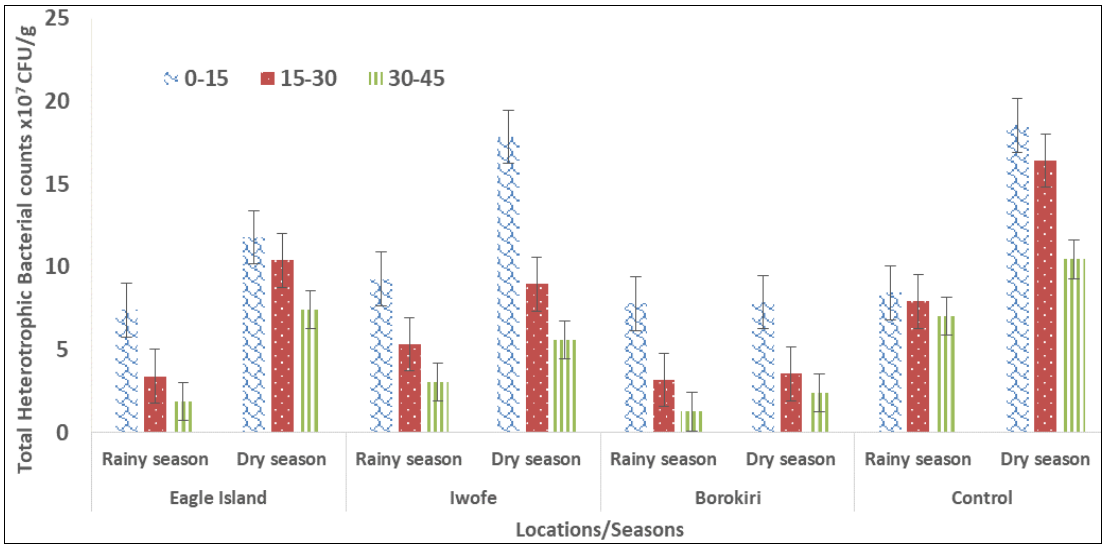


Fig 1: Total heterotrophic bacterial count of soil across the wetlands in wet and dry seasons

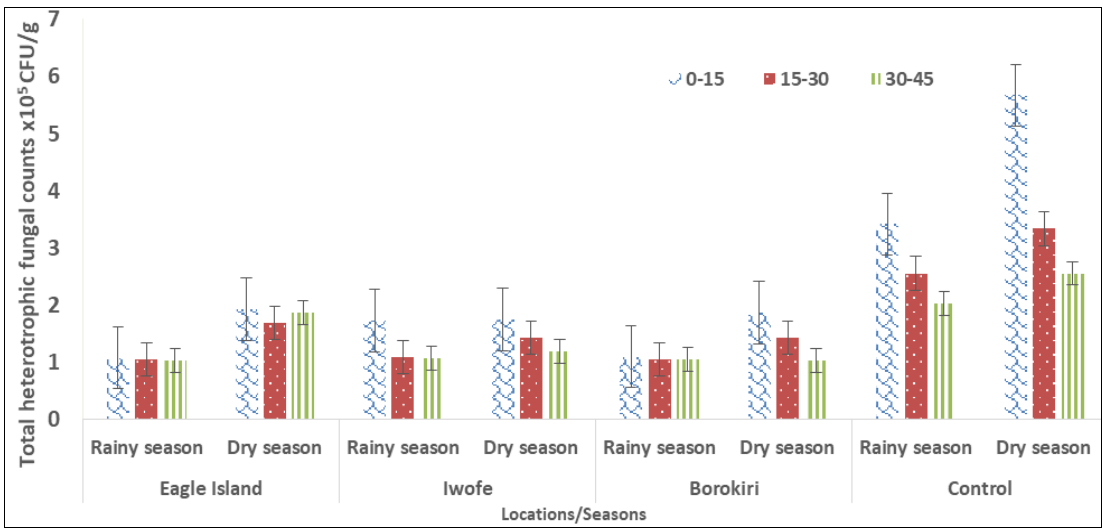


Fig 2: Fungal counts of soil across the wetlands in wet and dry seasons

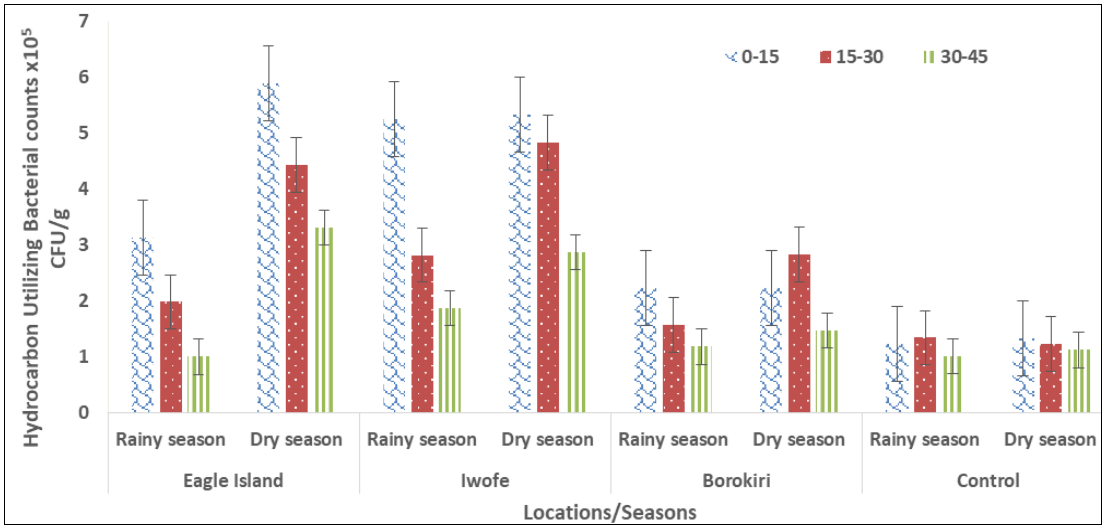


Fig 3: Hydrocarbon utilizing bacterial counts of soil across the different wetlands in wet and dry seasons

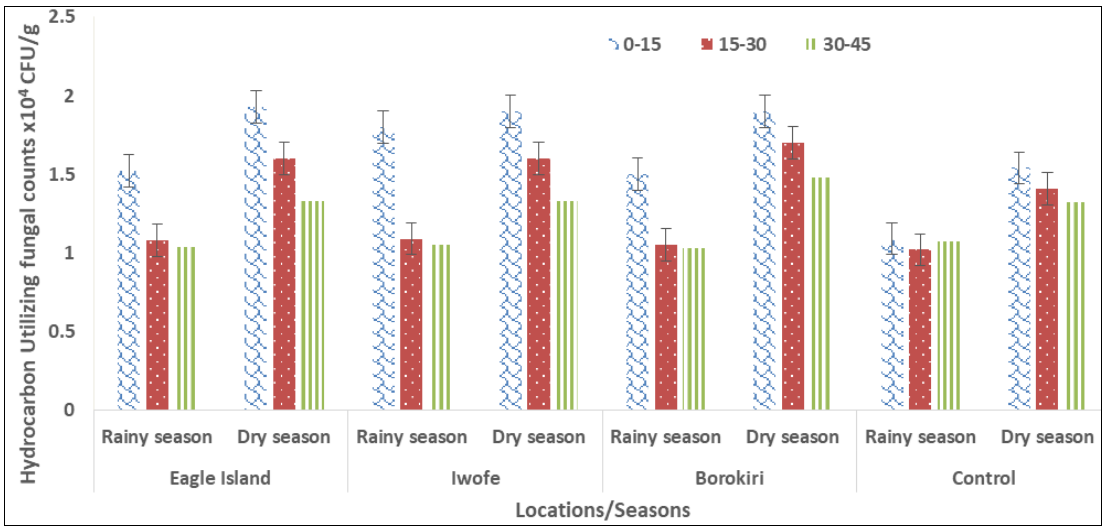


Fig 4: Hydrocarbon utilizing fungal counts of soil across the wetlands in wet and dry seasons

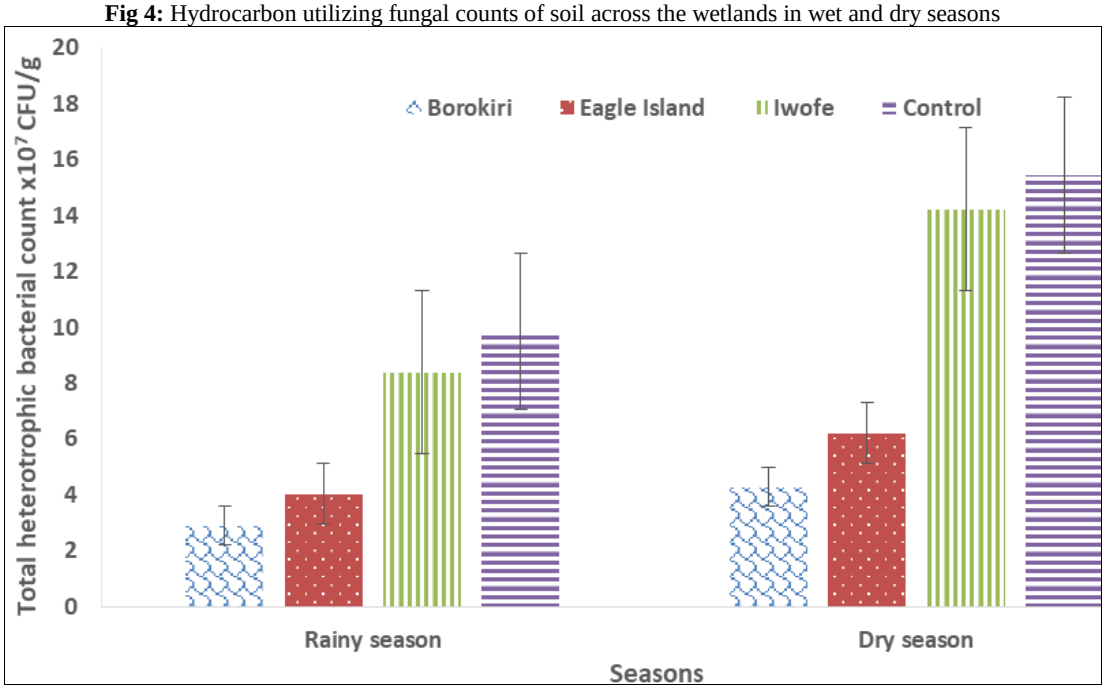


Fig 5: Comparison of the Total Heterotrophic Bacterial Count of the Wetland Sediments in Wet and Dry Season

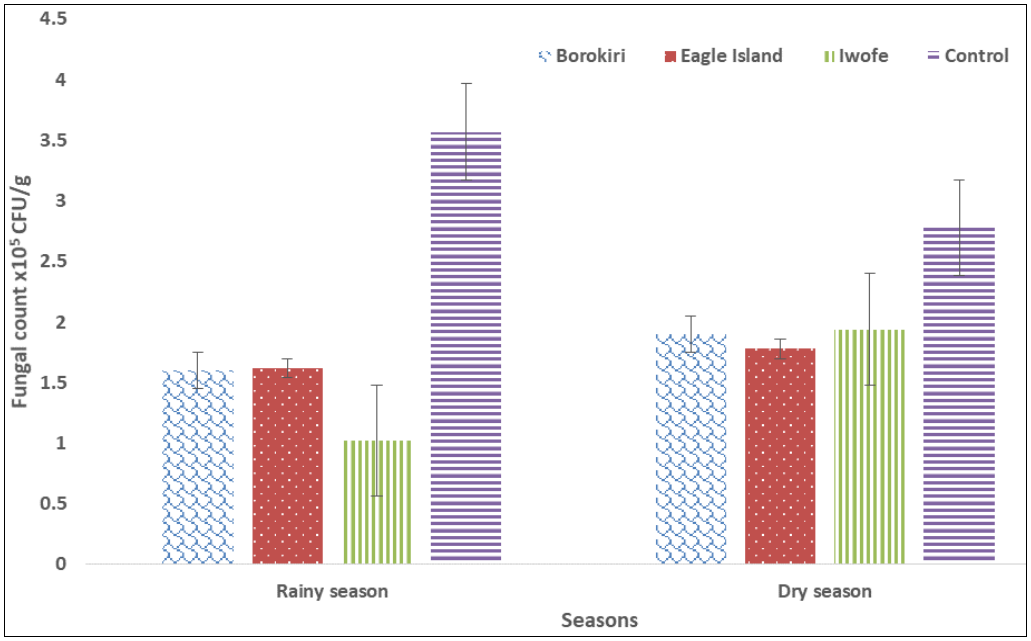


Fig 6: Comparison of the Fungal Count of the Wetland Sediments in Wet and Dry Seasons

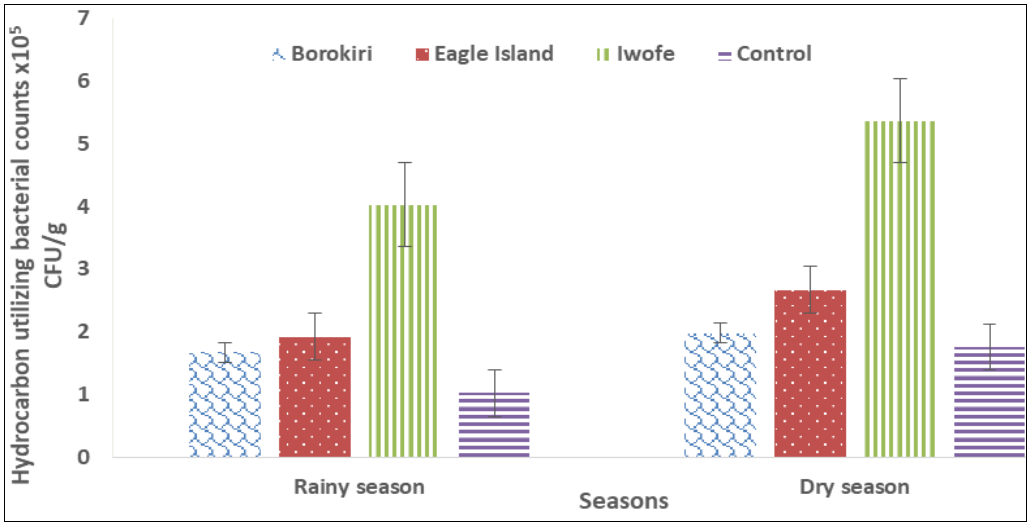


Fig 7: Comparison of the Hydrocarbon Utilizing Bacterial Count of the Wetland Sediments in Wet and Dry Seasons

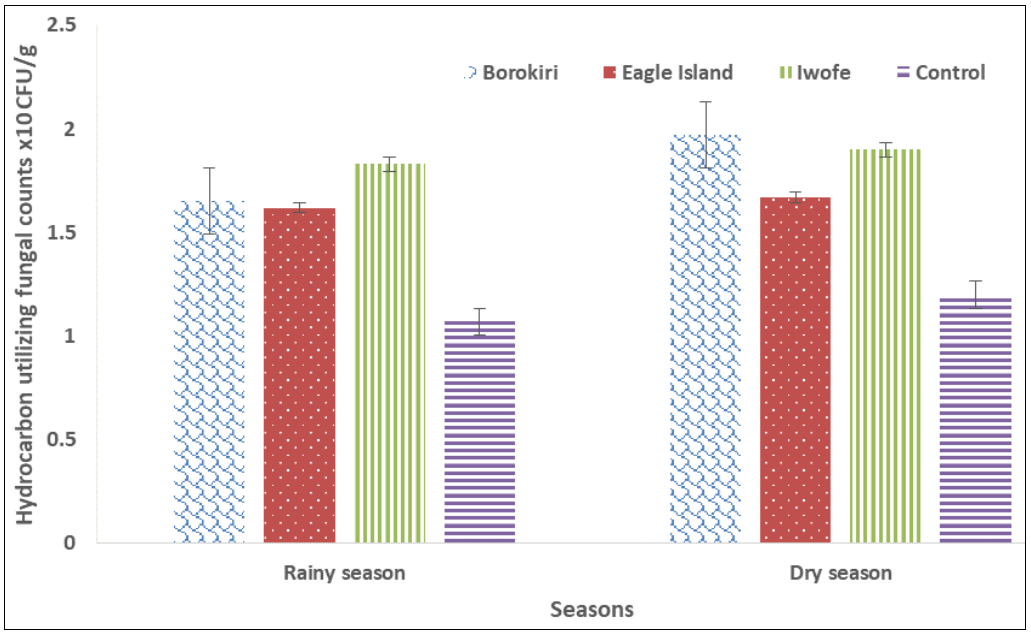


Fig 8: Comparison of the Hydrocarbon Utilizing Fungal Count of the Wetland Sediments in Wet and Dry Seasons

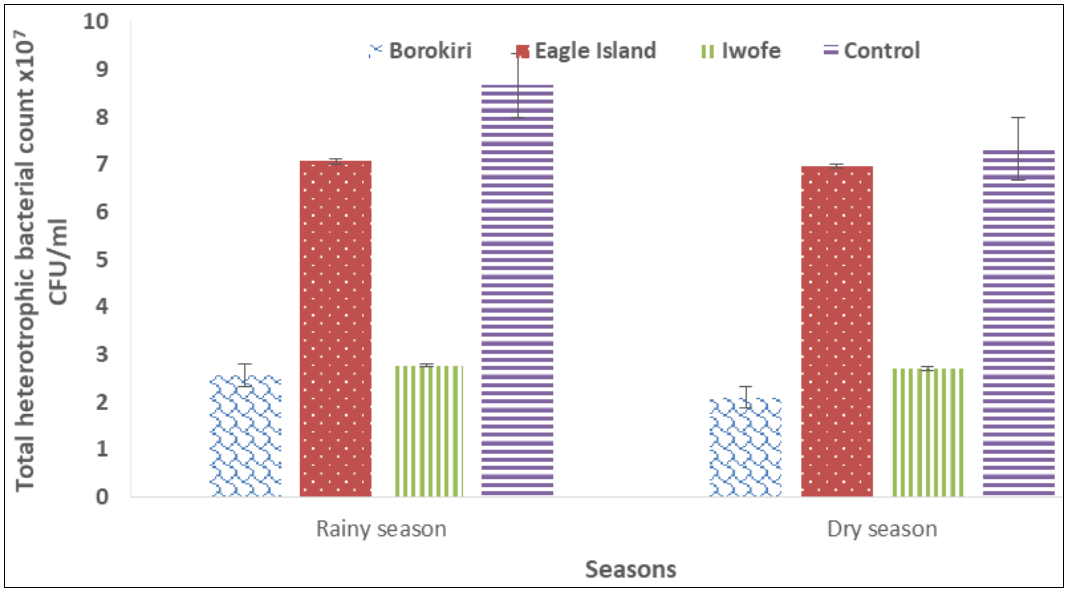


Fig 9: Comparison of the Total Heterotrophic Bacterial Counts of Surface Water Across the Wetlands in Wet and Dry Seasons

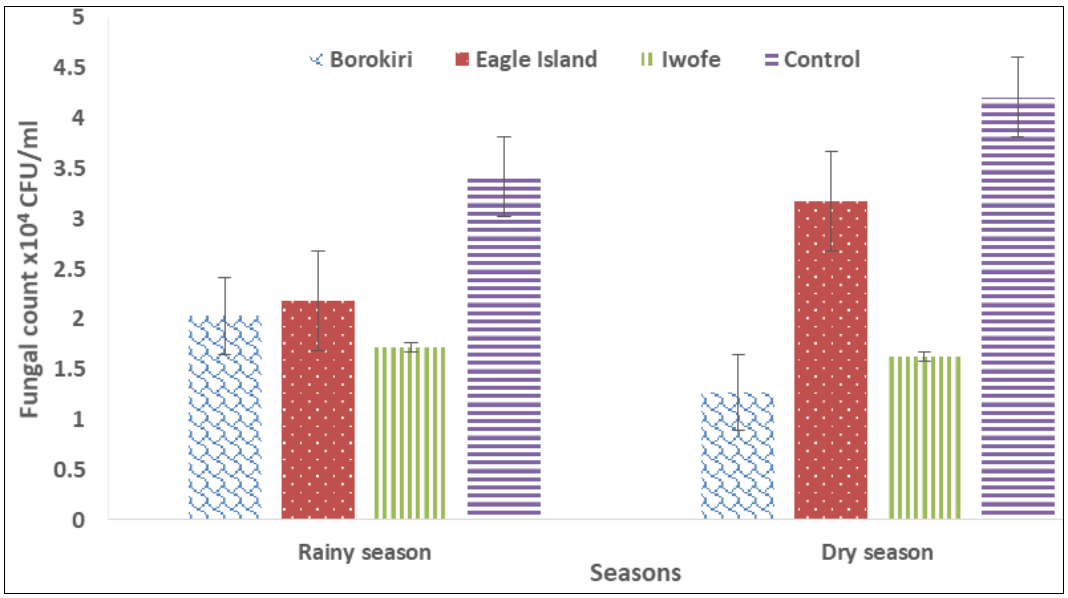


Fig 10: Comparison of the Fungal Counts of Surface Water across the Wetlands in Wet and Dry Seasons

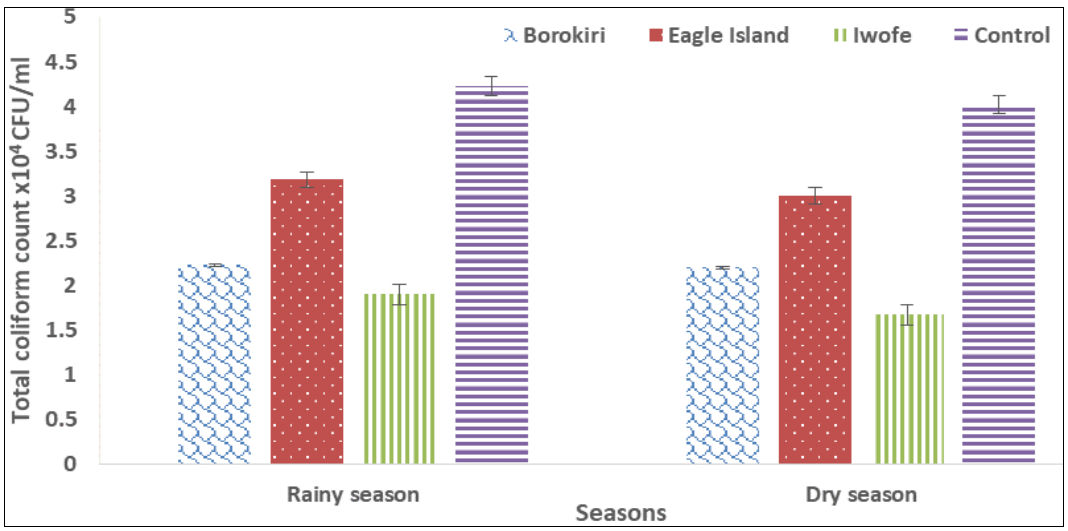


Fig 11: Comparison of the total coliform counts of surface water across the wetlands in wet and dry seasons

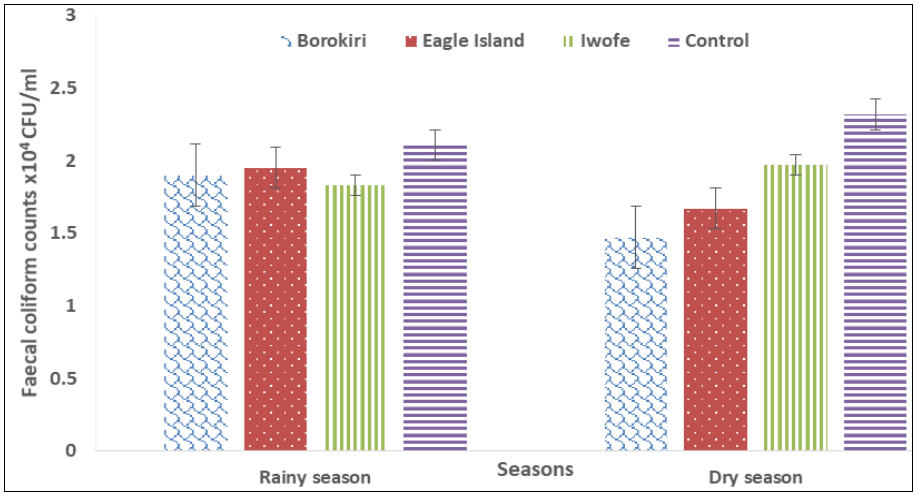


Fig 12: Faecal coliform counts of surface water across the different wetlands in wet and dry seasons

3.7 Total Petroleum Hydrocarbon Content of the Wetlands in Wet and Dry Seasons

The total petroleum hydrocarbon content of the wetland samples (soil, sediment and surface water) in wet and dry seasons is presented in Figs 3.13 and 3.14. In both seasons, the soil sample had the highest TPH content followed by the sediment and the surface water.

3.8 Polycyclic Aromatic Hydrocarbon Content of the Wetlands in Wet and Dry Seasons

The total petroleum hydrocarbon content of the wetland samples (soil, sediment and surface water) in wet and dry seasons is presented in Figs 3.15 and 3.16. In both seasons, the soil sample had the highest TPH content followed by the surface water and sediment.

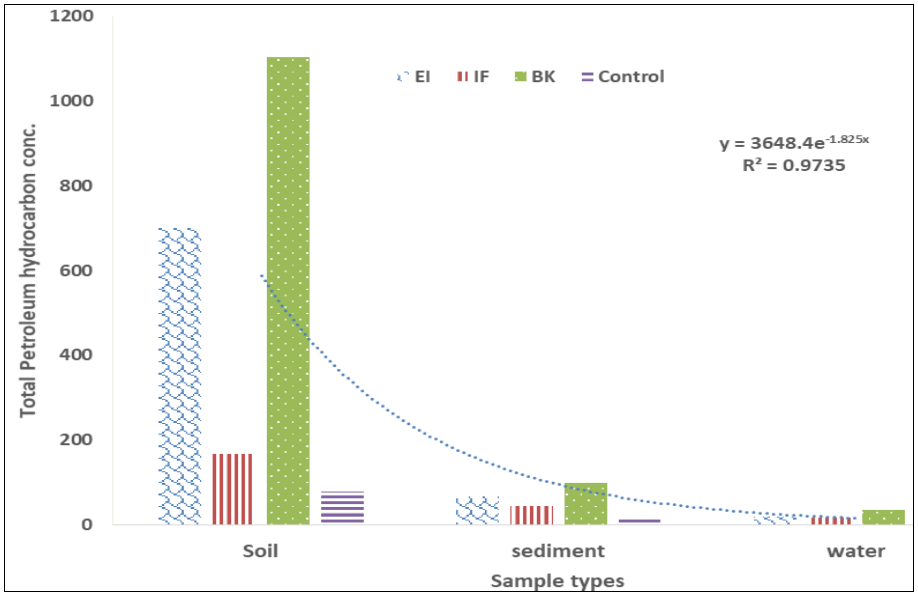


Fig 13: Total Petroleum Hydrocarbon Content of the Samples in Wet Season

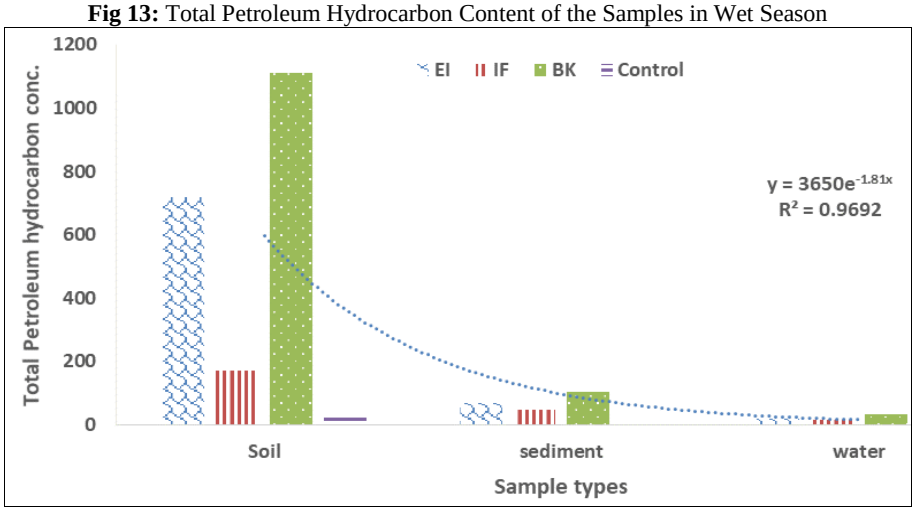


Fig 14: Total Petroleum Hydrocarbon Content of the Samples in Dry Season

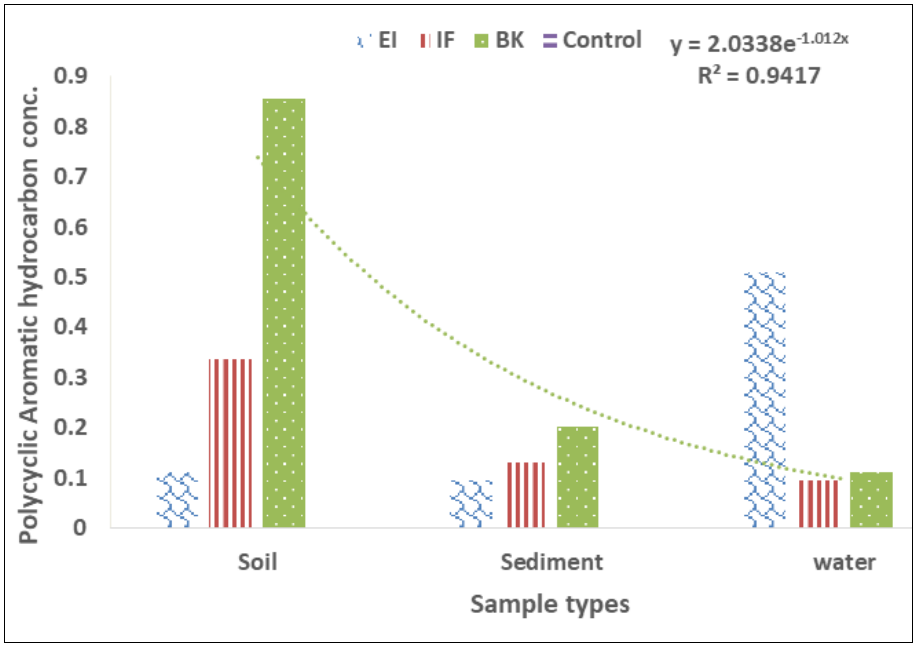


Fig 15: Polycyclic Aromatic Hydrocarbon Content of the Samples in Wet Season

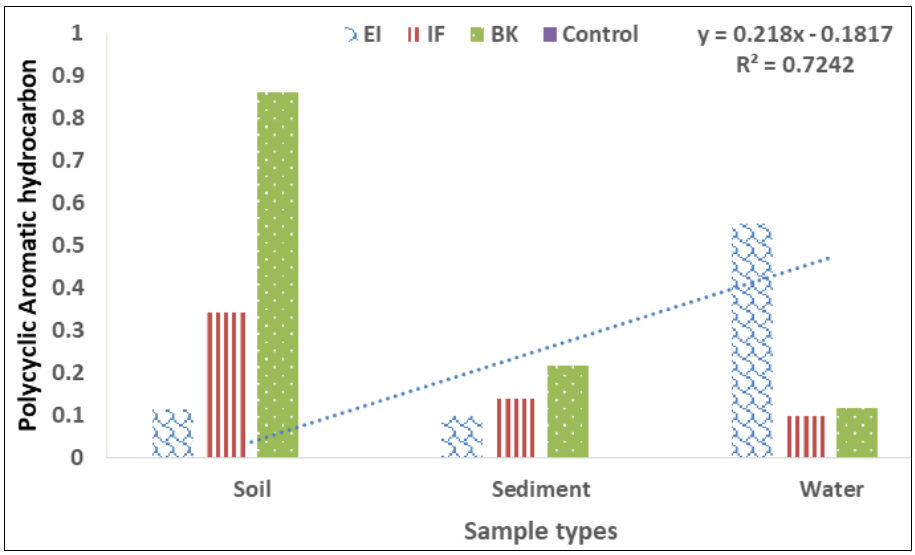


Fig 16: Polycyclic Aromatic Hydrocarbon Content of the Samples in Dry Season

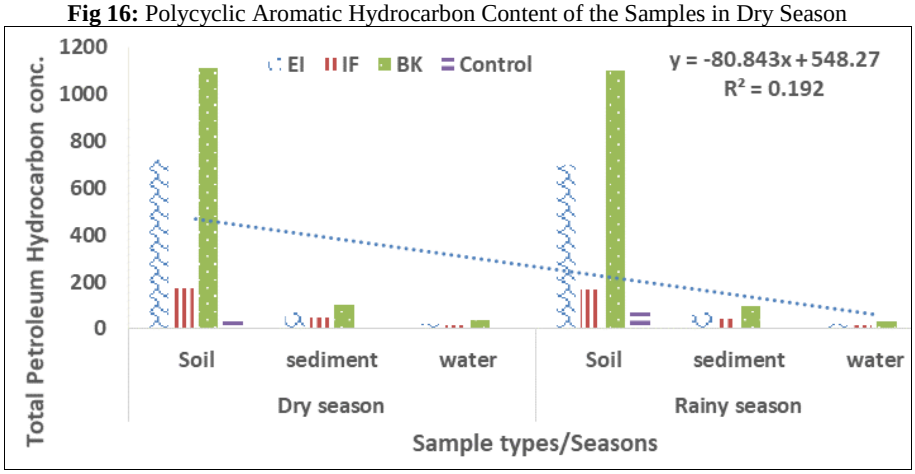


Fig 17: Comparing the total petroleum hydrocarbon content of the Samples in Wet and Dry Seasons

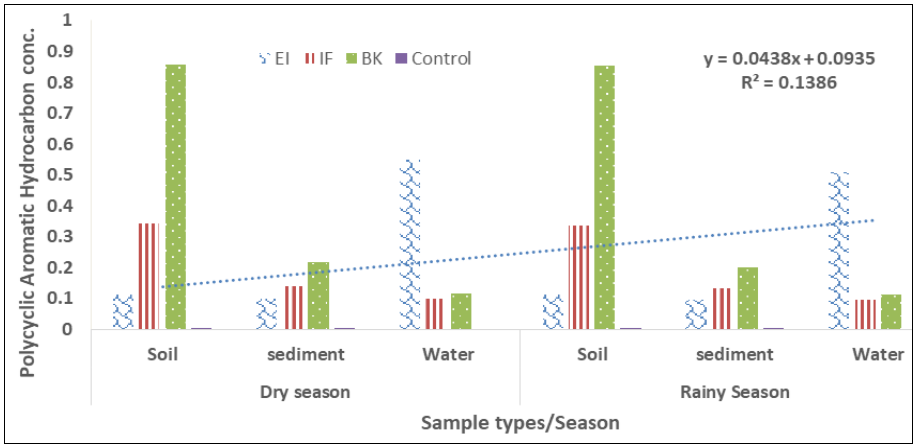


Fig 18: Comparing the polycyclic aromatic hydrocarbon content of the samples in wet and dry Seasons

4. Discussion

This study provided information on the effect of seasonal variation on the microbiology of three Coastal Wetlands (Iwofe, Eagle Island and Borokiri) 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 wetland soil, sediment and surface water. As observed in the study, significantly higher counts were observed during the dry season than the rainy seasons in the wetland soil and sediment and this conformed to a similar study carried out by Unimke *et al.* [30]. This could be due to more favourable environmental conditions such as temperature and oxygen levels in the dry season. Seasonal variation however, had little effect on the microbial population of the surface water. This study also showed that microorganisms in wetland soils decrease with an increase in soil depth which is in agreement with the Udotong *et al.* [31]. 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 subtilis*, *B. rigui*, *B. flexus*, *Lysinibacillus macroides*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, and *Proteus penneri*. The fungal isolates were of the genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus*. These isolates have the ability to utilize crude oil as their sole source of carbon and energy and the dominance of these organisms have been reported by different researchers as crude oil degraders [32, 33]. *Bacillus species* was the most occurring bacterial species at the three soil depths in all the wetland locations studied while *Penicillium species* and *Aspergillus species* were the most occurring fungal species. Assessment of the microbial population of the surface water in both seasons indicated very high microbial load of bacteria that are of public health importance such as *Escherichia coli*, *Salmonella enterica*, *Shigella flexneri* and *Vibrio parahaemolyticus.* Fungi that are known for producing aflatoxin were also isolated from the surface water. These microorganisms will continue to proliferate and decrease the shelf life of fishes and sea foods particularly when they die due to asphyxiation from the oil spill. Sea foods from these waters can serve as a point of conveying microbial infection to man. Fishes and sea foods gotten from these wetland surface waters are gradually being deprived of oxygen, as a result of the excess spill of petroleum which will result in suffocation causing their death in the river and on the wetland sediment. Oil spillage on wetlands could also introduce various concentrations of heavy metals, total petroleum hydrocarbons and polycyclic aromatic hydrocarbons causing death of fish and sea food. Crude oil spills on the surface water may result in elevation of the total petroleum hydrocarbons and poly aromatic hydrocarbon levels in aquatic life found in the water and the water. Consequently, consumption of sea foods and fishes from such sources may pose threat to human health. The study revealed a decrease in total heterotrophic bacterial and fungal count with increasing total petroleum hydrocarbon (TPH) content in the samples. The wetland with the highest TPH (Borokiri) had the least microbial population while the wetland with the least TPH had the highest microbial population (Iwofe). 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.

5. Conclusion

Isolation and identification of microorganisms from the wetland samples indicated an abundance of hydrocarbon utilizing bacteria, thus, indicating that the isolates possess the functional ability for hydrocarbon degradation and heavy metal transformation. These wetlands have been severely contaminated by crude oil, as shown by the high quantity of petroleum hydrocarbons recovered from the soil, sediment, and surface water. Such circumstances typically imply severe environmental degradation and ecological imbalance, which can lead to serious health problems in local residents like leukaemia, brain damage, mental deficiency, stunted growth, especially in children, anaemia, lungs and kidney cancer, liver necrosis, nephritis, and even death. As a result of the increased industrial and anthropogenic activities around the area, there is a consequent release of millions of toxic and recalcitrant chemicals into the wetland soil, sediment and surface water environment. These activities however, release polycyclic aromatic hydrocarbons and heavy metals (among many others), thus, causing serious health problems to humans, marine/aquatic animals and pollute the environment due to their recalcitrant and bio-accumulative nature.

Conflict of Interest

Not available

Financial Support
Not available

References

1. Keddy PA. Wetland Ecosystem: principles and conservation (2nd ed.). NewYork: Cambridge University Press; c2010. p. 497.
2. Fagorite VI, Odundun OA, Iwueke LE, Nwaigbo UN, Okeke OC. Wetlands; A review of their classification, significance and management for sustainable development. International Journal of Advanced Academic research. 2019;5(3):45-52.
3. Bassi N, Kumar MD, Sharma A, Pardha-Saradhi P. Status of wetlands in India: A review of extent, ecosystem benefits, threats and management strategies. Journal of Hydrology: Regional Studies. 2014;2:1-19.
4. Garg JK. Wetland assessment, monitoring and management in India using geospatial techniques. Journal of Environmental Management. 2015;148:112-123.
5. Mamat A, Zhang Z, Mamat Z, Zhang F, Yinguang C. Pollution assessment and health risk evaluation of eight (Metalloid) heavy metals in farmland soil of 146 cities in China. Environmental Geochemistry and Health. 2020;42(11):3949-3963.
6. Liu H, Wu G. Heavy metal contamination and ecological risk assessments in the sediments and zoobenthos of selected mangrove ecosystems, South China, Catena; c2009. p. 136–142.
7. Pezeshki SR, Hester MW, Lin Q, Nyman JA. The effects of oil spill and clean-up on dominant US Gulf coast marsh macrophytes: A review. Journal of Environmental Pollution; c2000. p. 129-139.
8. Vinas M, Sabate J, Espuny JM, Solanas MA. Bacterial Community Dynamics and Polycyclic Aromatic Hydrocarbon degradation during bioremediation of Heavily Creosote-Contaminated Soil. Applied and Environmental Microbiology. 2005;71(11):7008-7018.
9. Linden O, Palsson J. Oil Contamination in Ogoniland, Niger Delta. AMBIO. 2013;42:685-701.
10. Rahman KS, Rahman TJ, Lakshmanaperumalsamy P, Banat IM. Towards Efficient Crude Oil Degradation by a Mixed Bacterial Consortium. Journal of Bioresource Technology. 2002;85(3):257-261.
11. Benson NU, Udosen ED, Akpabio O. Inter-seasonal distribution and partitioning of heavy metals in subtidal sediment of Qua Iboe Estuary and associated Creeks, Niger Delta (Nigeria), Environmental Monitoring and Assessment. 2008;1(3):253-265.
12. Osuji LC, Onojake CM. Field reconnaissance and estimation of petroleum hydrocarbon and heavy metal contents of soils affected by the Ebocha-8 oil spillage in Niger Delta, Nigeria, Journal of Environmental Management. 2006;3(4):133-139.
13. Pepper IL, Gerba CP. Environmental sample collection and processing. In Environmental microbiology. Academic Press; c2015.p. 157-159.
14. Ugochukwu UC, Onuora OH, Onuorah AL. Water quality evaluation of Ekulu River using water quality index (WQI). Journal of Environmental Studies. 2019;4(1):4-12.
15. APHA. (American Public Health Association; American Water Works Association (AWWA); Water Environmental Federation (WEF). Standard methods for the examination of water and wastewater, 26th edition; c2012.
16. Foght J, Aislabie J. Enumeration of Soil Microorganisms. Monitoring and Assessing Soil Bioremediation, 2005, 261-280.
17. Prescott LM, Harley JP, Klein DA. Microbiology. 6th edition. McGraw Hill, London; c2005.
18. Pepper IL, Gerba CP. Environmental sample collection and processing. In Environmental microbiology. Academic Press; c2015. p. 157-159.
19. Ogbonna DN, Ideriah TJ. Effect of Abattoir Waste Water on Physico-chemical Characteristics of Soil and Sediment in Southern Nigeria. Journal of Scientific Research and Reports. 2014;3(12):1612-1632.
20. Okerentugba EU, Ezeronye AO. Studies on the effect of abattoir and industrial effluents on the heavy metals and microbial quality of Aba River Nigeria. African Journal of Biotechnology. 2003;4(3):266-272.
21. Edori OS, Odoemelam SA. Concentration, source apportionment and ring size analysis of polycyclic Aromatic Hydrocarbons (PAHs) in water from Kolo Creek, Niger Delta, Nigeria. Int. J Adv. Chem. Res. 2022;4(1):36-41.
DOI: 10.33545/26646781.2022.v4.i1a.45
22. Mills AL, Breuil C, Colwell. Enumeration of Petroleum degrading marine and estuarine microorganisms by most probable number method. Canadian Journal of Microbiology. 1978;12:234-248.
23. Chikere CB, Ekwuabu CB. Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil-impacted sites in Bodo, Ogoniland, Nigeria. African Journal of Environmental Science and Technology. 2014;8(6):401-406.
24. Cheesebrough M. Preparation of reagents and culture media. District Laboratory Practice in Tropical Countries, 2006, 394-401.
25. Prescott lM, Harley JP, Klein DA. Microbiology, 9th Edition, Mc Graw-Hill, New York; c2011. p. 1014.
26. Amadi EN, Kabir-Klin DB, Kpormon LB, Robinson VK. Microbial flora and nutritional composition of adult palm wine beetle (*Rhynchophorus phoenicis*). International Journal of Current Microbiology and Applied Sciences. 2014;3(11):189-192.
27. Chikere CB, Okpokwasili GC, Chikere BO. Bacterial diversity in a tropical crude oil-polluted soil undergoing bioremediation. African journal of biotechnology. 2009;8(11):53-60.
28. Massoud MS, Al-Abdah F, Al-Ghadban AN, Al-Sarawi M. Bottom sediments of the Arabian Gulf11 TPH and TOC contents as indicators of oil [pollution and implications for the effect and fate of the Kuwait oil slick. Environmental Pollution. 1996;92:270-284.
29. Aigberua OA, Okumoko PD. Distribution, spread and environmental risk status of Pb, Cd and Cr in soils of an open-air waste dumpsite along Tombia/Amassoma road in Yenagoa metropolis. Journal of Plant and Animal Ecology. 2019;9(7):32-54.
30. Bewick V, Cheek L, Ball J. Statistics review 12: survival analysis. Critical care. 2004;8:1-6.
31. Unimke AA, Mmuoegbulam AO, Bassey IU, Obot SE. Assessment of the Microbial Diversity of Spent-Oil Contaminated Soil in Calabar, Nigeria. Journal of Advances in microbiology. 2017;4(4):1-9.

32. Udotong IR, Ofonime UM, Udotong JR. Microbiological and Physicochemical Studies of Wetland Soils in Eket, Nigeria. International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering. 2008;8(2):346.
33. Chikere BO, Okpokwasili GC. Frequency of occurrence of microorganisms at a petrochemical out fall. Journal of Tropical Biosciences. 2004;4:12-18.
34. Olukunle OF, Boboye B. The molecular succession of bacterial community of crude oil polluted soil and water samples from the Niger Delta Nigeria. British Journal of Applied Science and Technology. 2013;3(4):777-781.
35. Williams JO, Dimbu P. Effect of Abbatoir waste water on soil microbial communities. Scholars Academic Journal of Biosciences. 2015;5:452-455.

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