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Characterization of culturable microbial community in oil contaminated soils in Greater Port Harcourt Area, Nigeria

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The study investigated microbial population dynamics in soils of Greater Port Harcourt Area, Nigeria. The study involved the quantification of petroleum pollutants and the enumeration and characterization of culturable fungi and bacteria. Gas Chromatograph FID method was used to quantify total petroleum hydrocarbons (TPH) in the soil while morphological and biochemical tests were used to characterize the microorganisms. The highest TPH was 9,759.37±883.36 ppm which exceeded the Department of Petroleum Resources' (DPRs') intervention value of 5,000 ppm. The percentage of hydrocarbon utilizing fungi (% HUF) ranged between 1.93±0.57 and 66.55±5.60 which were observed in sites A1 (Aluu) and CA (Agricultural control), respectively. The percentage of hydrocarbon utilizing bacteria (% HUB) ranged between 0.12±0.01 and 7.89±1.25 which was observed in sites A3 (Emuoha) and CU (Urban control), respectively. The most prevalent bacterial species includes *Bacillus subtilis, Micrococcus lylae, Staphylococcus aureus, Bacillus cereus* and *Alcaligen faecalis*. The most prevalent HUB isolate was *Bacillus subtilis.* The most prevalent fungal isolates were *Aspergillus niger, Aspergillus flavus* and *Candida torulopsis*. *A. niger* was the most prevalent among the HUF isolates which was identified in 12 sites. It was concluded that the study sites harboured TPH degrading microorganisms that are appropriate for bioremediation of TPH polluted sites.

Key words: Total petroleum hydrocarbons, soil pollution, biodiversity, fungi, bacteria.

INTRODUCTION

Rapid population growth and an immense industrial revolution, even though beneficial in the civilization of human living standards, have jeopardized the state of the environment (Zhao et al., 2017; Jacob et al., 2018; Liu et al., 2019) by introducing a variety of toxic substances such as total petroleum hydrocarbons (TPH), polycyclic

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aromatic hydrocarbons (PAHs), pesticides, heavy metals, synthetic pigments, and polychlorinated biphenyls (PCBs) (Bilal et al., 2017; Barrios-Estrada et al., 2018). In Nigeria, the Niger Delta region is a major centre of activities of oil mining and its' associated industrial sectors. This oil-rich region accounts for more than three quarters of Nigeria's total annual revenue. This industrial growth has led to environmental degradation and left vast footprints of hydrocarbons in the environment (Lindén and Pålsson, 2013). Exploration of oil in Nigeria started in the 1950s and large processing facilities were built to harvest this mineral resource. The eruption of oil spills is on the rise due to increased exploration and insufficient environmental management strategies and this has led to the accumulation of total petroleum hydrocarbons (TPH) over time in sensitive natural habitats. Such chemical spills have intensified, contaminating soils not just in industrial areas but also in the agricultural areas. The consequences of pollution have left undesirable environmental and socio-economic issues leading to loss of ecological resources, poverty and public health concerns (UNEP, 2011; Nkonya et al., 2016; Wali et al., 2019). Further, projections show that the global population may exceed 9 billion by 2050 and that agricultural production would have to rise by 70 to 100% to support the growing population (National Geographic Society, 2020). Yield improvements cannot be accomplished unless the ecosystem is controlled to protect the integrity of the soil ecosystem.

Microorganisms are ubiquitous and are an integral part of the environment since they play a vital role in maintaining processes including biogeochemical cycles in the ecosystem. Soil microorganisms' biogeography is essentially distinct from their counterparts found in animals and plants, and is thus still poorly understood (Whitman et al., 1998). It is vital to assess the impact of anthropogenic activities on the structure of the soil microorganism community in order to provide a basis for reference to the positive and negative impacts that may occur in soils. Microbial diversity of soil must be maintained at its optimum level in order to achieve long-term agricultural productivity. Also, knowledge of soil quality is important for the effective management of farms as it provides baseline data on strategies to maintain and improve soil fertility (Zhou et al., 2014). Soil microorganisms' metabolic activities are mainly driven by temperature and physicochemical parameters (Yang et al., 2020). Bacteria containing nirK, nirS, and nosZ-I genes often have a unique composition in farmland soils as compared to wetland soils, with nirK and nirS being particularly distinct from those containing nosZ-I (Bowen et al., 2020). A reduction in soil pH decreases the abundance of genes and changes the composition of nirK and nirS in agricultural and wetland soil, and raises the ratio of N_2O: (N_2+N_2O) in agricultural soils (Bowen et al., 2020). Agricultural practices have a significant influence on chemical and microbiological soil parameters affecting soil fertility (Bowen et al., 2020).

The patterns of microorganisms in soil polluted with petroleum products vary depending on the chemical composition of the soil and the type of petroleum products. *Escherichia coli, Pseudomonas* species*, Bacillus* species, *Proteus* species, and *Penicillin* species, have been identified to exist in soil contaminated with cyanide (Eze and Onyilide, 2015). The analysis shows the presence of microorganisms in soils contaminated with cyanide at a concentration of 3.0 mg/kg, showing that microorganisms can survive in cyanide contaminated habitats. Soil pollution affects the population and diversity of soil microorganisms. Microbial diversity is declining with an increase in contamination of the environment (Xie et al., 2016). The presence of low levels of microorganisms is related to increased intoxication of cadmium in soils (Xie et al., 2016). Exposure of microorganisms to the concentration of pollutants in soil is therefore causative to the development of adaptive characteristics among the various species found in contaminated soils. Acquisition of new genes that are responsible for resistance for toxicants is an option for microorganisms in the environment. Heavy metal contaminated soil in the marketplaces (Uyo, Umuabia, Sokoto and Oka) in Nigeria has been shown to influence the diversity and distribution of soil microorganisms (Akpoveta et al., 2010; Ogbemudia and Mbong, 2013; Eze et al., 2013; Imarhiagbe et al., 2017). Furthermore, the growth of microalgae (*Microcystis flos-aquae*) in crude oil contaminated media show an exponential growth and reduction of crude oil in the media, an indication of the potential of microorganisms for oil degradation in polluted environments (Ifeanyi and Ogbulie, 2016), and adaptation by shifts in microbial populations, species richness and diversity, thus the role played by microorganisms is diverse. The use of oil spills in Calabar Cross River State in Nigeria has been shown to influence the distribution of microorganisms in soil (Unimke et al., 2017). Some heterotrophic bacteria isolated from these soils included: *Pseudomonas* spp*., Bacillus* spp*., Klebsiella* species*, Proteus* spp*., Enterococcus faecalis* and *Flavobacterium* species (Unimke et al., 2017). The total hydrocarbon utilizing bacteria (THB) include *Bacillus* spp*.,* *Pseudomonas* spp., and *Micrococcus* species (Unimke et al., 2017). Highly prevalent genera were *Pseudomonas* spp., and *Bacillus* spp., indicating that oil degradation microbes are more abundant in oil contamination areas (Unimke et al., 2017). *Arthrobacter* species, strain YC-RL1, could use bisphenol A (BPA) as a carbon source to grow in contaminated soil (Ren et al., 2016). Sourced from soils that were contaminated with crude oil, *Planococcus maritimus* Isolate Y42 was able to use crude oil as its sole source of energy carbon (Yang et al., 2018). *Pseudomonas, Rhizobium, Rhodococcus, Sphingomonas, Enterobacter, Acinetobacter, Bacillus, Paenibacillus,* and *Variovorax* species were found in

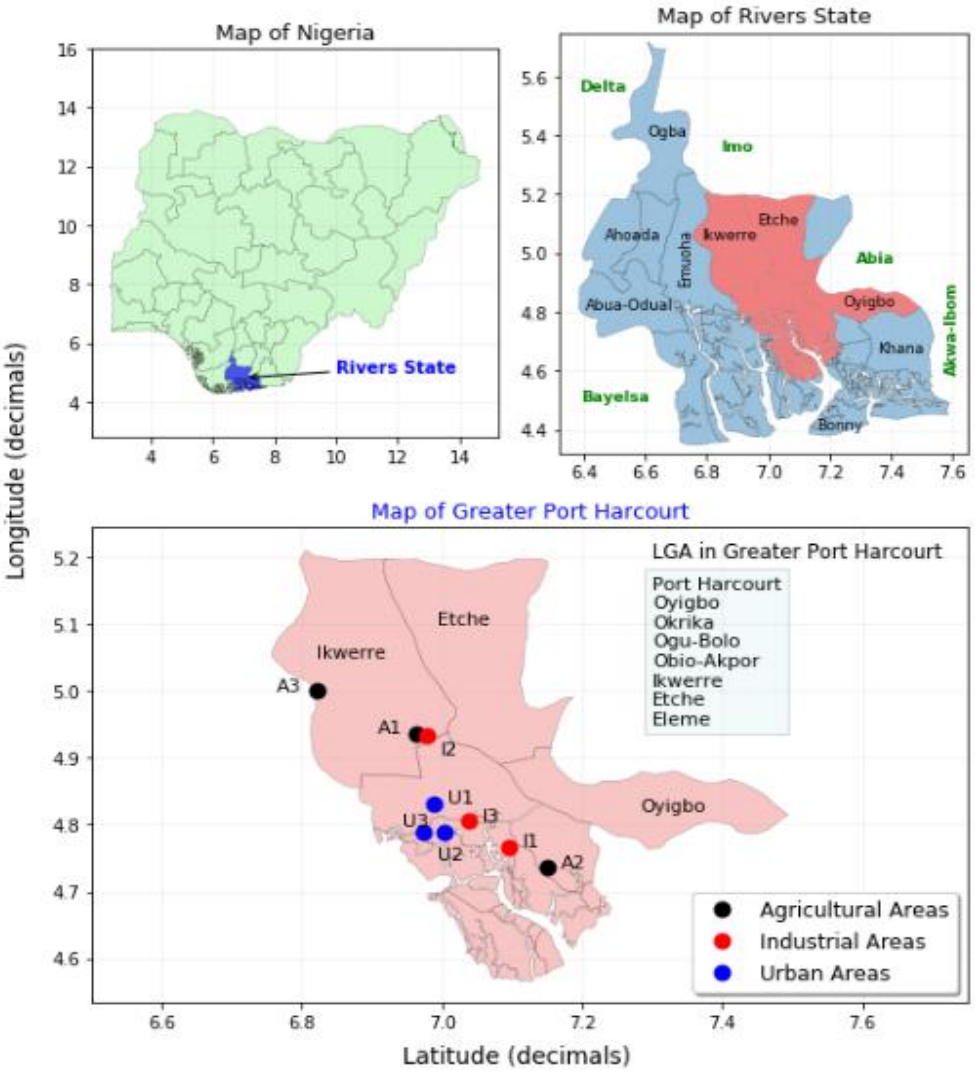

Figure 1. Location of sampling sites in selected areas in Greater Port Harcourt Area, Rivers State, Nigeria. The different dot colours show different economic activities (Black is agriculture, red is industry and blue is urban).

various petroleum contaminated soils and had high biodegradability on alkane mixtures with diverse lengths of the carbon chain ranging between C9 to C30 (Zheng et al., 2018). The microbial diversity in petroleum contaminated soils may be different in soils with similar or different types of contaminants, as most contaminated soils are also polluted by other industrial wastes and chemicals. These studies show that anthropological activities are a threat to soil ecosystem integrity and it is important to periodically monitor the concentration of pollutants in the soil and their effects on soil microorganisms.

The objective of this study was to determine microbial diversity in oil-contaminated soils in three broad sites; urban, industrial and agricultural. Morphological and biochemical experiments have been used to classify a variety of cultivable microbes. The findings of this study could be useful in the production of highly efficient isolates for bioremediation of soils contaminated with petroleum oil.

MATERIALS AND METHODS

Description of the study site

This study was conducted in nine selected test and three control sites in Port Harcourt, the Capital of Rivers State, Nigeria (Figure 1). The study sites were grouped into three areas, including urban (GRA phase 2, Diobu-Mile 1 and Mguoba), industrial (Eleme hosting NNPC Refinery, Agbada-SPDC-flow station) and agricultural (Aluu, Oquwi-Eleme, Emuoha-Eu). The study sites were characterized with different economic activities shown in Table 1.

Table 1. Codes and economic activities of study sites in Greater Port Harcourt Area, Nigeria.

Study site coding	Selected study areas	Coordinates N latitude E Longitude	Characteristic and main activities
Agricultural			
A1	Aluu	4° 56’ 11.160’ 6° 57’ 52.248	Flow station
A2	Eleme	4° 44’ 09.874’ 7° 08’ 58.494’	Village close to refinery
A3	Emuoha	5° 00’ 00.018’ 6° 49’ 13.032’	Flow station
CA	Control	5° 00’ 21.384’ 6° 49’ 00.000’	>1 km away from suspected areas
Industrial			
I1	Onne	4° 46’ 00.402’ 7° 05’ 43.092’	Hosts the NNPC Refinery
I2	Agbada	4° 56’ 03.444’ 6° 58’ 42.060’	Hosts SPDC- flow station in a rural setting
I3	Trans-Amadi	4° 48’ 20.455’ 7° 02’ 17.646’	Schlumberger/, Hallburton
CI	Control	4° 47’ 13.788’ 7° 07’ 44.620’	>1 km away from suspected areas
Urban			
U1	GRA Phase 2	4° 49’ 53.574’ 6° 59’ 45.552’	Inhabited areas Perecuma street
U2	Diobu-Mile 1	4° 47’ 20.382’ 7° 00’ 13.164’	Petroleum refinery
U3	gbuoba	4° 50’ 39.864’ 6° 58’ 20.232’	NTA
CU	Control	4° 49’ 17,040’ 6° 59’ 24.168’	>1 km away from suspected areas

Sampling

In the wet season (April to October 2018), composite samples were collected by random sampling from each of the three areas; urban, industrial, and agricultural. Five (5) samples were collected at random around each test field. The five individual samples were thoroughly mixed in a sterile jar by coning and quartering to achieve a homogeneous composite blend. A total of 12 composite samples; A1, A2, A3, I1, I2, I3 U1, U2 and U3 as test samples, and CA, CI and CU as control samples (Table 1), were collected simultaneously. The samples were obtained at a depth of 0 to 15 cm from the top of the soil using a regular auger three times during the rainy season. Homogenized composite samples (400 g) were then wrapped using a sterile wooden shovel into polyethylene bags. Samples were collected for microbial analysis using pre-sterilized materials to prevent sample contamination. The locations of the sampling sites were determined using the GPS and the measurements were recorded. Samples were taken to the

laboratory in an ice box for examination.

Laboratory analysis

Determination of total petroleum hydrocarbon (TPH) content of soil

The Hewlett Packard 5890 Series II Gas Chromatograph FID method was used. In this method, 1 g of well-mixed sample was weighed into Acetone rinsed beaker. Then, 1 g of anhydrous sodium sulphate was added to the soil sample and 5 ml of solvent (1:1 of dichloromethane and acetone) was added and stirred for 15 min using a magnetic stirrer and the ensuing mixture was poured into a round bottom flask. This was repeated once more by adding 5 ml of mixed solvent. It was stirred and permitted to stand/settle and then decanted into another round bottom flask. The solvent was concentrated with 1 ml hexane to exchange it and it was re-concentrated to 2 ml. The columns were eluted (washed off) with 10 ml n-hexane. 1 ml of the extract was pipetted into the column and 10 ml of n-hexane was used to collect the aliphatic components. The extract was concentrated to 1 ml and poured into a glass vial for Gas Chromatography.

Enumeration of total heterotrophic bacteria (THB)

Heterotrophic bacteria were enumerated by pour plate method (APHA, 1998). One gram of soil sample was weighed into 9 ml sterile diluent (0.85% NaCl) under aseptic condition (laminar bench floor). It was then homogenized using a laboratory vortex mixer (Model: 10101001, IP42) and serially diluted. Then 0.1 ml aliquot of the inoculum was collected using a sterile pipette, inoculated on Nutrient Agar (NA) medium. The inoculum was spread evenly using a sterile glass spreader stick. Plates were then incubated at 37°C for 24 h. Thereafter, colonies were counted and expressed as colony forming units (CFUs/mg of soil) value per gram of soil sample. Distinct colonies with different morphological patterns (color, size, shape, edge, elevation, surface and opacity) were picked and streaked or subculture on freshly prepared nutrient agar medium in order to obtain pure culture after 24 h of incubation at 37°C. The pure cultures were Gram stained for microscopic examination and were further subjected to biochemical tests.

Enumeration of hydrocarbon utilizing bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated by the pour plate method (APHA, 1998) method. 1 g of soil sample was weighed into a 9 ml sterile diluent (0.85% NaCl) under aseptic conditions. The sample was then homogenized using a laboratory vortex mixer (Model: 10101001, IP42) and serially diluted. Then 0.1 ml aliquot of the inoculum was inoculated on Mineral Salt Agar (MSA) medium containing g/l of $MgSO_4.7H_2O$ 0.42 g, KCl 0.29 g, K_2HPO_4 1.25 g, KH_2PO_4 0.83 g, $NaNO_3$ 0.42 g, NaCl 10 g and Agar Powder 18 g, using the spread technique. Sterile filter paper (Whatman 540) was soaked with crude oil and placed in the lid of petri dish. Plates were incubated in inverted position at room temperature for 5 days until there was observable growth. Thereafter, distinct colonies were purified by sub-culturing on a freshly prepared medium and incubated for 24 h, from which microscopic examination and biochemical tests.

Enumeration of total fungi

Total fungi were performed using a pour plate method (APHA, 1998). Under aseptic conditions, one gram of soil sample was weighed in a 9 ml sterile diluent (0.85 per cent NaCl). The sample was then homogenized using a vortex mixer (Model 10101001, IP42) and diluted in series using sterile pipettes. Thereafter, 0.1 ml of the inoculum aliquot was inoculated on Potato Dextrose Agar (PDA) mixed with an antibacterial reagent (Normocure™) to inhibit bacterial growth and allow only fungal growth. Then, the inoculated plates were incubated for 5 to 7 days at ambient temperature. To obtain colony forming unit per gram (CFU/g) of the soil, colonies were enumerated using a colony counter.

Enumeration of hydrocarbon utilizing fungi

Hydrocarbons utilizing fungi (HUF) were cultured using the pour plate method (APHA, 1998). Under aseptic conditions (laminar flow bench), 1 g of soil sample was weighed into a 9 ml sterile diluent (0.85% NaCl). The sample was then homogenized using a laboratory vortex mixer (Model: 10101001, IP42) and serially diluted using sterile pipettes. 0.1 ml aliquot of inoculum was then inoculated on Mineral Salt Agar (MSA) mixed with an antibacterial reagent (Normocure™) in order to inhibit the growth of bacteria and allow for only growth of fungi. Sterile filter paper (Whatman 540) was subsequently soaked with crude oil and put in the petri dish cover. At room temperature, the plates were then incubated in an inverted position for 5 to 7 days. Colonies were counted using a colony counter to get colony forming units per gram of soil. Cultural characteristics (colour and microscopic observations) of the isolates were then observed and purified by sub-culturing on freshly prepared medium and incubated again for 3 to 5 days. From the pure cultures, microscopic examination was done using lactophenol cotton blue stain and observed under ×400 magnification.

Determination of % hydrocarbon utilizing fungi and bacteria

Percent hydrocarbon utilizing fungi and bacteria were expressed as a fraction of the total heterotrophic viable count using the formula:

$$\%\ \text{HUF/HUB} = \frac{Hydrocarbon\ utilizing\ fungi/bacteria}{Total\ heterotrophic\ viable\ count} \times \frac{100}{1}$$

Characterization and identification of THB and TF fungi

The fungal and bacterial isolates were identified morphologically (color, size, shape, edge, elevation, surface and opacity). Further, bacterial isolates were identified biochemically and characterized according to the scheme of Bergey's manual of Determinative Bacteriology (Holt et al., 1994) [This was the locally available method and the characterization was limited to it] using standard procedures: Sucrose fermentation test, Indole test, Citrate utilization test, Catalase test, Oxidase test, Motility test, Methyl red test, Voges-Proskauer test, Triple sugar iron (TSI) agar test, Nitrate reduction test, Starch hydrolysis test, Glucose test and Lactose fermentation test.

RESULTS

Prevalence and characterization of microorganisms

Prevalence and diversity of THB in different sampling sites

Table 2 shows the ranks of prevalence, diversity of THB,

Table 2. Variation in prevalence of THB in soil from agricultural, industrial and urban areas in Greater Port Harcourt Area, Nigeria.

Identified microbe	Site of prevalence												P
Sampling site	**CA**	**A1**	**A2**	**A3**	**CI**	**I1**	**I2**	**I3**	**CU**	**U1**	**U2**	**U3**	
Bacillus subtilis	+	+	+	+	-	+	-	-	+	+	+	+	9
Micrococcus lylae	+	+	+	-	+	-	+	-	+	-	+	+	8
Staphylococcus aureus	+	+	-	+	-	+	-	-	+	+	+	+	8
Bacillus cereus	+	+	-	-	+	-	+	+	-	-	+	-	6
Alcaligen faecalis	-	+	+	+	+	-	+	-	+	-	-	-	6
Micrococcus kristinae	-	-	-	-	+	-	+	+	-	-	+	+	5
Pseudomonas aeruginosa	-	-	-	-	-	+	+	-	+	+	-	-	4
Bacillus thuringens	-	-	+	-	+	-	+	-	-	-	-	-	3
Micrococcus letus	+	-	-	+	-	+	-	-	-	-	-	-	3
Escherichia coli	-	-	-	-	-	-	-	+	-	+	-	-	2
Protues mirabilis	-	-	-	-	+	-	-	-	-	+	-	-	2
Flavobacterium breve	-	-	-	-	-	-	-	-	-	-	-	+	1
Serratia marscencens	-	-	-	-	-	-	-	-	-	+	-	-	1
Isolates per site	5	5	4	4	6	4	6	3	5	6	5	5	58

P stands for prevalence of species.

Table 3. Variation in prevalence of HUB in soil from agricultural, industrial and urbanized areas in parts of Rivers State, Nigeria.

Identified microbe	Site of prevalence												P
Sampling site	**CA**	**A1**	**A2**	**A3**	**CI**	**I1**	**I2**	**I3**	**CU**	**U1**	**U2**	**U3**	
Bacillus subtilis	-	+	+	+	+	-	-	+	+	+	+	+	9
Alcaligen faecalis	-	+	+	+	+	-	+	-	+	-	-	-	6
Bacillus cereus	+	+	-	-	-	+	+	-	-	-	-	-	3
Micrococcus kristinae	-	-	-	-	+	-	+	+	-	-	-	-	3
Micrococcus lylae	+	-	-	-	-	-	-	-	+	+	-	-	3
Bacillus thuringens	-	-	+	-	-	+	-	-	-	-	-	-	2
Flavobacterium breve	-	-	-	-	-	-	-	+	-	-	-	+	2
Pseudomonas aeruginosa	-	-	-	-	-	+	-	-	-	+	-	-	2
Staphylococcus aureus	-	-	-	+	-	-	-	-	-	-	+	-	2
Micrococcus letus	-	-	-	+	-	-	-	-	-	-	-	-	1
Isolates per site	2	3	3	4	3	3	3	3	2	3	2	2	33

P stands for prevalence of species.

microbial population and community composition in the study sites. *Bacillus subtilis* was most prevalent and was identified in 9 sites including CA (agricultural control), A1 (Aluu), A2 (Eleme), A3 (Emuoha), I1 (Onne), CU (urban control), U1 (GRA phase 2), U2 (Diobu-Mile 1), U3 (Mgbuoba). The least prevalent species was *Flavobacterium breve* which was isolated from samples from U3 (Mgbuoba). Agbada (I2) and industrial control (CI) sites showed the highest number (6) of isolates. There was variation in the number of isolates between control and contaminated sites.

Prevalence and diversity of HUB in sampling sites

Table 3 shows the ranks of prevalence and diversity of HUB in the sampling sites. The most prevalent isolate was *B. subtilis* which was prevalent in A1 (Aluu), A2 (Eleme), A3 (Emuoha), CI (industrial control), I3 (Trans-Amadi), CU (Urban control), U1 (GRA Phase 2), U2 (Diobu-Mile 1) and U3 (Mgbuoba). The highest diversity was observed in site A3 which had 4 isolates *B. subtilis, Alcaligen faecalis, Staphylococcus aureus* and *Micrococcus letus*. There was no difference in diversity

Table 4. Variation in prevalence of TF in soil from agricultural, industrial and urban areas in Greater Port Harcourt Area, Nigeria.

Identified microbe	Site of prevalence												P
Sampling sites	CA	A1	A2	A3	CI	I1	I2	I3	CU	U1	U2	U3	
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	12
Aspergillus flavus	-	+	-	+	+	+	-	+	+	-	-	-	6
Candida torulopsis	-	+	-	-	+	-	-	+	-	+	+	-	5
Mucor mucedo.	-	+	+	-	-	-	-	+	+	+	-	-	5
Saccharomyces cerevisiae	-	-	+	-	-	+	+	-	+	+	-	-	5
Paecilomyces spp.	+	-	+	+	-	-	-	-	-	-	-	-	3
Chrysosporium spp.	+	-	-	-	-	-	-	-	-	-	-	+	2
Cladiosporium spp.	-	-	-	-	-	-	+	-	-	-	+	-	2
Geotrichium spp.	-	-	+	-	-	-	-	-	-	-	-	+	2
Penicillium expanum	-	-	-	-	+	+	-	-	-	-	-	-	2
Rhizopus stolonifer	-	-	-	-	-	-	-	-	-	-	+	-	1
Aspergillus fumigatus	+	-	-	-	-	-	-	-	-	-	-	-	1
Isolates per site	4	4	5	3	4	4	3	4	4	4	4	3	46

P stands for prevalence of species.

Table 5. Variation in Prevalence of HUF Isolates in Soil from Agricultural, Industrial and Urban areas in Greater Port Harcourt Area, Nigeria.

Identified microbe	Site of prevalence												P
Sampling sites	CA	A1	A2	A3	CI	I1	I2	I3	CU	U1	U2	U3	
Aspergillus niger	+	+	+	+	+	+	+	+	+	+	+	+	*12*
Aspergillus flavus	-	+	-	-	-	-	-	+	+	-	-	-	*3*
Paecilomyces spp.	+	-	+	+	-	-	-	-	-	-	-	-	*3*
Mucor mucedo.	-	-	+	-	-	-	-	-	+	-	-	-	*2*
Penicillium expanum	-	-	-	-	+	+	-	-	-	-	-	-	*2*
Cladiosporium spp.	-	-	-	-	-	-	+	-	-	-	+	-	*2*
Saccharomyces cerevisiae	-	-	-	-	-	+	+	-	-	-	-	-	*2*
Aspergillus fumigatus	+	-	-	-	-	-	-	-	-	-	-	-	*1*
Candida torulopsis	-	+	-	-	-	-	-	-	-	-	-	-	*1*
Chrysosporium spp.	-	-	-	+	-	-	-	-	-	-	-	-	*1*
Geotrichium spp.	-	-	-	-	-	-	-	-	-	-	-	+	*1*
Rhizopus stolonifer	-	-	-	-	-	-	-	-	-	-	-	-	*0*
Isolates per site	*3*	*3*	*3*	*3*	*2*	*3*	*3*	*2*	*3*	*1*	*2*	*2*	*30*

P stands for prevalence of species.

(3) between all the industrial and the control site; however, there was a difference in diversity between the controls and contaminated sites in urban and agricultural areas.

Prevalence and diversity of TF in sampling sites

Table 4 shows the ranks of prevalence and diversity of TF in the sampling sites. The most prevalent fungal isolate includes *Aspergillus niger, Aspergillus flavus* and *Candida torulopsis* which were prevalent in 12, 6 and 5 sites, respectively (Table 3). The highest diversity was observed in A2 (Eleme) with 5 isolates *A. niger, Mucor mucedo, Saccharomyces cerevisiae, Paecilomyces* species, and *Geotrichium* species (Table 3). The list diversity was observed in A3 (Emuoha), I1 (Onne) and U3 (Mgbuoba) with 3 isolates each.

Prevalence and diversity of HUF in different sampling locations

Table 5 shows the different hydrocarbon utilizing fungi (HUF) that were isolated from the soil samples. *A. niger*

Table 6. Variation of population of THB, HUB, %HUB, TF, HUF and %HUF in soil from agricultural, industrial and urban areas in Greater Port Harcourt Area, Nigeria (Wet season).

Code	THB	HUB	% HUB	TF	HUF	% HUF	TPH (ppm)
A1	$1.30\times10^{6}\pm4.36\times10^{5}$	$1.49\times10^{4}\pm2.31\times10^{2}$	1.22±0.34	$1.45\times10^{5}\pm8.08\times10^{3}$	$2.77\times10^{3}\pm6.81\times10^{2}$	1.93±0.57	3,307.27±125.51
A2	$7.07\times10^{7}\pm4.93\times10^{6}$	$6.83\times10^{5}\pm5.77\times10^{3}$	0.64±0.47	$3.27\times10^{4}\pm2.52\times10^{3}$	$3.70\times10^{3}\pm1.85\times10^{3}$	11.18±4.91	6,198.49±598.56
A3	$5.73\times10^{7}\pm1.15\times10^{6}$	$7.10\times10^{4}\pm4.36\times10^{3}$	0.12±0.01	$8.23\times10^{3}\pm2.52\times10^{2}$	$3.10\times10^{3}\pm2.65\times10^{2}$	37.63±2.62	5,253.17±1,014.27
CA	$6.17\times10^{7}\pm1.53\times10^{6}$	$1.73\times10^{4}\pm4.58\times10^{2}$	0.20±0.15	$6.18\times10^{3}\pm2.25\times10^{2}$	$4.10\times10^{3}\pm2.65\times10^{2}$	66.55±5.60	453.61±233.01
I1	$1.37\times10^{7}\pm1.10\times10^{6}$	$2.47\times10^{4}\pm1.33\times10^{3}$	0.14±0.08	$6.17\times10^{5}\pm4.04\times10^{5}$	$6.48\times10^{3}\pm1.04\times10^{2}$	29.99±42.10	6,543.74±1022.19
I2	$7.43\times10^{6}\pm4.51\times10^{5}$	$1.24\times10^{5}\pm7.51\times10^{3}$	1.16±0.87	$1.32\times10^{4}\pm1.06\times10^{3}$	$2.27\times10^{3}\pm5.03\times10^{2}$	17.39±5.38	4,151.89±688.19
I3	$3.37\times10^{7}\pm5.13\times10^{6}$	$3.97\times10^{4}\pm1.15\times10^{3}$	0.12±0.02	$7.50\times10^{4}\pm1.73\times10^{3}$	$6.67\times10^{3}\pm2.50\times10^{3}$	8.92±3.45	9,759.37±883.36
CI	$7.17\times10^{7}\pm2.52\times10^{6}$	$3.88\times10^{4}\pm3.46\times10^{2}$	0.27±0.23	$5.21\times10^{4}\pm8.08\times10^{2}$	$4.57\times10^{4}\pm3.70\times10^{4}$	6.74±5.96	173.45±18.75
U1	$4.57\times10^{7}\pm4.59\times10^{7}$	$3.67\times10^{3}\pm1.15\times10^{2}$	0.49±0.79	$1.44\times10^{4}\pm5.13\times10^{2}$	$3.40\times10^{3}\pm1.11\times10^{3}$	23.40±6.98	5,908.56±1,252.00
U2	$1.88\times10^{7}\pm8.27\times10^{6}$	$2.88\times10^{4}\pm1.06\times10^{3}$	0.48±0.46	$6.60\times10^{4}\pm2.00\times10^{3}$	$5.20\times10^{3}\pm4.00\times102$	7.87±0.85	2,810.28±530.53
U3	$1.96\times10^{7}\pm2.95\times10^{6}$	$5.27\times10^{4}\pm1.15\times10^{3}$	0.27±0.03	$6.23\times10^{4}\pm2.52\times10^{3}$	$3.45\times10^{4}\pm2.44\times10^{4}$	55.88±40.62	5,566.89±528.33
CU	$3.33\times10^{6}\pm4.16\times10^{5}$	$2.60\times10^{5}\pm2.59\times10^{4}$	7.89±1.25	$7.70\times10^{4}\pm4.00\times10^{3}$	$2.90\times10^{3}\pm2.00\times102$	16.52±22.47	467.05±401.73

Data are Mean ± standard deviations (Significant coefficients = 0.05).

was the most prevalent among the hydrocarbon utilizing fungal isolates which was identified in all the 12 test and control locations. There was no difference (3) in number of isolates between agricultural control site and contaminated sites. There was difference in number of isolates between urban and industrial control sites and contaminated sites (Table 5). The sites with the highest number of isolates were CA (agricultural control), A1 (Aluu), A2 (Eleme), A3 (Emuoha), I1 (Onne), I2 (Agbada) and CU (urban control).

Population and distribution of microorganisms

Population of THB, HUB, %HUB, TF, HUF and *%HUF*

The population and distribution of microorganisms was done in wet season. In the wet season, the % HUB ranged between 0.12±0.01 and 7.89±1.25 which was observed in sites A3 (Emuoha) and CU (urban control) respectively (Table 6). In the dry season, the %HUB ranged between 0.23±0.01 and 4.13±0.86 which were observed in sites A1 (Aluu) and CA (agricultural control) respectively. All sites had values of HUB below 10% in both the wet and the dry seasons. In the wet season, %HUF ranged between 1.93±0.57 and 66.55±5.60 which were observed in A1 (Aluu) and CA (agricultural control) respectively. In the dry season, %HUF ranged between 0.74±0.12 and 13.20±0.75 observed in locations A1 (Aluu) and CA (agricultural control), respectively (Table 6). All values of %HUF in the dry season were below the threshold value of 10%. The %HUF in A2 (Eleme), A3 (Emuoha), CA (agricultural control), CU (urban control), I1 (Onne), I2 (Agbada), U1 (GRA Phase 2) and U3 (Mgbuoba) were above the 10% threshold value in the wet season (Table 7).

DISCUSSION

There are differences in responses and distribution of fungi and bacteria in soils polluted with different contaminants. The difference in response of microorganisms to concentrations of different pollutants between fungi and bacteria can be attributed to difference in strategies of evasion or accommodation of pollutants in the environment (Zanardo et al., 2018). For example, similarity in bacteria richness and diversity in Pb contaminated sites while in non-contaminated sites show difference in fungal richness and diversity (Zanardo et al., 2018). The present study showed that %HUB was lower as related to %HUF. The discrepancy in distribution between fungi and bacterial richness and diversity can be attributed to their different strategies to avoid or tolerate high concentrations of pollutants (Zanardo et al., 2018). *B. subtilis* and *Alkaligen faecalis* were the

Table 7. Variation of population of THB, HUB, %HUB, TF, HUF and %HUF in soil from agricultural, industrial and urban areas in Greater Port Harcourt Area, Nigeria (Dry season).

Code	THB	HUB	% HUB	TF	HUF	% HUF	TPH (ppm)
A1	$6.87\times10^{6}\pm7.02\times10^{5}$	$1.57\times10^{4}\pm2.08\times10^{3}$	0.23±0.01	$2.93\times10^{4}\pm4.51\times10^{3}$	$2.20\times10^{2}\pm7.00\times10^{1}$	0.74±0.12	3,307.27±125.51
A2	$6.13\times10^{6}\pm1.10\times10^{6}$	$3.47\times10^{4}\pm1.53\times10^{3}$	0.58±0.12	$3.33\times10^{3}\pm1.53\times10^{2}$	$3.67\times10^{2}\pm3.51\times10^{1}$	11.00±0.87	6,198.49±598.56
A3	$6.50\times10^{6}\pm3.27\times10^{6}$	$2.20\times10^{4}\pm2.00\times10^{3}$	0.45±0.33	$4.70\times10^{3}\pm2.00\times10^{2}$	$2.77\times10^{2}\pm2.52\times10^{1}$	5.88±0.44	5,253.17±1,014.27
CA	$4.00\times10^{5}\pm3.61\times10^{4}$	$1.67\times10^{4}\pm4.73\times10^{3}$	4.13±0.86	$3.00\times10^{3}\pm2.00\times10^{2}$	$3.97\times10^{2}\pm4.73\times10^{1}$	13.20±0.75	453.61±233.01
I1	$6.37\times10^{6}\pm4.04\times10^{5}$	$2.47\times10^{4}\pm1.53\times10^{3}$	0.39±0.01	$4.20\times10^{4}\pm9.54\times10^{3}$	$5.07\times10^{2}\pm2.08\times10^{1}$	1.26±0.34	6,543.74±1022.19
I2	$6.17\times10^{6}\pm4.51\times10^{5}$	$2.07\times10^{4}\pm2.08\times10^{3}$	0.33±0.02	$5.30\times10^{3}\pm3.61\times10^{2}$	$2.27\times10^{2}\pm4.04\times10^{1}$	4.27±0.63	4,151.89±688.19
I3	$4.37\times10^{5}\pm2.52\times10^{4}$	$1.07\times10^{4}\pm1.15\times10^{3}$	2.44±0.17	$3.67\times10^{3}\pm4.04\times10^{2}$	$3.20\times10^{2}\pm2.65\times10^{1}$	8.75±0.44	9,759.37±883.36
CI	$6.80\times10^{6}\pm4.58\times10^{5}$	$2.33\times10^{4}\pm1.53\times10^{3}$	0.34±0.01	$5.23\times10^{3}\pm5.69\times10^{2}$	$3.80\times10^{2}\pm1.00\times10^{1}$	7.32±0.89	173.45±18.75
U1	$6.77\times10^{6}\pm9.87\times10^{5}$	$1.73\times10^{4}\pm1.53\times10^{3}$	0.26±0.02	$3.53\times10^{3}\pm3.06\times10^{2}$	$3.33\times10^{2}\pm2.52\times10^{1}$	9.51±1.50	5,908.56±1,252.00
U2	$7.73\times10^{6}\pm1.12\times10^{6}$	$3.43\times10^{4}\pm2.08\times10^{3}$	0.45±0.06	$5.60\times10^{3}\pm3.61\times10^{2}$	$3.60\times10^{2}\pm1.00\times10^{1}$	6.45±0.58	2,810.28±530.53
U3	$6.13\times10^{6}\pm9.45\times10^{5}$	$3.50\times10^{4}\pm4.58\times10^{3}$	0.59±0.15	$3.97\times10^{3}\pm3.06\times10^{2}$	$4.50\times10^{2}\pm5.29\times10^{1}$	11.33±0.72	5,566.89±528.33
CU	$7.00\times10^{6}\pm1.06\times106$	$2.13\times10^{4}\pm1.53\times10^{3}$	0.31±0.02	$4.67\times10^{3}\pm2.52\times10^{2}$	$3.13\times10^{2}\pm4.16\times10^{1}$	6.70±0.57	467.05±401.73

Data are Mean ± standard deviations (Significant coefficients = 0.05).

most prevalent HUB in the current study sites. The least prevalent HUB was *Micrococus letus* which was prevalent only in site A3 (Emuoha). The most prevalent HUF were *A. niger*, *A. flavus* and *Paecilomyces* species. The least prevalent isolates included *Aspergillus fumigatus, Candida torulopsis, Chrysosporium* species and *Geotrichium* species. Hydrocarbon utilizing microorganisms were diverse among all sites of study. Hydrocarbon utilizing bacteria are abundant in the environment but not limited to oil polluted sites (Okoh, 2006). However, this happens when there are optimal conditions as the observations showed a declined trend in populations of fungi and bacteria across the wet and dry seasons. High populations were favoured in the wet season as compared to the dry season. Presence of poisonous materials from pollution is a key slow down to microorganism activities and hence a cause to diverse microorganism species that have capacity to degrade hydrocarbons (Akoachere et al., 2008) and other pollutants in soil.

Atlas and Cerniglia (1999) identified *Pseudomonas* and *Bacillus* spp. as the dominant isolated bacterial genera in oil contaminated environment. *Micrococcus lylae* and *B. subtilis* were the utmost prevalent in 8 and 9 out of 12 study and control sites, respectively. *B. subtilis* has been identified in oil contaminated sites and has been attributed to bioremediation of the contaminated sites (Al-Dhabaan, 2019). Other bacterial species isolated from oil polluted sites (Atlas and Cerniglia, 1999) include, *Micrococcus, Flavobacterium, Enterococcus*, *Proteus*, and *Klebsiella* spp. Oil contaminated sites are dominated by Gram negative bacteria (Bartha, 2009; Agency for Toxic Substances and Disease Registry {ATSDR}, 2000; Singer and Finnerty, 2004). This was not the case in the present study, as Gram negative and Gram-positive bacteria were equally distributed among the study sites, where the dominant Gram-positive bacteria was *Bacillus* and *Micrococcus* while the dominant Gram-negative bacteria were *Pseudomonas* sp. *Pseudomonas aeruginosa* was isolated from two sites I1 (Onne) and U1 (GRA Phase 2) hence was less prevalent among the study sites. *P. aeruginosa* has been isolated in diesel polluted areas (Chikere et al., 2019). *Bacillus cereus* has also been associated with bioremediation of petroleum hydrocarbons (Al-Dhabaan, 2019). Additionally, *P. aeruginosa* is an efficient degrader of diesel. *Acinetobacter, Myroids, Pseudomonas*, and *Bacillus* are degraders in the spill sites of the long-chain hydrocarbon fraction (Chikere et al., 2019; Wang et al., 2019). There are variations in microbial community structure in the hydrocarbons polluted soils, where the community shows higher similarity between sites polluted with similar contaminants (Avidano et al., 2005). The presence of various chemical pollutants in the environment hardly affects bacterial density but rather the structure of the community (Avidano et al., 2005).

Characterizing soil chemical properties, metabolic fingerprinting, enzymatic activities, and bacterial community structure could be useful soil health assessment tools (Avidano et al., 2005).

CONCLUSION AND RECOMMENDATION

The study concludes that the most prevalent bacterial species includes *B. subtilis, Micrococcus lylae, S. aureus, B. cereus* and *A. faecalis*. The most prevalent HUB isolate was *Bacillus subtilis.* The most prevalent fungal isolates were *A. niger, A. flavus* and *C. torulopsis*. *A. niger* was the most prevalent among the HUF isolates which was identified in 12 contaminated and control sites. The findings conclude that the sites of study harboured TPH tolerant bacteria and fungi and are appropriate for the selection of microorganism's habitats for bioremediation of TPH polluted areas. Therefore, contamination of soils in the study areas negatively affected microbial populations, diversity and species richness. Microbes have adapted to tolerate the presence of petroleum hydrocarbons or can even use them for nourishment. Some implications are useful, such as the use of bacteria to clean up metal and/or hydrocarbon-contaminated sites. Overall, this study adds to our understanding of patterns of microbial succession in different anthropogenic activities where agricultural soils were the most affected. Further, this research contributes to our understanding of patterns of hydrocarbon use and microbial succession in different oil-polluted soils with various human activities (urban, industrial and agricultural).

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR) (2000). Toxicological profile for total petroleum hydrocarbons (TPH). Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.

Akoachere JTK, Akenji TN, Yongabi NF, Nkwelang G, Ndip NR (2008). Lubricating oil- degrading bacteria in soils from filling stations and auto-mechanic workshops in Buea, Cameroon: Occurrence and characteristics of isolates. African Journal of Biotechnology 7:14.

Akpoveta OV, Osakwe SA, Okoh BE, Otuya BO (2010). Physicochemical Characteristics and Levels of Some Heavy Metals in Soils around Metal Scrap Dumps in Some Parts of Delta State, Nigeria. Journal of Applied Science and Environmental Management 14(4):57-60.

Al-Dhabaan FA (2019). Morphological, biochemical and molecular identification of petroleum hydrocarbons biodegradation bacteria isolated from oil polluted soil in Dhahran, Saud Arabia. Saudi Journal of Biological Sciences 26(6):1247-1252.

Atlas RM, Cerniglia CE (1999). Bioremediation of petroleum pollutants. Biological Science 45:332-350.

American Public Health Association (APHA) (1998). Standard Methods for Examination of Water and Waste Water. American Public Health Association, American Water Works Association and Water Pollution Control Federation, 20th Edition, Washington DC, USA.

Avidano L, Gamalero E, Cossa GP, Carraro E (2005). Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. Applied Soil Ecology 30(1):21-33.

Barrios-Estrada C, de Jesús Rostro-Alanis M, Muñoz- Gutiérrez BD, Iqbal HM, Kannan S, Parra-Saldivar R (2018). Emergent contaminants: endocrine dis- ruptors and their laccase-assisted degradationea review. Science of the Total Environment 612:1516e1531.

Bartha R (2009). Biotechnology of petroleum alkanes. In RM Atlas (ed.), Petroleum pollutant biodegradation. Microbial microbiology. Macmillan Publishing Co., Ecology 12:155-172.

Bilal M, Asgher M, Parra-Saldivar R, Hu H, Wang W, Zhang X, Iqbal HM (2017). Immobilized ligninolytic enzymes: an innovative and environmental responsive tech- nology to tackle dye-based industrial pollutantsea review. Science of the Total Environment 576:646e659.

Bowen H, Maul JE, Cavigelli MA, Yarwood S (2020). Denitrifier abundance and community composition linked to denitrification activity in an agricultural and wetland soil. Applied Soil Ecology 151. https://doi.org/10.1016/j.apsoil.2020.103521

Chikere CB, Tekere M, Adeleke R (2019). Enhanced microbial hydrocarbon biodegradation as stimulated during field-scale landfarming of crude oil-impacted soil. Sustainable Chemistry and Pharmacy 14:100177.

Eze VC, Onyilide DM (2015). Microbiological and Physicochemical Characteristics of Soil receiving Cassava Effluent in Elele, Rivers State, Nigeria. Journal of Applied and Environmental Microbiology 3(1):20-24.

Eze VC, Omeh YN, Ugweje CD (2013). Eze VC, Omeh YN, Ugweje CD (2013). Microbiological and Physicochemical Assessment of Soil Contaminated with Lairage Effluent in Umuahia, Abia State, Nigeria. Journal of Pharmacy and Biological Sciences 8(2):50-56.

Holt JG, Krieg NR, Sneath, PHA, Stanley JT, Williams ST (1994). Bergey's Manual of Determinative Bacteriology. 9th Ed. Williams and Wilkins Company, Baltimore, USA pp. 71-561.

Ifeanyi VO, Ogbulie JN (2016). Biodegradation of Crude Oil by Microalgae Microcystis flos-aquae. Nigerian Journal of Microbiology 30(2):3459-3463

Imarhiagbe EE, Osarenotor O, Obayagbona ON, Eghomwanre AF, Nzeadibe BN (2017). Evaluation of Physicochemical, Microbiological and Polycyclic Aromatic Hydrocarbon Content of Top Soils from Oka Market Waste Collection Site, Benin City, Nigeria. Journal of Applied Sciences and Environmental Management http://dx.doi.org/10.4314/jasem.v21i1.12

Jacob D, Kotova L, Teichmann C, Sobolowski SP, Vautard R, Donnelly C, Koutroulis AG, Grillakis MG, Tsanis IK, Damm A, Sakalli A, van Vliet MTH (2018). Climate Impacts in Europe Under +1.5°C Global Warming. Earth's Future 6(2):264-285.

Lindén O, Pålsson J (2013). Oil contamination in Ogoniland, Niger Delta.
Liu Y, Chen X, Liu J, Liu T, Cheng J, Wei G, Lin Y (2019). Temporal and spatial succession and dynamics of soil fungal communities in restored grassland on the loess plateau in China. Land Degradation & Development 30:1273-1287.
National Geographic Society (2020). The Future of Food, National Geographic Magazine, https://www.nationalgeographic.com/foodfeatures/feeding-9-billion/
Nkonya E, Mirzabaev A, von Braun J (2016). Economics of land degradation and improvement - a global assessment for sustainable development. Springer Open. https://doi.org/10.1007//978-3-319-19168-3. Springer, New York.
Ogbemudia FO, Mbong EO (2013). Soil reaction (pH) and heavy metal index of dumpsites within Uyo municipality Merit Research. *Journal of Environmental Science and Toxicology* 1(4):82-85.
Okoh AI (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. Biotechnology and Molecular Biology Review 1:38-50.
Ren L, Jia Y, Ruth N, Shi Y, Wang J, Qiao C, Yan Y (2016). Biotransformations of bisphenols mediated by a novel Arthrobacter sp. strain YC-RL1. Applied Microbiology and Biotechnology 100(4):1967e1976.
Singer ME, Finnerty WR (2004). Microbial metabolism of straight-chain and branched alkanes. In R. M. Atlas (ed.), “Petroleum microbiology”. Macmillan Publishing Co., New York.
United Nations Environment Programme (UNEP) (2011). In: Environmental Assessment of Ogoniland. United Nations Environment Programme. Retrieved from. http://www.unep.org
Unimke AA, Mmuoegbulam1 AO, Bassey IU, Obot SE (2017). Assessment of the Microbial Diversity of Spent-Oil Contaminated Soil in Calabar, Nigeria. Journal of Advances in Microbiology 4(4):1-9.
Wali E, Nwankwoala HO, Ocheje JF, Onyishi CJ (2019). Oil spill incidents and wetland loss in Niger Delta: implication for sustainable development goals. International Journal of Environment and Pollution Research 7:1-20.
Wang M, Deng B, Fu X, Sun H, Xu Z (2019). Characterizations of microbial diversity and machine oil degrading microbes in machine oil contaminated soil. Environmental Pollution 255:113190.
Whitman WB, Coleman DC, Wiebe WJ (1998). The diversity and biogeography of soil bacterial communities. Proceedings of the National Academy of Sciences of the United States of America 95(12):6578-6583.
Xie Y, Fan J, Zhu W, Amombo E, Lou Y, Chen L, Fu J (2016). Effect of Heavy Metals Pollution on Soil Microbial Diversity and Bermudagrass Genetic Variation. Frontiers in Plant Science 7:755.
Yang P, Luo Y, Gao Y, Gao X, Gao J, Wang P, Feng B (2020). Soil properties, bacterial and fungal community compositions and the key factors after 5-year continuous monocropping of three minor crops. PLoS ONE 15(8):e0237164.
Yang R, Liu G, Chen T, Zhang W, Zhang G, Chang S (2018). The complete genomic sequence of a novel cold-adapted bacterium, Planococcus maritimus Y42, isolated from crude oil-contaminated soil. Standards in Genomic Sciences 13:23e34.
Zanardo M, Rosselli R, Meneghesso A, Sablok G, Stevanato P, Engel M, Altissimo A, Peserico L, Dezuani V, Concheri G, Schloter M, Squartini A (2018). Response of Bacterial Communities upon Application of Different Innovative Organic Fertilizers in a Greenhouse Experiment Using Low-Nutrient Soil Cultivated with Cynodon dactylon. Soil Systems pp. 1-11.
Zhao B, Liu A, Wu G, Li D, Guan Y (2017). Characterization of heavy metal desorption from road-deposited sediment under acid rain scenarios. Journal of Environmental Sciences 51:284-293.
Zheng J, Feng JQ, Zhou L, Mbadinga SM, Gu JD, Mu BZ (2018). Character- ization of bacterial composition and diversity in a long-term petroleum contaminated soil and isolation of high-efficiency alkane-degrading strains using an improved medium. World Journal of Microbiology and Biotechnology 34(2):34e44.
Zhou W, Lv T, Chen Y, Westby AP, Ren W (2014). Soil Physicochemical and Biological Properties of Paddy-Upland Rotation: A Review. Hindawi pp. 1-8.