Frontiers in Environmental Microbiology
2024, Vol. 10, No. 1, pp. 12-28
https://doi.org/10.11648/j.fem.20241001.13

Research Article

Seasonal Population Trends of Microbial Communities in Oil Tainted Soils in Greater Port Harcourt Area, Nigeria

Wanjala Paul Muyoma[1, *], Boadu Kwasi Opoku[2], Eliud Nalianya Wafula[1], Etela Ibisime[3]

[1]School of Pure and Applied Sciences, Bomet University College, Bomet County, Kenya

[2]Industrial Chemistry Unit, Department of Chemistry, School of Physical Sciences, University of Cape Coast, Cape Coast, Ghana

[3]Department of Animal Science, University of Port Harcourt, Port Harcourt, Nigeria

Abstract

The utilization of oil in industries has devastating effect to the environment. Industrial effluents and oil spills are continuously contaminating the soil. Further, seasonality influences the distribution of pollutants in soil. Consequently, soil microbial biota and ecological processes are affected. This study assessed the effect of seasonality on soil fungal and bacterial communities in oil contaminated soils in 12 selected sites in Greater Port Harcourt Area. Standard analytical procedures were used to obtain bio-physicochemical data from the soil samples and t test was used to analyse data. The levels of total petroleum hydrocarbons (TPH) were above 5000 ppm (DPR recommended limit). There was significant difference ($p \leq 0.000$) between the means of TPH in wet and dry seasons. Seasonality influenced % HUF and % HUB in the soils of urban, industrial and agricultural sites. Generally, the seasonality trends showed that there was a declining population of THB, HUB, TF, and HUF from the wet season to the dry season. However, the results show that there was a stable trend in % HUB as compared to oscillations observed in % HUF in oil tainted soils across a seasonal divide. We recommend characterization of the microorganism to identify the best candidate for bioremediation of oil tainted soils across a seasonal divide.

Keywords

Soil, Fungi and Bacteria, Community Structure, Total Petroleum Hydrocarbons, Season

1. Introduction

A significant driver of soil formation, organic matter turnover, and nutrient cycling is soil microbiota [33]. According to Alkorta, Epelde, and Garbisu [1], soil moisture content affects the solubility, mobility, and bioavailability of soil contaminants as well as the amount of oxygen that is available. Cycles of wetting and drying can change the organic pollutants' bioavailability in soil [1]. Floods and periods of heavy precipitation cause more water erosion, which spreads soil contaminants away from contaminated areas [1]. Variations in precipitation amounts and frequency have an impact on soil microbial activity and eventually on the integrity of the soil ecosystem [1]. In wet seasons, the pH of soils is

*Corresponding author: piwanjala@buc.ac.ke (Wanjala Paul Muyoma)

Received: 21 December 2023; **Accepted:** 6 January 2024; **Published:** 20 February 2024

altered by frequent flooding and drainage [35]. During rainy season pH of acid soils increases while pH of alkaline soils decreases [35]. Because of its high pH, rainwater encourages the exchange of ions between phosphate and OH^- in soils that release P into floodwater [35]. Increased Fe^{3+} concentrations in rainfall result in the formation of mixed Fe^{2+} -Fe^{3+} hydroxide, which has a greater specific surface area and enhances the adsorption of soluble P [34, 37]. As most inorganic P is attached to calcium (Ca) compounds, alkaline soils benefit greatly from the retention of P due to Ca compounds [35, 46].

Reduced rainfall can have a significant impact on the biomass of microorganisms but little on the makeup of communities [22]. Rainfall variations can affect the carbon cycle in terrestrial ecosystems, which is why the robustness of biological processes in soil depends on the population of low-lying microorganisms in these ecosystems. Regarding the impact of seasonality on microbial diversity in soil ecosystems, there are conflicting and ambiguous results [28, 40] as there is more than only rainfall as an influencing factor, but complex interactions between microorganisms and biotic and abiotic components. Reductions in rainfall have the potential to directly or indirectly alter the biomass and community of microorganisms. Lower soil moisture reduces the mobility of solutes and limits the availability of substrate for microorganisms. This directly prevents the growth of decomposer bacteria, which in turn results in less carbon and nitrogen from detritus entering the soil ecosystem [26]. Conversely, several microbes exhibit tolerance towards damp environments, such as Actinobacteria, Gram-positive bacteria and Arbuscular mycorrhizal fungi [45]. This suggests that variations in seasonality and precipitation patterns affect the kinds of microorganisms that live in soil [5, 20].

The amount of water or available moisture regulates how often soil particles and microbes collide [5]. Reduced moisture affects soil total microorganism population [7]. Reduced rainfall has an impact on microorganisms' capacity to get the necessary nutrients from the soil [7, 11]. Nevertheless, not all regions exhibit a correlation between decreased rainfall and the populations of microorganisms [22]. This is explained by the population of soil microorganisms' evolutionary tolerance to extreme temperatures and water stress [6, 24]. Greater resistance to decreased rainfall can be shown by fungal communities than by bacteria [48]. This is explained by the fact that fungus have filamentous structures, which enable them to access and utilize substrates found in soil at extremely low moisture levels [36]. Additionally, the ability of hyphae to access available nutrients and stronger cell walls in fungus influence their responses to decreased rainfall [36]. Fungi's thick cell walls help them resist the environment's high osmotic pressure [14]. This also holds true for bacteria, as Gram-positive bacteria are able to present themselves more effectively in a variety of seasonal conditions due to their stronger cell walls and more developed osmoregulatory mechanisms than Gram-negative bacteria. For instance, Acidobacteria and Actinobacteria are resistant to drought and barely impacted by precipitation levels [14]. The entire decline in the number of microorganisms can also be ascribed to the warming impacts of the dry spell [10, 12], indicating that soil microorganisms can be altered in accordance with various habitats. For instance, a decrease in rainfall can have an impact on soil microorganisms in a forest while having no effect on the population of microorganisms in a nearby grassland [20]. The factors influencing the populations of microorganisms in various environments may differ depending on how those ecosystems are structured [4]. Therefore, reduced microorganism populations in soil can negatively affect soil carbon dynamics by causing a decline in carbon levels [9, 19, 47]. Therefore, changes in microbe populations in contaminated environments may be impacted by seasonal variations.

Rainfall's effect on soil microbes is still unknown [8]. Reductions in rainfall lower the biomass of soil microorganisms, but have less of an impact on fungi than on bacteria [43]. Rainfall can significantly affect microbial biomass but has little effect on the makeup of microorganism communities [22]. Rainfall increases reduce soil respiration and soil organic carbon, which are positively correlated with changes in the biomass of microorganisms [7]. In certain ecosystems, various microorganism communities react differently to varying levels of precipitation [5, 20, 27]. The description of the distribution patterns among microorganism populations in relation to changes in rainfall patterns is still somewhat unclear [28, 40] particularly in polluted soils. The relationship between the composition of the soil microorganisms and studies comparing the functional characteristics of microbial communities from various habitats with seasonal variations have shown the ecosystem function [17, 18, 30, 29, 44], there aren't many research looking into how changes in the microbial ecosystem affect a specific location [16, 31]. These studies factored community shifts following contamination of soil with Total Petroleum Hydrocarbons (TPH) but not in a seasonal change perspective. In addition, some of the results in these studies contradict each other, also within the same field of study [21]. In general, the effects of the season on the structure of the microbial soil community are still poorly understood, particularly in oil contaminated soils.

Therefore, the aim of this study is to assess the seasonal dynamics of soil microbial communities in oil tainted soil in Greater Port Harcourt Area, Nigeria. It is challenging to forecast the dynamics of microorganisms in the environment since the distribution and abundance of soil microorganisms depend on both biotic and abiotic factors [13, 32]. A decrease in solute mobility limits the availability of substrates, which prevents bacteria from growing [7]. Seasonal variations in soil oxygen availability have a domino effect on the population of microorganisms in the community, primarily decomposers [15]. Anaerobic conditions cause a reduction in the breakdown of cell wall components, but aerobic and anaerobic conditions cause equivalent degradation of chemicals that are easily leachable and hydrolyzable [15].

2. Materials and Methods

2.1. Description of the Study Site

In Port Harcourt, the capital of Rivers State, Nigeria, nine chosen test and three control sites were used for this study (Figure 1). The study locations were divided into three categories: agricultural (Aluu, Oquwi- Eleme, Emuoha- Eu), industrial (Eleme, which houses the NNPC Refinery, Agbada-SPDC- flow station), and urban (GRA phase 2, Diobu- Mile 1 and Mguoba).

As seen in Table 1, the study sites were characterized by a variety of economic activities. From AccuWeather's weather forecasts, the mean monthly temperature (℃) and precipitation (mm) were determined.

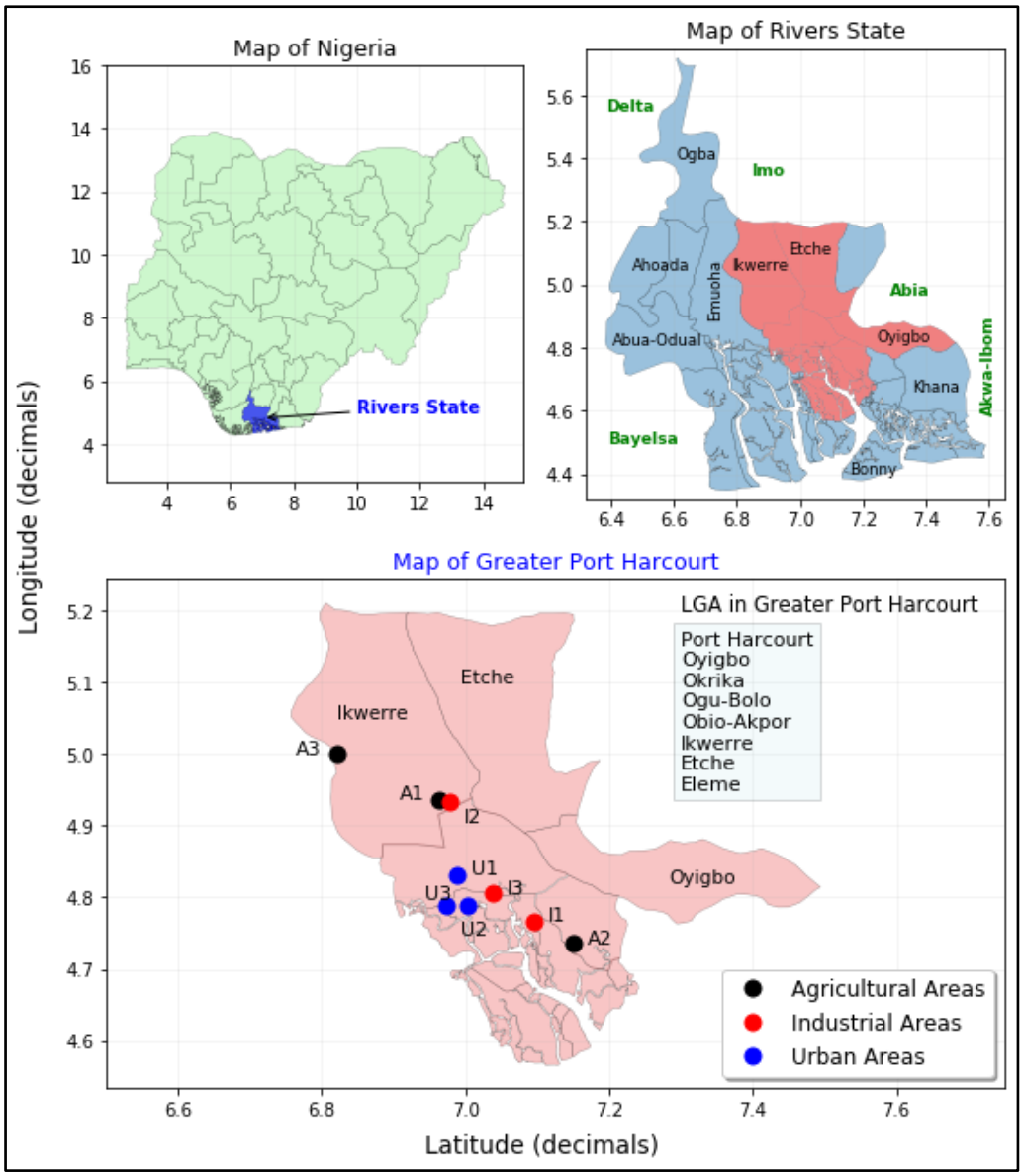

Figure 1. *Sample site locations in chosen research areas in the Greater Port Harcourt region of Rivers State, Nigeria. [38].*

Table 1. *Table displaying economic activity and study regions [38].*

No	Selected Study Sites	Study Site Coding (Locations)	Coordinates N latitude E Longitude	Characteristic and main activities
		Agricultural Area		
1	Aluu	A1	4 °56' 11.160' 6 °57' 52.248	Flow station

No	Selected Study Sites	Study Site Coding (Locations)	Coordinates N latitude E Longitude	Characteristic and main activities
2	Eleme	A2	4 °44' 09.874' 7 °08' 58.494'	Village close to refinery
3	Emuoha	A3	5 °00' 00.018' 6 °49' 13.032'	Flow station
4	Control	CA	5 °00' 21.384' 6 °49' 00.000'	>1 km away from suspected areas
		Industrial Area		
1	Onne	I1	4 °46' 00.402' 7 °05' 43.092'	Hosts the NNPC Refinery
2	Agbada	I2	4 °56' 03.444' 6 °58' 42.060'	Hosts SPDC- flow station in a rural setting
3	Trans-Amadi	I3	4 °48' 20.455' 7 °02' 17.646'	Schlumberger/, Hallburton
4	Control	CI	4 °47' 13.788' 7 °07' 44.620'	>1 km away from suspected areas
		Urban Area		
1	GRA Phase 2	U1	4 °49' 53.574' 6 °59' 45.552'	Inhabited areas Perecuma street
2	Diobu-Mile 1	U2	4 °47' 20.382' 7 °00' 13.164'	Petroleum refinery
3	Mgbuoba	U3	4 °50' 39.864' 6 °58' 20.232'	NTA
4	Control	CU	4 °49' 17,040' 6 °59' 24.168'	>1 km away from suspected areas

2.2. Sampling

Using random sampling, composite samples were gathered from the urban, industrial, and agricultural sectors in both the dry season (December 2018 to February 2019) and the wet season (April to September 2018). Using a random strategy, five (5) distinct samples were gathered from each test field and season. To create a uniform composite mixture, the five separate samples were thoroughly mixed by quartering and coning in a sterile container. Using a conventional auger, a total of 12 composite samples were taken three times every season from the topsoil at a depth of 0 to 15 cm. The test samples were A1, A2, A3, I1, I2, I3 U1, U2, and U3, and the control samples were CA, CI, and CU (Table 1). Afterwards, 400 g of homogenized composite samples were placed in polyethylene bags with the use of a sterile wooden shovel. To avoid sample contamination, pre-sterilized items were used for the collection of samples for microbiological analysis. A GPS was used to locate the sampling sites, and Table 1 contains the GPS data that were obtained. Samples were brought to the lab so they could be examined.

2.3. Laboratory Analysis

2.3.1. Determination of Total Petroleum Hydrocarbon (TPH) Content of Soil

The FID technique for the Hewlett Packard 5890 Series II Gas Chromatograph was employed. Using this procedure, a beaker washed with acetone was filled with 1 g of well-mixed material. Subsequently, 1 g of anhydrous sodium sulphate and 5 ml of a solvent (1:1 of dichloromethane and acetone) were added to the soil sample. The mixture was then agitated for 15 minutes using a magnetic stirrer before being transferred into a round-bottom flask. This was done again with the addition of 5 milliliters of mixed solvent. After stirring and allowing it to stand or settle, it was poured into a different round-bottom flask. The solvent was concentrated to 2 ml after being exchanged for 1 ml of hexane. Ten milliliters of n-hexane were used to elute (wash off) the columns. The aliphatic components were collected using 10 ml of n-hexane after 1 ml (1 ml) of the extract was pipetted into the column. For gas chromatography, the extract was concentrated to 1 ml and put into a glass container.

2.3.2. Enumeration of Total Heterotrophic Bacteria (THB)

Heterotrophic bacteria were enumerated by pour plate method [2, 3]. Under aseptic conditions (laminar bench floor), one gram of soil sample was weighed into nine milliliters of sterile diluent (0.85% NaCl). After that, it was serially diluted and homogenized with a lab vortex mixer (Model: 10101001, IP42). Next, using a sterile pipette, a 0.1 ml aliquot of the inoculum was collected and inoculated on Nutrient Agar (NA) medium. A sterilized glass spreader stick was used to distribute the inoculum uniformly. After that, plates were incubated for 24 hours at 37 ℃. Subsequently, the colonies were tallied and reported as the value of colony forming units (CFUs/mg of soil) per gram. Subsequently, the colonies were tallied and reported as the value of colony forming units (CFUs/mg of soil) per gram. After being incubated for 24 hours at 37 ℃, distinct colonies with varying morphological patterns (color, size, form, edge, elevation, surface, and opacity) were selected and streaked or subcultured on newly prepared nutritional agar medium in order to obtain pure culture. The pure cultures underwent biochemical testing after being Gram stained for microscopic inspection.

2.3.3. Enumeration of Hydrocarbon Utilizing Bacteria

Hydrocarbon utilizing bacteria (HUB) were enumerated by the pour plate method [2, 3] method. Under aseptic circumstances, one gram of soil sample was weighed into a nine milliliter sterile diluent (0.85% NaCl). After that, the material was serially diluted and homogenized using a lab vortex mixer (Model: 10101001, IP42). 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. Crude oil was soaked on sterile filter paper (Whatman 540), which was then put inside the petri dish lid. The plates were cultured for five days at room temperature in an inverted orientation until discernible growth appeared. Following a 24-hour incubation period and sub-culturing on a newly prepared medium, discrete colonies were purified in preparation for microscopic inspection and biochemical testing.

2.3.4. Enumeration of Total Fungi

HUF (hydrocarbon-using fungus) was cultivated via the pour plate method [2, 3]. One gram of soil sample was weighed in nine milliliters of sterile diluent (0.85% NaCl) while the environment was aseptic. After homogenizing the material with a vortex mixer (Model 10101001, IP42), sterile pipettes were used to dilute the sample in series. After that, 0.1 ml of the inoculum aliquot was added to Potato Dextrose Agar (PDA) together with NormocureTM, an antibacterial reagent, to prevent bacterial development and promote fungal growth instead. The infected plates were then allowed to incubate at room temperature for a duration of 5 to 7 days. Using a colony counter, colonies were counted in order to determine the colony forming unit per gram (CFU/g) of the soil.

2.3.5. Enumeration of Hydrocarbon Utilizing Fungi

HUF (hydrocarbon-using fungus) was cultivated via the pour plate method [2, 3]. One gram of soil sample was weighed into a 9 ml sterile diluent (0.85% NaCl) in an aseptic setting (laminar flow bench). After homogenizing the material with a lab vortex mixer (Model: 10101001, IP42), sterile pipettes were used to serially dilute the sample. Then, in order to prevent the growth of bacteria and promote the growth of fungi exclusively, a zero-point one milliliter (0.1 milliliter) aliquot of inoculum was inoculated on Mineral Salt Agar (MSA) combined with an antibacterial reagent (Normocure^{TM}). After being immersed in crude oil, sterile filter paper (Whatman 540) was placed within the petri dish cover. The plates were then incubated for five to seven days at room temperature with the lids on. To calculate colony forming units per gram of soil, colonies were counted using a colony counter. After that, the isolates' cultural traits (color and microscopic observations) were examined, and they were refined by subculturing on newly made medium and re-incubating for three to five days. Using lactophenol cotton blue dye, a microscopic analysis was performed on the pure cultures under a ×400 magnification.

2.3.6. Determination of % Hydrocarbon Utilizing Fungi and Bacteria

The following formula was utilized to express the percentage (%) of hydrocarbon-utilizing fungi and bacteria as a fraction of the overall heterotrophic viable count.

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

2.3.7. Data Analysis

Statistical analyses were performed by SPSS statistical package. Data analysis was done using descriptive analysis to summarize the data set as a representation of the entire or a sample of a population. The measure of central tendency that was done is the mean, while measures of variability include standard deviation at significance level $p < 0.05$. The t-test, was used to determine whether the means of TPH between the two seasons are different to each other. Seasonal variation was analyzed using a six-point moving average [trend] (April, July, September and December 2018 and January and February 2019) as a starting point using python statistical package.

3. Results

3.1. Variation in the Levels of TPH Across the Wet and Dry Seasons

The concentrations of TPH in the study sites were done to ascertain the levels of contamination and were above the ≤5000ppm (DPR recommended threshold values) p = 0.150 and 0.001 for wet and dry season respectively. Total Petroleum Hydrocarbon (TPH) concentrations in soils of all the control sites were within the recommended limit. Table 2 shows the mean variations of TPH among the study sites and also the study areas. There was significant difference ($p \leq 0.000$) between the means of TPH in wet and dry seasons. Generally, in all the study areas, the mean TPH was higher in the dry season as compared to the wet season. Also, the test sites had higher concentrations as compared to the control sites which were deemed non-contaminated.

Table 2. *Seasonal variation in Total Petroleum Hydrocarbon (TPH) content in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Study area	Study site	Mean ± Std. D TPH Wet (n=3)	Mean ± Std. D	Mean ± Std. D TPH Dry (n=3)	Mean ± Std. D
	A1	3791.70 ±613.63		4558.83 ±189.89	
Agricultural	A2	3146.81 ±776.81	4552.78 ±1736.25	4602.67 ±44.28	5707.65 ±1693.28
	A3	6719.84 ±447.31		7961.45 ±15.27	
Agricultural Control	CA	2020.30 ±526.58	2020.30 ±526.58	2328.59 ±44.88	2328.59 ±44.88
	I1	5610.41 ±790.82		9616.70 ±303.81	
Industrial	I2	6068.55 ±467.26	6868.33 ±1656.72	7678.42 ±430.87	9028.19 ±1058.28
	I3	8926.04 ±671.50		9789.45 ±283.31	
Industrial Control	CI	3324.12 ±261.31	3324.12 ±261.31	3451.93 ±499.68	3451.93 ±499.68
	U1	7741.89 ±503.99		8601.39 ±169.46	
Urban	U2	9213.61 ±65.32	8377.48 ±709.36	10603.92 ±152.69	9654.64 ±882.19
	U3	8176.95 ±198.90		9758.62 ±170.52	
Urban Control	CU	2713.72 ±449.26	2713.72 ±449.26	3232.54 ±643.83	3232.54 ±643.83
Mean N=27		6599.53 ±2120.44		8130.16 ±2140.41	
Df = 52					
T = -2.64					
P = 0.011					
Mean difference = -1530.63					

3.2. Changes in the Populations of THB Due to Seasonality

There was a decreasing trend in THB population in most of the study sites; (Emuoha) A3, (Industrial Control) C1, (Eleme) A2, (GRA-Phase 2) U1, (Trans-Amadi) I3, (Mgbuoba) U3, (Diobu-Mile 1) U2 and (Onne) I1 across the wet and the dry seasons. There was a stable population in (Onne) I1, (Agbada) I2 and (Aluu) A1 across the wet and dry seasons (Table 3, Figure 2). The highest decrease was observed between September and December. A stable population was observed the dry season in December, January and February as opposed to oscillations in populations in the wet season; April, June and September (Figure 2). In (GRA-Phase 2) U1 a sharp decrease was observed from April to July where there was slight increase from July to September then followed by a decrease in population in the month of September to February (Figure 2).

Table 3. *Seasonal variation in populations of total heterotrophic bacteria (THB) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Sampling site	**WET**			**Mean**	**DRY**			**Mean**
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CI	6.90×10^{7}	7.20×10^{7}	7.40×10^{7}	7.17×10^{7}	7.20×10^{6}	6.90×10^{6}	6.30×10^{6}	6.80×10^{6}
I1	1.32×10^{7}	1.30×10^{7}	1.50×10^{7}	1.37×10^{7}	6.80×10^{6}	6.30×10^{6}	6.00×10^{6}	6.37×10^{6}
I2	7.40×10^{6}	7.00×10^{6}	7.90×10^{6}	7.43×10^{6}	6.60×10^{6}	6.20×10^{6}	5.70×10^{6}	6.17×10^{6}
I3	3.50×10^{7}	2.80×10^{7}	3.80×10^{7}	3.37×10^{7}	4.60×10^{5}	4.40×10^{5}	4.10×10^{5}	4.37×10^{5}
CA	6.20×10^{7}	6.00×10^{7}	6.30×10^{7}	6.17×10^{7}	4.30×10^{5}	4.10×10^{5}	3.60×10^{5}	4.00×10^{5}
A1	1.10×10^{6}	1.00×10^{6}	1.80×10^{6}	1.30×10^{6}	7.60×10^{6}	6.80×10^{6}	6.20×10^{6}	6.87×10^{6}
A2	7.30×10^{7}	6.50×10^{7}	7.40×10^{7}	7.07×10^{7}	7.00×10^{6}	6.50×10^{6}	4.90×10^{6}	6.13×10^{6}
A3	5.80×10^{7}	5.60×10^{7}	5.80×10^{7}	5.73×10^{7}	9.30×10^{6}	7.30×10^{6}	2.90×10^{6}	6.50×10^{6}
CU	3.20×10^{6}	3.00×10^{6}	3.80×10^{6}	3.33×10^{6}	8.20×10^{6}	6.60×10^{6}	6.20×10^{6}	7.00×10^{6}
U1	9.80×10^{7}	1.20×10^{7}	2.70×10^{7}	4.57×10^{7}	7.90×10^{6}	6.30×10^{6}	6.10×10^{6}	6.77×10^{6}
U2	1.00×10^{7}	2.64×10^{7}	2.00×10^{7}	1.88×10^{7}	9.00×10^{6}	7.30×10^{6}	6.90×10^{6}	7.73×10^{6}
U3	1.80×10^{7}	1.78×10^{7}	2.30×10^{7}	1.96×10^{7}	7.20×10^{6}	5.80×10^{6}	5.40×10^{6}	6.13×10^{6}
Temp (℃)	27.00	26.00	26.00	26.50	28.00	28.00	28.00	28.00
ppt (mm)	126.00	242.00	635.00	334.33	0.00	0.00	0.00	0.00

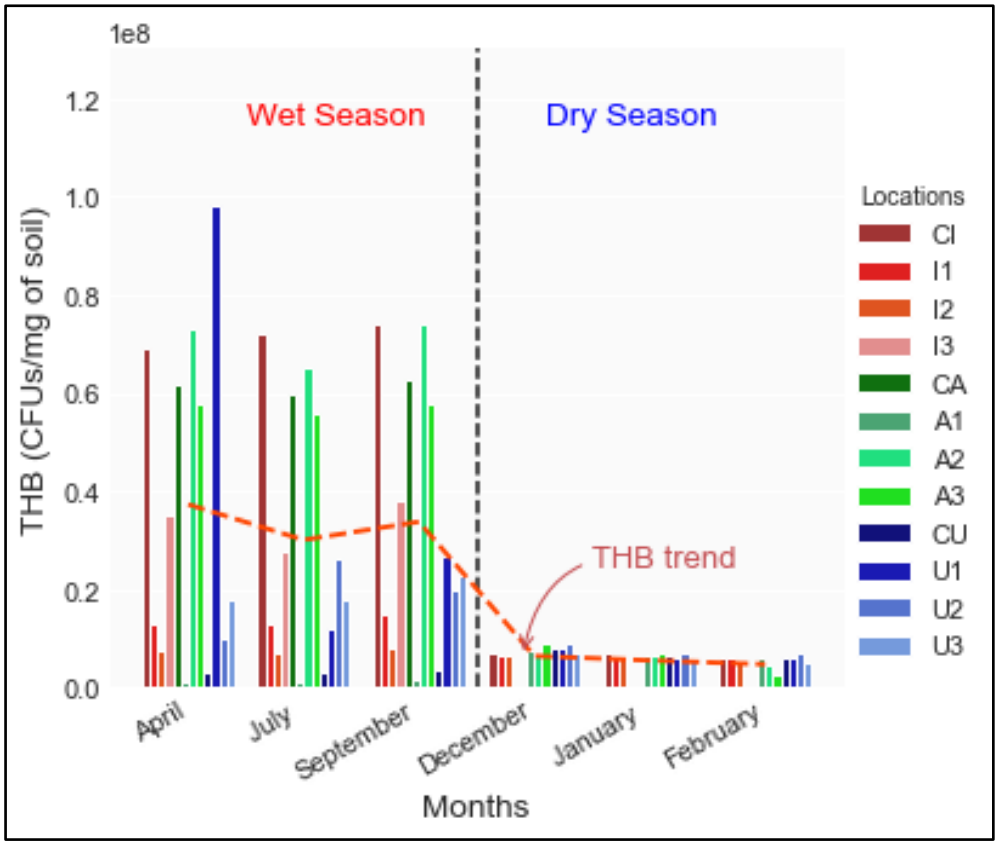

Figure 2. *Seasonal trend in population of total heterotrophic bacteria (THB) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

3.3. Seasonal Variation in Populations of Hydrocarbon Utilizing Bacterial (HUB)

The highest population of HUB was observed in Eleme (A2), Urban Control (CU) and Agbada (I2) in the wet season with populations of 6.80×10^5, 2.31×10^5 and 1.20×10^5 CFU/g of soil respectively (Table 4). There was an overall decreasing trend in HUB from wet to dry season (Table 4). A stable population was observed in Industrial Control (CI), Agbada (I2), Trans-Amadi (I3), Agricultural Control (CA), Aluu (A1), (Emohua) A3, GRA-Phase 2 (U1), Diobu Mile 1 (U2) and Mgbuoba (U3) in the wet and dry seasons. There was no significant variation on populations of HUB in the wet and dry season in Industrial Control (CI), Agbada (I2), Trans Amadi (I3), Agricultural Control (CA), Aluu (A1), Emuoha (A3), GRA Phase 2 (U1), Diobu Mile 1 (U2) and Mgbuoba (U3) (Table 4, Figure 3).

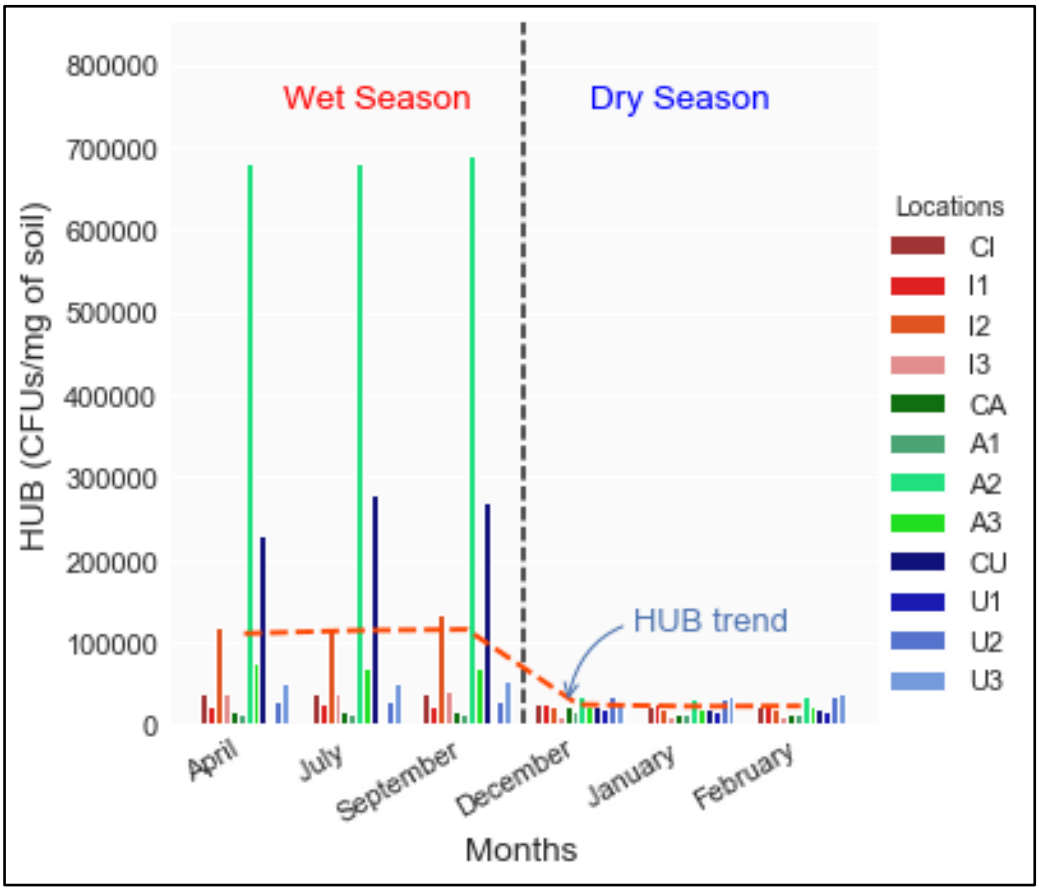

Figure 3. *Seasonal trend in populations of hydrocarbon utilizing bacteria (HUB) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Table 4. *Seasonal variation in populations of hydrocarbon utilizing bacteria (HUB) in wet and dry seasons in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Sampling site	**WET**			**Mean**	**DRY**			**Mean**
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CI	3.90×10^4	3.90×10^4	3.8×10^4	3.88×10^4	2.50×10^4	2.30×10^4	2.20×10^4	2.33×10^4
I1	2.38×10^4	2.62×10^4	$2.4+\times10^4$	2.47×10^4	2.60×10^4	2.50×10^4	2.30×10^4	2.47×10^4
I2	1.20×10^5	1.20×10^5	$1.30+\times10^5$	1.24×10^5	2.30×10^4	2.00×10^4	1.90×10^4	2.07×10^4
I3	3.90×10^4	3.90×10^4	$4.1+\times10^4$	3.97×10^4	1.20×10^4	1.00×10^4	1.00×10^4	1.07×10^4
CA	1.74×10^4	1.77×10^4	1.68×10^4	1.73×10^4	2.20×10^4	1.50×10^4	1.30×10^4	1.67×10^4

Sampling site	WET			Mean	DRY			Mean
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
A1	1.48×10^{4}	1.48×10^{4}	1.52×10^{4}	1.49×10^{4}	1.80×10^{4}	1.50×10^{4}	1.40×10^{4}	1.57×10^{4}
A2	6.80×10^{5}	6.80×10^{5}	6.90×10^{5}	6.83×10^{5}	3.60×10^{4}	3.30×10^{4}	3.50×10^{4}	3.47×10^{4}
A3	7.60×10^{4}	6.90×10^{4}	6.80×10^{4}	7.10×10^{4}	2.20×10^{4}	2.00×10^{4}	2.40×10^{4}	2.20×10^{4}
CU	2.31×10^{5}	2.80×10^{5}	2.70×10^{5}	2.60×10^{5}	2.30×10^{4}	2.10×10^{4}	2.00×10^{4}	2.13×10^{4}
U1	3.60×10^{3}	3.60×10^{3}	3.80×10^{3}	3.67×10^{3}	1.90×10^{4}	1.60×10^{4}	1.70×10^{4}	1.73×10^{4}
U2	2.84×10^{4}	2.80×10^{4}	3.00×10^{4}	2.88×10^{4}	3.60×10^{4}	3.20×10^{4}	3.50×10^{4}	3.43×10^{4}
U3	5.20×10^{4}	5.20×10^{4}	5.40×10^{4}	5.27×10^{4}	3.00×10^{4}	3.60×10^{4}	3.90×10^{4}	3.50×10^{4}
Temp (℃)	27.00	26.00	26.00	26.50	28.00	28.00	28.00	28.00
ppt (mm)	126.00	242.00	635.00	334.33	0.00	0.00	0.00	0.00

3.4. Seasonal Variation in Percent Hydrocarbon Utilizing Bacteria (% HUB)

There were mixed trends of increasing and decreasing in % HUB populations among the study locations between the wet and the dry seasons. Percent HUB decreased in Industrial Control (CI), Onne (I1), Agbada (I2), Aluu (A1), Eleme (A2), Emuoha (A3), Urban Control (CU), GRA Phase 2 (U1), Di-obu Mile 1 (U2) and Mgbuoba (U3) from the wet season to the dry season (Table 5, Figure 4). The highest decrease was observed in Urban Control (CU) (Figure 4). There was an increase in % HUB in Agricultural Control (CA) and Trans-Amadi (I3) from wet season to dry season (Table 4, Figure 4). All the levels of % HUB in all the study locations were below 10% and are grouped as pristine (Table 5).

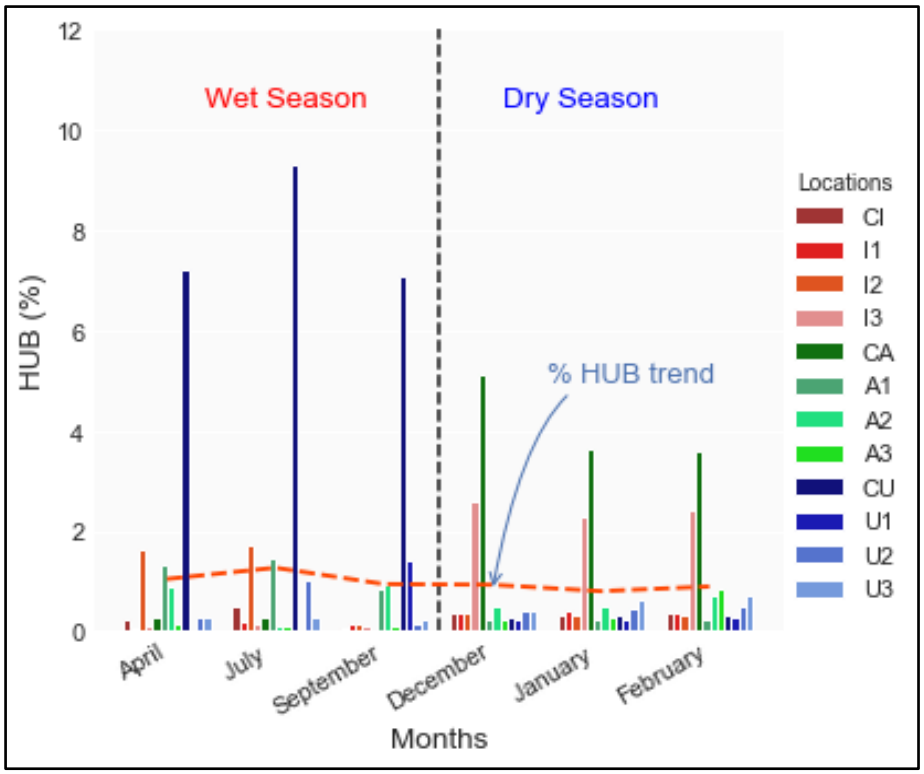

Figure 4. *Seasonal trend in percent hydrocarbon utilizing bacteria (% HUB) in soil from agricultural, industrial and urbanized areas in Greater Port Harcourt Area, Rivers State, Nigeria.*

Table 5. *Seasonal variation in percent HUB in wet and dry seasons in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Sampling site	**WET**			**Mean**	**DRY**			**Mean**
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CI	0.26	0.50	0.05	0.27	0.35	0.33	0.35	0.34
I1	0.05	0.20	0.16	0.14	0.38	0.40	0.38	0.39
I2	1.62	1.71	0.16	1.16	0.35	0.32	0.33	0.33
I3	0.11	0.14	0.11	0.12	2.61	2.27	2.44	2.44
CA	0.28	0.30	0.03	0.20	5.12	3.66	3.61	4.13
A1	1.35	1.48	0.84	1.22	0.24	0.22	0.23	0.23
A2	0.90	0.10	0.93	0.64	0.51	0.51	0.71	0.58
A3	0.13	0.12	0.12	0.12	0.24	0.27	0.83	0.45
CU	7.22	9.33	7.11	7.89	0.28	0.32	0.32	0.31
U1	0.04	0.03	1.41	0.49	0.24	0.25	0.28	0.26
U2	0.28	1.01	0.15	0.48	0.40	0.44	0.51	0.45
U3	0.28	0.29	0.23	0.27	0.42	0.62	0.72	0.59
Temp (℃)	27.00	26.00	26.00	26.50	28.00	28.00	28.00	28.00
ppt (mm)	126.00	242.00	635.00	334.33	0.00	0.00	0.00	0.00

3.5. Seasonal Variation in Populations of Total Fungal (TF)

Total fungal counts were done in all soil samples and the findings recorded in Table 6. There was a decreasing trend in fungal populations among all sites from the wet to dry season (Figure 5). In Onne (I1) there was an increase in population from April to July and a stable population from July to September where there was a decrease at the onset of the dry season (Figure 5). There was almost stable fungal population among study locations Industrial Control (CI), Agbada (I2), Trans Amadi (I3), Agricultural Control (CA), Emuoha (A3) and GRA-Phase 2 (U1) (Table 6, Figure 5). The highest fungal populations were observed in locations Onne (I1) and Eleme (A2) (Table 6).

Table 6. *Seasonal variation in populations of total fungal (TF) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Sampling site	**WET**			**Mean**	**DRY**			**Mean**
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CI	5.14×10^{4}	5.30×10^{4}	5.20×10^{4}	5.21×10^{4}	4.60×10^{3}	5.40×10^{3}	5.70×10^{3}	5.23×10^{3}
I1	1.50×10^{5}	8.60×10^{5}	8.40×10^{5}	6.17×10^{5}	3.30×10^{4}	4.10×10^{4}	5.20×10^{4}	4.20×10^{4}
I2	1.40×10^{4}	1.36×10^{4}	1.20×10^{4}	1.32×10^{4}	5.60×10^{3}	4.90×10^{3}	5.40×10^{3}	5.30×10^{3}
I3	7.60×10^{4}	7.60×10^{4}	7.30×10^{4}	7.50×10^{4}	4.10×10^{3}	3.30×10^{3}	3.60×10^{3}	3.67×10^{3}
CA	6.43×10^{3}	6.00×10^{3}	6.10×10^{3}	6.18×10^{3}	3.20×10^{3}	2.80×10^{3}	3.00×10^{3}	3.00×10^{3}
A1	1.54×10^{5}	1.40×10^{5}	1.40×10^{5}	1.45×10^{5}	2.50×10^{4}	2.90×10^{4}	3.40×10^{4}	2.93×10^{4}
A2	3.50×10^{4}	3.30×10^{4}	3.00×10^{4}	3.27×10^{4}	3.30×10^{3}	3.20×10^{3}	3.50×10^{3}	3.33×10^{3}
A3	8.50×10^{3}	8.00×10^{3}	8.20×10^{3}	8.23×10^{3}	4.50×10^{3}	4.90×10^{3}	4.70×10^{3}	4.70×10^{3}

Sampling site	WET			Mean	DRY			Mean
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CU	8.10×10^4	7.70×10^4	7.30×10^4	7.70×10^4	4.40×10^3	4.70×10^3	4.90×10^3	4.67×10^3
U1	1.40×10^4	1.43×10^4	1.50×10^4	1.44×10^4	3.20×10^3	3.60×10^3	3.80×10^3	3.53×10^3
U2	6.80×10^4	6.60×10^4	6.40×10^4	$6.60+10^4$	5.20×10^3	5.70×10^3	5.90×10^3	5.60×10^3
U3	6.50×10^4	6.00×10^4	6.20×10^4	$6.23+10^4$	3.70×10^3	3.90×10^3	4.30×10^3	3.97×10^3
Temp (℃)	27.00	26.00	26.00	26.50	28.00	28.00	28.00	28.00
ppt (mm)	126.00	242.00	635.00	334.33	0.00	0.00	0.00	0.00

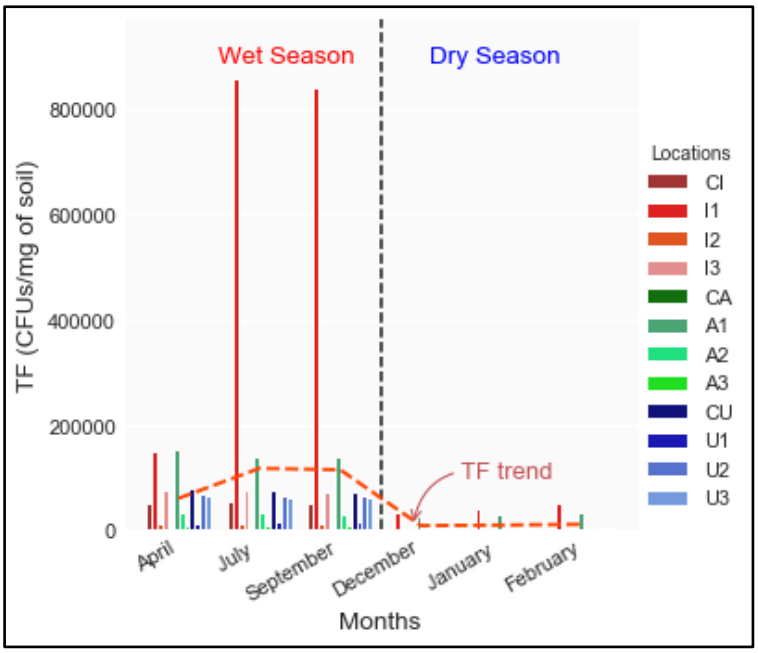

Figure 5. *Seasonal trend in populations of total fungi (TF) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

3.6. Seasonal Variation in Population of Hydrocarbon Utilizing Fungal (HUF)

The highest mean HUB observed in the wet season was in Industrial Control (CI) and Mgbuoba (U3) with 4.57×10^4 and 3.45×10^4 CFU/g of soil (Table 7). There was a decreasing trend in fungal population in all the study areas between the wet and the dry season (Table 7, Figure 6). There was a decrease in fungal population between the wet and the dry season (Figure 6).

Table 7. *Seasonal variation in populations of hydrocarbon utilizing fungi (HUF) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Sampling site	WET			Mean	DRY			Mean
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CI	3.00×10^3	6.60×10^4	6.80×10^4	4.57×10^4	3.80×10^2	3.90×10^2	3.70×10^2	3.80×10^2
I1	6.40×10^3	6.45×10^3	6.60×10^3	6.481×0^3	5.30×10^2	5.00×10^2	4.90×10^2	5.07×10^2

Sampling site	WET			Mean	DRY			Mean
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
I2	1.80×10^3	2.20×10^3	2.80×10^3	2.27×10^3	2.20×10^2	1.90×10^2	2.70×10^2	2.27×10^2
I3	3.80×10^3	7.80×10^3	8.40×10^3	6.67×10^3	3.40×10^2	2.90×10^2	3.30×10^2	3.20×10^2
CA	3.90×10^3	4.00×10^3	4.40×10^3	4.10×10^3	4.50×10^2	3.60×10^2	3.80×10^2	3.97×10^2
A1	2.00×10^3	3.00×10^3	3.30×10^3	2.77×10^3	1.70×10^2	1.90×10^2	3.00×10^2	2.20×10^2
A2	5.80×10^3	2.30×10^3	3.00×10^3	3.70×10^3	3.30×10^2	3.70×10^2	4.00×10^2	3.67×10^2
A3	3.20×10^3	2.80×10^3	3.30×10^3	3.10×10^3	2.50×10^2	2.80×10^2	3.00×10^2	2.77×10^2
CU	2.70×10^3	2.90×10^3	3.10×10^3	2.90×10^3	2.80×10^2	3.00×10^2	3.60×10^2	3.13×10^2
U1	2.20×10^3	3.60×10^3	4.40×10^3	3.40×10^3	3.60×10^2	3.10×10^2	3.30×10^2	3.33×10^2
U2	4.80×10^3	5.20×10^3	5.60×10^3	5.20×10^3	3.70×10^2	3.50×10^2	3.60×10^2	3.60×10^2
U3	6.40×10^3	4.70×10^4	5.00×10^4	3.45×10^4	4.30×10^2	4.10×10^2	5.10×10^2	4.50×10^2
Temp (℃)	27.00	26.00	26.00	26.50	28.00	28.00	28.00	28.00
ppt (mm)	126.00	242.00	635.00	334.33	0.00	0.00	0.00	0.00

3.7. Seasonal Variation in Percent Hydrocarbon Utilizing Fungi (% HUF)

There was increasing trend in population of % HUF from April to September. Locations Agricultural Control (CA), Mgbuoba (U3) and Onne (I1) recorded the highest levels of fungal population increase in the wet season with 66.55%, 55.88% and 29.99% respectively (Table 8). The minimal % value of HUF in wet season was 1.93% in Aluu (A1) and ranged from 1.93 % to 66.55 % in Agricultural Control (CA). There was a steady population of % HUB in the dry season ranging from 0.74% in Aluu (A1) to 13.20% in Control Agriculture (CA) (Table 8).

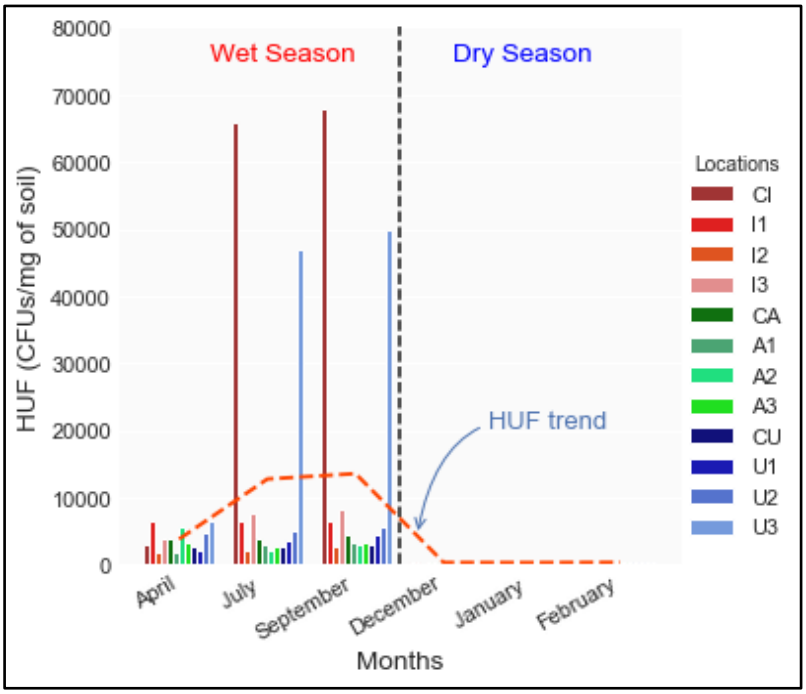

Figure 6. *Seasonal trend in populations of hydrocarbon utilizing fungi (HUF) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Table 8. *Seasonal variation in % hydrocarbon utilizing fungi (% HUF) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

Sampling site	**WET**			**Mean**	**DRY**			**Mean**
Month	**April**	**July**	**September**	**Mean Wet**	**December**	**January**	**February**	**Mean Dry**
CI	5.88	1.25	13.08	6.74	8.26	7.22	6.49	7.32
I1	4.20	7.20	78.57	29.99	1.61	1.22	0.94	1.26
I2	12.85	16.00	23.33	17.39	3.93	3.88	5.00	4.27
I3	5.00	10.26	11.51	8.92	8.29	8.79	9.17	8.75
CA	60.93	66.60	72.13	66.55	14.06	12.86	12.67	13.20
A1	1.29	2.14	2.36	1.93	0.68	0.66	0.88	0.74
A2	16.57	6.97	10.00	11.18	10.00	11.56	11.43	11.00
A3	37.65	35.00	40.24	37.63	5.56	5.71	6.38	5.88
CU	3.33	3.76	42.47	16.52	6.36	6.38	7.35	6.70
U1	15.71	25.17	29.33	23.40	11.25	8.61	8.68	9.51
U2	7.05	7.80	8.75	7.87	7.12	6.14	6.10	6.45
U3	9.00	78.00	80.64	55.88	11.62	10.51	11.86	11.33
Temp (℃)	27.00	26.00	26.00	26.50	28.00	28.00	28.00	28.00
ppt (mm)	126.00	242.00	635.00	334.33	0.00	0.00	0.00	0.00

3.8. Seasonal Trends in THB, HUB, TF, HUF, % HUB and % HUF

Figure 2 shows trend in THB populations over the wet and the dry seasons. Generally, there was a slight decrease in THB from April to July then an increase from July to September. There was a sharp decrease from September through the dry season to February. A stable population of THB was observed over the dry season. Figure 3 shows the trends in HUB population in the wet and the dry seasons. A constant population was observed from April to September which was followed by a sharp decline in population actress the dry season. A stable population of HUB was observed over the dry season. Figure 5 shows the overall TF trends observed in the wet and the dry seasons. There was an increase in population from April to July which was followed by a constant population from July to September and finally a decline in the TF populations from September to December. There was a stable population of TF in the dry season. Figure 6 shows trend in change of HUF population in the wet and the dry seasons. There was increase in population from April to July and a constant population from July to September which was followed by a decline in population from September to December. There was a stable population of HUF in the dry season. Figure 7 shows the trend changes in % HUB. The results show that there was a constant population all through the wet and the dry seasons. A stable trend was observed in wet and dry seasons. Figure 7 shows the general trend in changes in % HUF. There was an increase from April to September followed by a decrease from September to December. There was a stable population from December to February.

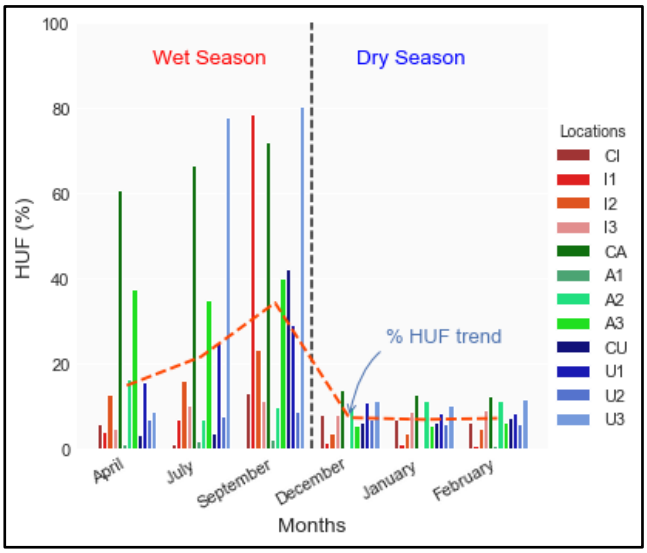

Figure 7. *Seasonal trend in percent hydrocarbon utilizing fungi (% HUF) in soil from agricultural, industrial and urban sites in Greater Port Harcourt Area, Rivers State, Nigeria.*

4. Discussion of Results

The present study evaluates the impact of the season on fungal and bacterial communities in the Greater Port Harcourt Area, Rivers State, Nigeria, in oil tainted soils. The findings indicate that the season has been found to influence the abundance and community structure of fungi and bacteria in the environment. Findings show that seasonality had major role in the resilience of microorganism population and diversity in oil tainted soils of the study sites [42]. Figures 2 and 4 show that the populations of THB and TF increase in the wet season and decrease in the dry season. The findings are in agreement with the findings of Salazar, Sulman, and Dukes [25], who show that climate has a significant role in microbial biomass. The results of this study also support the hypothesis that rainfall reductions significantly lower soil microorganism populations, with fungi being less affected than bacteria. Rainfall also has a minor impact on the composition of microorganism communities but a major impact on the quantity of microorganisms [22] as compared to influence by physicochemical parameters. Rainfall increases reduce soil respiration and soil organic carbon, which are positively correlated with shifts in the populations of microorganisms.

Figures 6 and 7 respectively show a contrast between % HUB and % HUF in oil tainted soils across a seasonal divide. From this observation, it is clear that the community structure of fungi and that of bacteria respond differently to seasonality in oil tainted soils. In this respect, microorganism communities respond variedly to different levels of precipitations in specified ecosystems with varied biotic and abiotic components [5, 20, 27]. These results are close to the findings of Ren et al. [22], which show that decreases in rainfall significantly reduces bacterial abundance by 1.0 %, but minimally suppresses fungal abundance. In comparison, in a separate situation, the relative abundance percentages for bacteria and fungi are both identical between non-contaminated soil and contaminated soil [39]. In a different scenario, microbial biomass is varied in different seasons being highest in spring following this sequence: spring> autumn> winter [17]. Different studies have utilized the components of microorganisms to show abundance in different seasons. The quantity of total bacteria actinomycin and fungal phospholipids fatty acid analysis (PLFAs) significantly varies with the seasons and follows the sequence order of; summer > autumn > spring > winter [44]. Siles and Margesin [30] also indicate greater diversity of microorganisms in autumn than spring and this is correlated with increased nutrient content observed in autumn compared to spring and the presumed high capacity of soil microbial communities to respond to distinct environmental changes in functional terms [30]. Therefore, soil and tillage factors have an effect on microorganisms (activity and biomass) and on the community structure [29]. Further, microbial biomass and respiration is higher in warmer as compared to cold season in turf grass ecosystem [43]. It is suggested that the primary determinant of seasonal dynamics of soil microbial biomass and operation in turf grass systems, located in the humid and warm zone, is the availability of nitrogen rather than climate [43]. Different ecosystems therefore harbour numerous microorganisms with different survival capacities. For instance, winter microbe communities demonstrate a greater potential for cellulose degradation than summer cultures [18].

Therefore, as much as the findings of the current study show decline in populations across the wet to the dry seasons, there is still much uncertainty surrounding description of patterns of microorganism communities to change in rainfall patterns in context of different contaminants in the environment [28, 40, 41]. Since distribution and abundance of soil microorganisms is dependent on both biotic and abiotic factors, it is advisable to consider both precipitation and physicochemical parameters of soil, as a decrease in solute mobility constrains the availability of nutrients therefore it inhibits flourishment of microorganisms in soil. However, some microorganisms are tolerant to water stress [23, 45] while others are not. The primary impact of seasonal variation on the microbial soil population largely depends on changes occurring at the spatial scale to which the microorganisms are most susceptible. In general, the analysis of individual components yields more data than the general analysis.

5. Conclusions

In conclusion, seasonal changes affect microbial community structure, soil properties and microbial populations. We observed a distinct seasonal shift for microbial populations in oil tainted soils. Seasonality influenced % HUF and % HUB in the soils of urban, industrial and agricultural sites of Greater Port Harcourt Area. However, the results show that there was a stable trend in % HUB as compared to oscillations observed in % HUF in oil tainted soils across a seasonal divide. Generally, the seasonality trends showed that there was a declining population of THB, HUB, TF, and HUF from the wet season to the dry season. We recommend characterization of the microorganism to identify the best candidate for bioremediation of oil tainted soils across a seasonal divide (Wanjala et al., BUC Conference).

Acknowledgments

In accordance with the World Bank's directive to establish the African Center of Excellence at Port Harcourt University in Nigeria, this study was conducted as part of the PhD program at the World Bank African Centre of Excellence for Oilfield Chemicals Research. The authors further thank Professor Ikechukwu O. Agbagwa of the University of Port Harcourt and the Regional Universities Forum for Capacity Building in Agriculture (RUFORUM) for their ongoing financial assistance and mentoring under the Carnegie Post-Doctoral fellowship.

Funding

Financial assistance and mentoring of this research was under the Carnegie Post-Doctoral fellowship, which included grants supporting the research work.

Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Alkorta, I., Epelde, L., and Garbisu, C., (2017). Environmental parameters altered by climate change affect the activity of soil microorganisms involved in bioremediation. FEMS Microbiology Letters. 364(19), 1–9. https://doi.org/10.1093/femsle/fnx200

[2] American Public Health Association [APHA]., (1995). Standard Method for Examination of Water and Waste Water. 19th Edition, Published by E. and F. N. Poan, Washington D.C.; 2-56.

[3] 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.

[4] Bai, R., Xi, D., He, J. Z., Hu, H. W., Fang, Y. T., and Zhang, L. M., (2015). Activity, abundance and community structure of anammox bacteria along depth profiles in three different paddy soils. Soil Biology and Biochemistry. 91, 212–221. https://doi.org/10.1016/j.soilbio.2015.08.040

[5] Beier, C., Beierkuhnlein, C., Wohlgemuth, T., Penuelas, J., Emmett, B., Korner, C., de Boeck, H. J., Christensen, J. H., Leuzinger, S., Janssens, I. A., and Hansen, K., (2012). Precipitation manipulation experiments - challenges and recommendations for the future. Ecology Letters 15, 899–911.

[6] Bradford M, Davies C, Frey S et al., (2008). Thermal adaptation of soil microbial respiration to elevated temperature. Ecol Lett; 11: 1316–27.

[7] Brockett, B. F. T., Prescott, C. E., and Grayston, S. J., (2012). Soil moisture is the major factor influencing microbial community structure and enzyme activities across seven biogeoclimatic zones in western Canada. Soil Biology and Biochemistry. 44(1), 9–20. https://doi.org/10.1016/j.soilbio.2011.09.003

[8] Buckeridge, K. M., Banerjee, S., Siciliano, S. D., and Grogan, P., (2013). The seasonal pattern of soil microbial community structure in mesic low arctic tundra. Soil Biology and Biochemistry, 65, 338–347. https://doi.org/10.1016/j.soilbio.2013.06.012

[9] Canarini, A., Kiaer, L. P., and Dijkstra, F. A., (2017). Soil carbon loss regulated by drought intensity and available substrate: a meta-analysis. Soil Biology and Biochemistry. 112, 90–99.

[10] Chen, J., Luo, Y. Q., Xia, J. Y., Jiang, L. F., Zhou, X. H., Lu, M., Liang, J. Y., Shi, Z., Shelton, S., Cao, J. J., (2015). Stronger warming effects on microbial abundances in colder re- gions. Scientific Reports 5, 18032.

[11] Chen, X., Deng, Q., Lin, G., Lin, M., and Wei, H., (2018). Science of the Total Environment Changing rainfall frequency affects soil organic carbon concentrations by altering non-labile soil organic carbon concentrations in a tropical monsoon forest. Science of the Total Environment, 644, 762–769. https://doi.org/10.1016/j.scitotenv.2018.07.035

[12] Classen, A. T., M. K. Sundqvist, J. A. Henning, G. S. Newman, J. A. M. Moore, M. A. Cregger, L. C. Moorhead, and C. M. Patterson, (2015). Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead? Ecosphere 6(8): 130. http://dx.doi.org/10.1890/ES15-00217.1

[13] Davidson, E. A., and Janssens, I. A., (2006). Temperature sensitivity of soil carbon decomposition and feedbacks to climate change. Nature 440, 165–173.

[14] Evans, S. E., and Wallenstein, M. D. (2014). Climate change alters ecological strategies of soil bacteria. Ecology Letters 17, 155-164.

[15] Geisseler, D., Linquist, B. A., and Lazicki, P. A., (2017). Soil Biology and Biochemistry Effect of fertilization on soil microorganisms in paddy rice systems e A. Soil Biology and Biochemistry, 115, 452–460. https://doi.org/10.1016/j.soilbio.2017.09.018

[16] Itziar Alkorta, Lur Epelde, Carlos Garbisu, (2017). Environmental parameters altered by climate change affect the activity of soil microorganisms involved in bioremediation, FEMS Microbiology Letters, Volume 364, Issue 19, fnx200, https://doi.org/10.1093/femsle/fnx200

[17] Jiang, X., Shi, X., & Wright, A. L. (2013). European Journal of Soil Biology Seasonal variability of microbial biomass associated with aggregates in a rice-based ecosystem. European Journal of Soil Biology, 56, 84–88. https://doi.org/10.1016/j.ejsobi.2013.03.004

[18] Koranda, M., Kaiser, C., Fuchslueger, L., Kitzler, B., Sessitsch, A., Zechmeister-Boltenstern, S., & Richter, A. (2013). Seasonal variation in functional properties of microbial communities in beech forest soil. Soil Biology and Biochemistry, 60, 95–104. https://doi.org/10.1016/j.soilbio.2013.01.025

[19] Liu, L. L., Wang, X., Lajeunesse, M. J., Miao, G. F., Piao, S. L., Wan, S. Q., Wu, Y. X., Wang, Z. H., Yang, S., Li, P., Deng, M. F., (2016). A cross-biome synthesis of soil respiration and its determinants under simulated precipitation changes. Global Change Biology 22, 1394 -1405.

[20] Nielsen, U. N., and Ball, B. A., (2015). Impacts of altered precipitation regimes on soil communities and biogeochemistry in arid and semi-arid ecosystems. Glob. Chang. Biol. 21, 1407e1421. https://doi.org/10.1111/gcb.12789

[21] Pratt B, Riesen R, Johnston CG (2012) PLFA analyses of microbial communities associated with PAH-contaminated riverbank sediment. Microbial Ecology 64: 680-691. https://doi.org/10.1007/s00248-012-0060-8

[22] Ren, C., Chen, J., Lu, X., Doughty, R., Zhao, F., Zhong, Z., … Ren, G. (2018). Responses of soil total microbial biomass and community compositions to rainfall reductions. Soil Biology and Biochemistry, 116(September 2017), 4–10. https://doi.org/10.1016/j.soilbio.2017.09.028

[23] Riah-Anglet, W., Trinsoutrot-Gattin, I., Martin-Laurent, F., Laroche-Ajzenberg, E., Norini, M. P., Latour, X., and Laval, K., (2015). Soil microbial community structure and function relationships: a heat stress experiment. Applied Soil Ecology. 86, 121–130.

[24] Romero-Olivares, A. L., Allison, S. D., Treseder, K. K., (2017). Soil microbes and their re- sponse to experimental warming over time: a meta-analysis of field studies. Soil Biology and Biochemistry 107, 32–40.

[25] Salazar, A., Sulman, B. N., and Dukes, S. (2018). Microbial dormancy promotes microbial biomass and respiration across pulses of drying-wetting stress. Soil Biology and Biochemistry, 116 (September 2017), 237–244. https://doi.org/10.1016/j.soilbio.2017.10.017

[26] Schrama, M., and Bardgett, R. D., (2016). Grassland invasibility varies with drought effects on soil functioning. Journal of Ecology 104, 1250–1258.

[27] Serna-Chavez, H. M., Fierer, N., and van Bodegom, P. M., (2013). Global drivers and patterns of microbial abundance in soil. Global Ecology and Biogeography. 22, 1162–1172.

[28] Shen, C. et al. (2016). Dramatic increases of soil microbial functional gene diversity at the treeline ecotone of Changbai Mountain. Front. Microbiol. 7, 1184, https://doi.org/10.3389/fmicb.2016.01184 (2016).

[29] Shi, Y., Lalande, R., and Hamel, C. (2013). Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. Biol Fertil Soils. https://doi.org/10.1007/s00374-013-0773-y

[30] Siles, J. A., and Margesin, R. (2017). Seasonal soil microbial responses are limited to changes in functionality at two Alpine forest sites differing in altitude and vegetation. Scientific Reports, (February), 1–16. https://doi.org/10.1038/s41598-017-02363-2

[31] Siles, J. A., and Margesin, R. (2018). Insights into microbial communities mediating the bioremediation of hydrocarbon-contaminated soil from an Alpine former military site. Applied microbiology and biotechnology, 102(10), 4409–4421. https://doi.org/10.1007/s00253-018-8932-6

[32] Singh, S., and Kumar, M., (2010). Heavy metal load of soil, water and vegetables in peri-urban Delhi, Environmental Modeling and Assessment. vol. 120, no. 1-3, pp. 79–91, 2006.

[33] Sotomayor-Ramírez D, Espinoza Y, Acosta-Martinez V (2009) Land use effects on microbial biomass C, beta -glucosidase and beta -glucosaminidase activities, and availability, storage, and age oforganic C in soil. Biol Fertil Soils 45: 487–497.

[34] Szilas, C. P., Borggard, O. K., and Hansedn, H. C. B., (1998). Potential iron and phosphate mobilization during flooding of soil material. Water Air Soil Pollut. 106, 97–109.

[35] Tian, J., Dong, G., Karthikeyan, R., Li, L., and Harmel, R. D., (2017). Phosphorus Dynamics in Long-Term Flooded, Drained, and Reflooded Soils. Water. 9, 531. https://doi.org/10.3390/w9070531

[36] Torsvik, V., and Ovreas, L., (2008). Microbial diversity, life strategies, and adaptation to life in extreme soils. In: Microbiology of Extreme Soils, vol. 13. Springer, Berlin, Heidelberg, pp. 15e43.

[37] Vig, A. C., and Dev, G., (1984). Phosphorus adsorption characteristics of some acid and alkaline soils. J. Indian Soc. Soil Sci. 32, 235-239.

[38] Wanjala Muyoma Paul, Odokuma Lucky, Etela Ibisime, Ramkat Rose, Blessing Adanta Odogwu, Boadu Kwasi Opoku, Koranteng-Addo Epton Joseph (2021). Impact of Nitrogen Fertilizer Use on Soil Ecosystem Integrity in the Greater Port Harcourt Region, Nigeria. International. Journal of Biochemistry, Biophysics & Molecular Biology. Vol. 6, No. 2, 2021, pp. 42-51. https://doi.org/10.11648/j.ijbbmb.20210602.13

[39] Wu, B., Hou, S., Peng, D., Wang, Y., Wang, C., Xu, F., & Xu, H. (2018). Response of soil micro-ecology to different levels of cadmium in alkaline soil. Ecotoxicology and Environmental Safety, 166(May), 116–122. https://doi.org/10.1016/j.ecoenv.2018.09.076

[40] Xi, N. X. and Bloor, J. M. G., (2016). Interactive effects of precipitation and nitrogen spatial pattern on carbon use and functional diversity in soil microbial communities. Applied Soil Ecology 100, 207-210.

[41] Xia, L., Lam, S. K., Yan, X., and Chen, D., (2017). How does recycling of livestock manure in agroecosystems affect crop productivity, reactive nitrogen losses and soil carbon balance? Environ. Sci. Technol. 51 (13), pp 7450–7457 1–28. https://pubs.acs.org/doi/10.1021/acs.est.6b06470

[42] Xie, Y., Fan, J., Zhu, W., Amombo, E., Lou, Y., Chen, L., and Fu, J., (2016). Effect of Heavy Metals Pollution on Soil Microbial Diversity and Bermudagrass Genetic Variation. Front. Plant Sci. 7: 755. https://doi.org/10.3389/fpls.2016.00755

[43] Yao, H., Bowman, D., and Shi, W., (2011). Seasonal variations of soil microbial biomass and activity in warm- and cool-season turfgrass systems. Soil Biology and Biochemistry, 43(7), 1536–1543. https://doi.org/10.1016/j.soilbio.2011.03.031

[44] Ze, W. U., Wen-xiong, L. I. N., & Lin, W. U. (2016). Effect ts of sea asonal variatio v ons on soil s mic crobial c commu unity comp osition of two typical zonal vegetati v ion type es in the e Wuyi Mount ains. J. Mt. Sci., (2), 1–10.

[45] Zeglin, L. H., Bottomley, P. J., Jumpponen, A., Rice, C. W., Arango, M., …… Myrold, D. D., (2013). Altered precipitation regime affects the function and composition of soil microbial communities on multiple time scales. Ecology 94, 2334–2345.

[46] Zhang, J., Li, M., Liu, S., Liu, Y. J., Zhang, L. Q., Cao, Q., and Sun, D. Z. (2011). Seasonal variations and bioavailability of inorganic phosphorus in soils of Yeyahu Wetland in Beijing, China. Intl. J. Sediment Res., 26, 181–192.

[47] Zhou, J., Gu, B., Schlesinger, W. H., and Ju, X., (2016). Significant accumulation of nitrate in Chinese semi-humid croplands. Scientific Reports, 6, 1-8. https://doi.org/10.1038/srep25088

[48] Zumsteg, A., Baath, E., Stierli, B., Zeyer, J., and Frey, B., (2013). Bacterial and fungal community responses to reciprocal soil transfer along a temperature and soil moisture gradient in a glacier forefield. Soil Biology and Biochemistry, 61, 121–132. https://doi.org/10.1016/j.soilbio.2013.02.017