Seasonal Influences On The Physicochemistry And Microbiology Of Soils In Industrial Areas In Port Harcourt Area

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Abstract: The seasonal influence on the physicochemistry and microbiology of soils in industrial areas in Port Harcourt was investigated. The study sites were categorized into four: high (Trans-Amadi, A), moderate (Waterlines, B), very low (Choba, C) and low (Mile 1, D) industrial areas. The control (Emohua, E) was taken as non-industrial area. Soil samples were collected at the depth of 0-3 using auger into polythene bags and transported to the laboratory for analyses. The mean seasonal variation of total heterotropic bacterial (THB) and hydrocarbon utilizing bacterial (HUB), total fungal (TF) and hydrocarbon utilizing fungal (HUF) counts during the dry season showed that the highly industrialized area (A) had more HUB (27.5%) than the none industrialized area (E) which had 0.8%. The HUF was higher (0.9%) in "A" than in "E" (0.5%). The % HUF indicated that "A" with value of 0.9% was higher than "C" (0.6%). Bacterial genera isolated included *Staphylococcus, Serratia Micrococcus, Escherichia coli, Pseudomonas, Citrobacter,* and *Bacillus* while fungal, genera characterized were *Mucor, Aspergillus, Candida, Penicillium, Rhizopus, Trichosporon* and *Sacharomyces.* Total petroleum hydrocarbon, oil and grease, total organic carbon, sulphate and magnesium concentrations were also higher in industrialized areas in both seasons. Similarly heavy metals analyses indicated that zinc, nickel, iron and chromium were significantly *(P<0.05)* higher in industrialized areas than others. The physiochemistry and heavy metals were generally higher in the soil during dry season. There was interplay between soil micro flora and physiochemical parameters. Industrial activities impacted negatively on microbial abundance and diversity in soil environment.
[R. Wosu Kinika and L.O. Odokuma. **Seasonal Influences on the Physicochemistry and Microbiology of Soils in Industrial Areas in Port Harcourt Area.** *Nat Sci* 2016;14(1):30-39]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 5. doi:10.7537/marsnsj140116.05.

Key words: Soil environment, microbial abundance and diversity, industrialized areas, Port Harcourt, seasonal variation, soil micro flora.

1. Introduction

Port Harcourt is perceived as being synonymous with oil producing areas in Nigeria, with majority of the oil industries citied in the area. The industries consist of mostly European and United States owned companies that are operating in joint venture with the Nigerian National Petroleum Corporation (NNPC) which has ownership of over 55-60% interest in the venture. These industries include the Shell Petroleum Development Company (SPDC) of Nigeria Ltd, Mobil, Chevron Nigeria Ltd, Total Nigeria Limited, Nigeria Agip Oil Company (NAOC), Texaco Nigeria Limited and the subsidiaries (both local and international). Contributing to the menace of indiscriminate discharges of industrial effluents in receiving soil and water bodies is the improper disposal of domestic wastes, particularly in urban centres of most developing countries. Open and indiscriminate dumping of solid wastes in drainages and river banks is one of the most critical problems facing the city of Ibadan (Omoleke, 2004). Excessive production of organic matter leads to the buildup of 'sludge' and the mineralization process consumes all dissolved oxygen from the water column, which causes fish kills (Osibanjo *et al*., 2011).

Industrial soils are specific system developed under the impact of a wide range of natural and artificial factors of disturbance. Their stability strongly depends on the evolution of the biotic component represented by soil microbial community that promotes the majority of the main biosphere cycles in which the soil is involved. All sector of industry and business leave on imprint on the environment as a result of their use of energy or raw materials and the production of waste or effluents that will be found in the natural environment (Henry and Heinke, 1996).

Soil, like any other natural resource, is not just of public health and economic value, but it is home for life. Many case studies from heavily concentrated industrial soils around the world, particularly (but by no means exclusively) in developing countries, indicate the possible environmental and health impacts of high levels of soil contamination resulting from the activities of these industries.

Microbial populations are a quick and effective indication of changing environmental parameters in

the form of shifts in microbial diversity and abundance (species richness and evenness). Changes in the relative abundance of particular groups of microorganisms indicate that the environment has been altered (Rosello-Mora and Amann, 2001; Tate, 2000; Lenat-Boron and Boron, 2012). Microorganisms are able to do this because they are much more sensitive to selective pressures (in the form of a changing environment), and are able to adapt quickly (EPA, 2012).

In as little as 20 minutes, some microbes are capable of 4 simultaneous mutations in every gene and doubling in cell count. Consequently, a microbial population can produce a next generation in only a few hours as compared to weeks, months or years for higher organisms.

Different microbial groups inhabit various niches within an ecosystem and therefore differ in their sensitivity to nutritional and environmental change (Giller *et al.,* 1998). In both field and laboratory experiments, structural changes in different microbial communities under heavy metal stress were observed (Frostegard et al., 1993b; Pennanen *et al*., 1996; Moffet *et al*., 2003; Abaye *et al*., 2005; Macdonald *et al*., 2007). A healthy soil has several physical, chemical and biological properties: it needs to incorporate adequate organic matter, have a good structure, and be home to adverse group of organisms.

Agricultural soil health is linked to human health, as poor soils yield fewer crops with decreased nutritional value. The anthropogenic influences, stemming from increased industrialization, oil exploration activities, sabotage and illegal refining of petroleum (Nwaichi *et al*., 2010) in oil-rich region, on biogeochemical cycles could impede on soil quality evaluation (Akpahwe and Solomon, 2012). Soil is a living resource, but once contamination exceeds a certain threshold, the soil may be considered 'functionally dead'. Pollution by heavy metals and many organic contaminants is practically irreversible (European commission, 2012).

Heavy metal contamination result in reduction of microbial biomass disturbs the community structure (Doelman, 1986). Metal exposure may lead to the establishment of tolerant microbial populations, which are often represented by several Gram-positive genera such as *Bacillus, Arthrobacter* and *Corynebacterium* or Gram-negative bacteria such as *Pseudomonas, Ralstonia* or *Burkholderia* (Okerentugba and Ezeronye, 2003; Piotrowaka – Seget *et al*., 2005). Community structure is an important aspect of the microbial biomass as the microbial community structure is the parameter controlling microbial activity (Ramsey *et al*., 2005) and influences ecosystem functioning (Gadd, 2008; Kiikkila, 2003).

Contamination can seriously affect soil's ability to perform some of its key functions in the ecosystem (Brevik *et al*., 2013; Onyema *et al.,* 2013). Ecosystem system functioning is incomplete without soil microorganism, as they affect the chemical, biological and physical characteristic of soil. Because microbial community regulates decomposition processes and nutrient cycling, it is of keen interest to understand how its structure is affected by industrial pollution stress.

The study therefore, evaluates the impact of industrial activities on seasonal variation of microbial diversity and abundance in some soils in the Port Harcourt areas of Rivers State.

2. Materials And Methods

2.1 Samples collection

Soil samples were collected from five different areas within Port Harcourt and its environments. These study areas includes Trans-Amadi area, Mile 1 area, Waterline area, Emuoha area, and Choba area of Port Harcourt, Rivers State, Nigeria. Periodic sampling of the various areas was undertaken, the sites were sampled in dry (December – February) and wet (March – May), 2015.

At each site, three replicate bulk samples were taken, consisting of 30 randomly selected sub-samples from surface soil (0-30cm depth) using a manual auger into polythene bags. The samples were transported to the laboratory for microbiological, gas chromatographic and physicochemical analyses within 24-48h.

2.2 Experimental design

Five (5) experimental treatments setup showing sample code, study area and study area description are as presented in Table 1.

Table 1: Description of experimental treatment design

Sample code	Study area	Study area description
MIA	Mile 1area	Low Industrial area
TMA	Trans-Amadi area	High Industrial area
EMA	Emuoha area	None Industrial area (Pristine control)
WLA	Waterlines area	Moderate Industrial area
CBA	Choba area	Very low Industrial area

*MIA: Mile 1 area, TMA: Trans-Amadi area, EMA: Emuoha area, WLA: Waterlines area, CBA: Choba area

2.4 Sample processing

Soil slurry was prepared by adding one gram (1g) of the soil samples aseptically to each test tube containing nine milliliters (9ml) of sterile saline (0.85% w/v) as diluents.

The test tubes were vigorously shaken to dislodge the microorganisms that might adhere to the soil particles. The content of the tubes were diluted (10-fold). From each dilution of 10^{-3} to 10^{-6}, 0.1ml was plated on sterile Nutrient agar, Saboraud dextrose agar and Mineral salt agar for microbial enumeration.

2.5 Microbiological analyses

2.5.1 Enumeration of total culturable heterotrophic bacterial and fungal counts

Aliquots (0.1ml) of the serially diluted soil samples were spread-plated out in duplicates on nutrient agar plates following the method previously described by Chikere *et al*. (2009). The plates were incubated at 35^0C for 24 to 48h for total culturable heterotrophic bacteria count (TCHBC). Also, for total heterotrophic fungi count (THFC), the same procedures were followed but 1ml of lactic acid was added in the Saboraud dextrose agar medium to inhibit the growth of bacteria.

2.5.2 Enumeration of hydrocarbon utilizing bacterial and fungal counts

Hydrocarbon utilizing bacterial count (HUBC) was enumerated using a modified mineral salt medium of Mills *et al*. (1978). It contained: $MgSO_4.7H_2O$, 0.40g; KCl, 0.28g; KH_2PO_4, 0.80g; Na_2HPO_4, 1.20g; NH_4NO_3, 0.40g, NaCl, 15g; agar No. 2, 20g in 1 liter of de-ionized water.

The pH was adjusted to 7.1 and media autoclaved at 121^0C for 15 min. Soil slurry was prepared and used for serial 10-fold dilution by mixing 1g of wet soil with 9ml of sterile saline suspension. Crude oil was added by soaking a 9cm Whatman No. 1 filter paper with 10 ml of fresh Bonny light crude oil. The flooded filter paper was then placed on the lid of the agar plate and incubated for 7 days at 25±8 ^{0}C in an inverted position following the method of Abu and Ogiji (1996).

For hydrocarbon utilizing fungal count (HUFC), the same procedure was followed except that 1ml of lactic acid was added in the modified mineral salt medium to inhibit the growth of bacteria. The filter papers placed on the lid of the agar plate served as a source of energy and carbon and supplied the hydrocarbons by vapour-phase transfer to inverted inoculums.

2.6 Physicochemical analyses

The American Public Health Association (APHA) method was adopted following standard analytical procedures (FEPA, 1991; APHA, 1998; AOAC, 1990). The parameters analyzed were pH, alkalinity, phosphate content, nitrate content, total organic carbon, total petroleum hydrocarbon and heavy metals such as copper (Cu), zinc (Zn), iron (Fe), lead (Pb), chromium (Cr) and nickel (Ni).

2.7 Identification of isolates

Bacterial isolates were examined for colonial morphology and biochemical characteristics. Test employed include Gram staining, motility test, catalase test, citrate utilization, indole test, voges proskauer test, oxdase test and sugar fermentation test. Confirmatory identities of the bacteria were made using the *Bergey's Manual of Determinative Bacteriology* (Holt *et al*., 1994; Cheesbrough (2004).

2.8 Statistical Analysis

The statistical tool- Two ways analysis of variance (ANOVA) was used to analyze the data obtained from the study to determine the level of significance (Zar, 1999). A value of $P<0.05$ was accepted as significant and $P>0.05$ was considered are not.

3. Results

The result of seasonal variation of the total culturable heterotrophic bacterial counts (TCHBC), total culturable heterotrophic fungal counts (TCFC), hydrocarbon utilizing bacterial count (HUBC) and hydrocarbon fungal count (HUFC) during the dry and wet seasons are as in Figs 1−4 while Fig. 5 indicated the results of the total petroleum hydrocarbon concentration in the investigated soil during the study period.

Results obtained for the seasonal variation and percentage occurrence of all and single bacteria isolates in the soil environment during the dry and wet seasons are as represented in Tables 2 and 3 while that of fungal population are as shown in Tables 4 and 5 respectively.

Seasonal variations were observed in the concentrations of various soil parameters, including those whose concentrations were related or unrelated to petroleum effluent discharge. While most of the parameters showed higher concentrations during dry season when water volume is decreased to its maximum, phosphate and nitrate which is components of agricultural fertilizers commonly used by farmers in the Port Harcourt area, showed reduced concentration in the wet season.

Phosphate level in the soil has been associated with oil operations because of the changes in concentration during the dry season which suggests an additional source of nutrients in the soil. Results of physicochemical analysis during the dry and wet seasons are presented in Table 6 while the seasonal variation heavy metals concentration in soil during the study is given in Table 7.

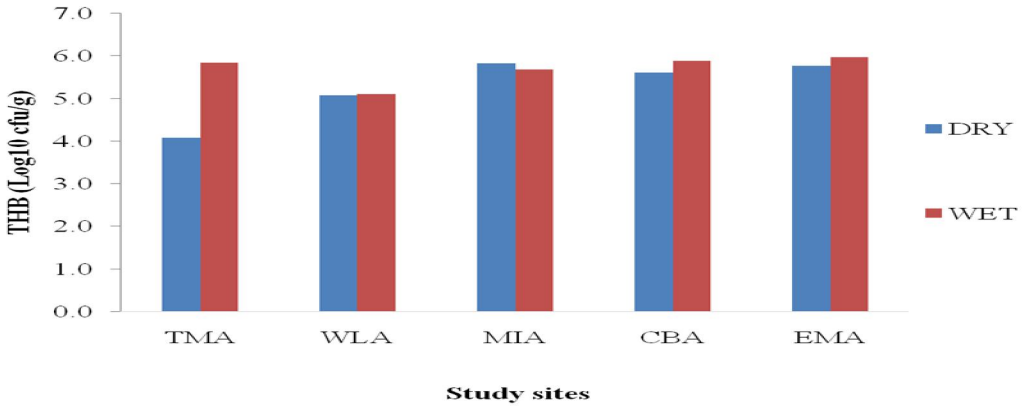

Fig.1: Seasonal variation of total heterotrophic bacterial count during the study season

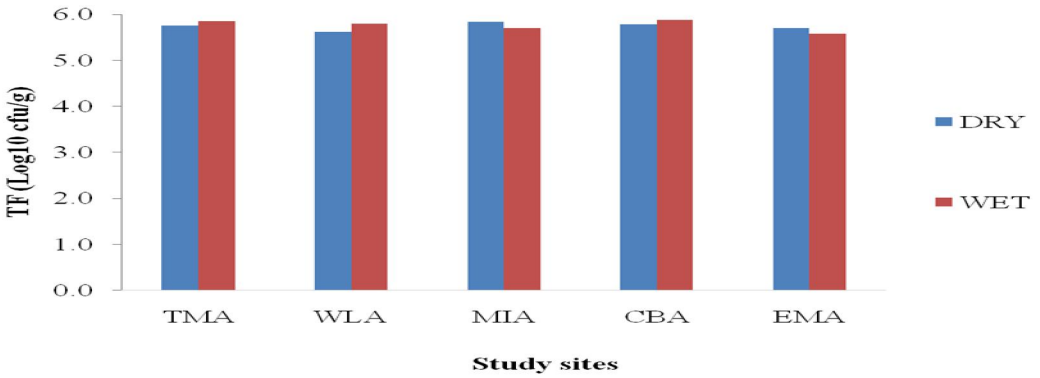

Fig.2: Seasonal variation of total fungal count during the tudy season

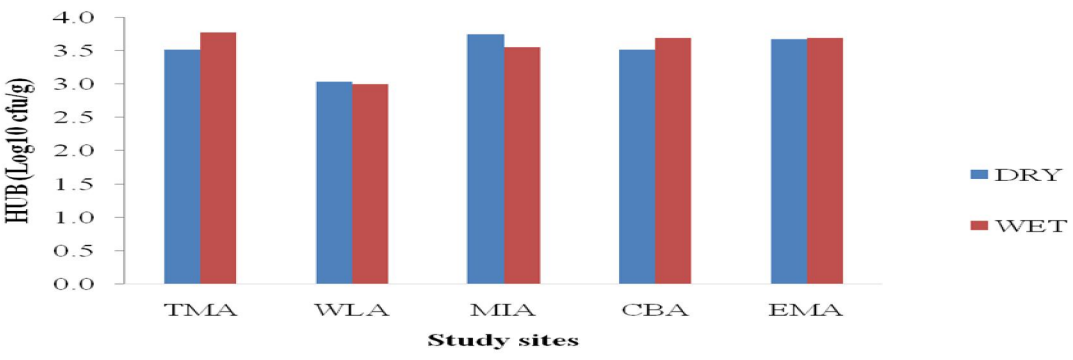

Fig.3: Seasonal variation of hydrocarbon utilizing bacterial count during the study seasons

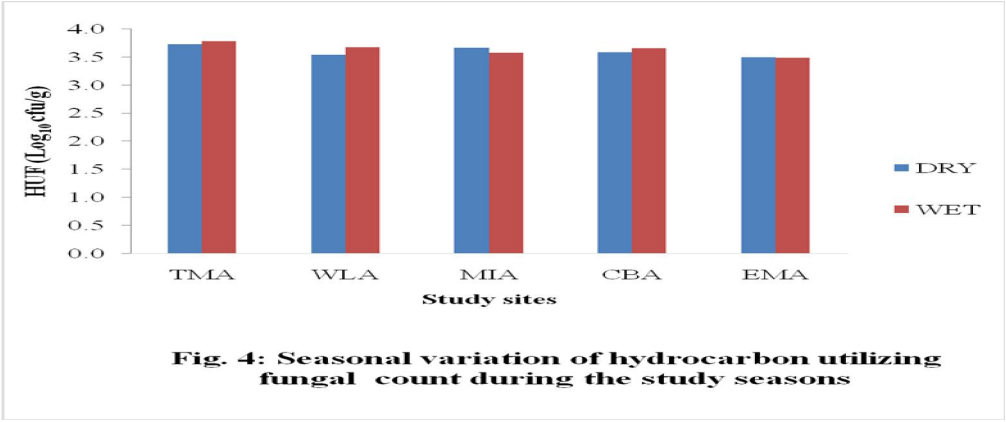

Fig. 4: Seasonal variation of hydrocarbon utilizing fungal count during the study seasons

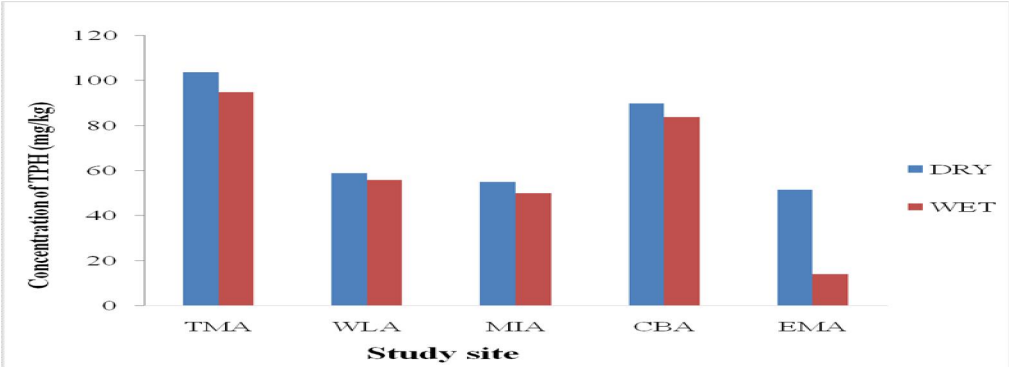

Fig. 5: Seasonal variation of total petroleum hydrocarbon (TPH) during the study season

Table 2: Seasonal variation in bacterial population during the dry season

Isolate	TMA	WLA	MIA	CBA	EMA	Occurrence/ frequency	% occurrence of all isolate
Citrobacter	+	+	-	-	-	2	40
E. coli	+	-	+	-	+	3	60
Micrococcus	+	-	-	-	-	1	20
Pseudomonas	-	-	-	+	-	1	20
Staphylococus	+	+	+	+	+	5	100
No. of Organisms	**4**	**2**	**2**	**2**	**2**		
% of single isolate	**80**	**40**	**40**	**40**	**40**		

CBA, Choba area,M1A, Mile 1 area,TMA, Trans-Amadi area, WLA, Waterlines area, EMA, Emuoha area, Mg/kg: milligram per kilogram, − (negative), + (positive)..

Table 3: Seasonal variation in bacterial population during the wet season

Isolate	TMA	WLA	MIA	CBA	EMA	Occurrence/ Frequency	% occurrence of all isolate
Bacillus	+	-	-	+	+	3	42.9
Citrobacter	+	+	-	-	-	2	28.6
E. coli	+	-	+	-	+	3	42.9
Micrococcus	+	-	+	-	-	2	28.6
Pseudomonas	-	+	-	+	-	2	28.6
Serratia	+	-	-	-	-	1	14.3
Staphylococus	+	+	+	+	+	5	71.4
No. of Organism	**6**	**3**	**3**	**3**	**3**		
% of single isolate	**85.7**	**42.9**	**42.9**	**42.9**	**42.9**		

Table 4: Seasonal variation in fungal population during the dry season

Isolate	TMA	WLA	MIA	CBA	EMA	Occurrence/Frequency	% occurrence of all isolate
Aspergillus	+	+	-	+	-	3	60
Candida	-	-	+	-	-	1	20
Mucor	-	+	+	-	-	2	40
Penicillum	-	-	-	-	+	1	20
Saccharomyces	-	-	-	+	-	1	20
% of single isolate	**1**	**2**	**2**	**2**	**1**		
	20	**40**	**40**	**40**	**20**		

Table 5: Seasonal variation in fungal population during the wet season

Isolates	TMA	WLA	MIA	CBA	EMA	Occurrence/Frequency	% occurrence of all isolate
Aspergillus	+	+	-	+	-	3	42.9
Candida	+	-	+	-	-	2	28.6
Mucor	-	-	+	-	-	1	14.3
Penicillum	-	-	-	+	+	2	28.6
Rhizopus	-	+	+	-	-	2	28.6
Saccharomyces	-	-	-	+	-	1	14.3
Trichosporon	+	-	-	-	-	1	14.3
% of single isolate	**3**	**2**	**3**	**3**	**1**		
	42.9	**28.6**	**42.9**	**42.9**	**14.3**		

Table 6: Seasonal variation of physiochemical parameters during the dry and wet season

Study sites	pH		TOC (mg/kg)		O/G (mg/kg)		NO_3 (mg/kg)		PO_4 (mg/kg)		SO_4 (mg/kg)		Ca (mg/kg)		Na (mg/kg)	
	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Dry	DRY	WET	DRY	WET
TMA	4.35	4.38	0.546	0.536	950.0	940.0	5.0	4.5	13.0	12.8	25.0	24.5	0.986	0.977	0.052	0.051
WLA	6.71	6.72	0.059	0.056	7.0	6.8	3.0	2.9	12.0	11.6	17.0	16.6	0.532	0.522	0.038	0.037
MIA	4.91	4.93	0.429	0.419	4.5	5.2	4.2	4.1	8.0	7.6	20.0	19.7	1.186	1.184	0.081	0.080
CBA	6.91	6.98	1.619	1.616	4.0	3.7	3.9	3.5	10.4	10.1	21.0	20.2	1.346	1.344	0.064	0.063
EMA	6.90	6.93	0.215	0.214	4.0	3.7	2.7	2.8	10.5	9.7	12.9	11.9	0.847	0.838	0.840	0.830

CBA,Choba area,**M1A,** Mile 1 area,**TMA,** Trans-Amadi area, **WLA,** Waterlines area, **EMA,** Emuoha area, **Mg/kg:** milligram per kilogram.

Table 7: Seasonal variation heavy metals concentration in soil during the study seasons

	Copper (mg/kg)		Zinc (mg/kg)		Iron (mg/kg)		Lead (mg/kg)		Chromium (mg/kg)		Nickel (mg/kg)	
Study sites	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry	Dry
TMA	15.7	15.5	6.6	6.4	13066	12065	35.7	34.6	15.8	14.9	9.2	9.1
WLA	12.65	12.45	5.9	5.7	41.25	40.22	50	48.3	8.7	8.6	11.5	10.6
MIA	14	13	17.2	16.8	980	978	15.6	14.5	14.5	14.2	4.3	3.8
CBA	1.4	1.38	5	4	22	21	4.6	4.5	1.3	1	1.9	1.7
EMA	9.12	9.11	2.8	2.6	23.7	23.5	22.3	2.2	1.3	1.2	0.9	0.7

CBA, Choba area, **M1A,** Mile 1 area, **TMA,** Trans-Amadi area, **WLA,** Waterlines area, **EMA,** Emuoha area, **Mg/kg:** milligram per kilogram

4. Discussion

Fig. 1 indicated that Choba (CBA) had total heterotrophic bacteria (THB) counts of 4.10 and 7.80 x 10^5 cfu/g, Mile 1 (MIA) had 6.80 and 4.90 cfu/g while the Trans-Amadi (TMA) had 1.20 x 10^4 and 7.00 x 10^5 cfu/g, Waterlines (WLA) gave values of 1.20 and 1.30 x 10^5 cfu/g while counts of 5.90 and 9.40 x 10^5 cfu/g were obtained for Emuoha (EMA). Total heterotrophic fungi (THF) counts (fig. 2) had 6.00 and 7.00 x 10^5 for dry and wet seasons in CBA, 6.90 and 5.00 x 10^5 cfu/g (MIA), 5.60 and 7.00 x 10^5 cfu/g (TMA) respectively during the study period.

In WLA, 4.20 and 6.20 x 10^5 cfu/g was obtained while counts of 5.00 and 3.80 x 10^5 cfu/g were recorded in EMA for both seasons.The hydrocarbon utilizing bacteria (HUB) counts (fig. 3) were 3.30 and 4.90 x 10^3 cfu/g (CBA), 5.60 and 3.60 x 10^3 cfu/g (MIA), 3.30 and 6.00 x 10^3 cfu/g (TMA), 1.10 and 1.00 x 10^3 cfu/g (WLA) and 4.70 and 4.90 x 10^3 cfu/g in EMA were recorded in both dry and wet seasons.

The hydrocarbon utilizing fungi (HUF) counts (fig. 4) indicated values of 3.80 and 4.50 x 10^3 cfu/g (CBA), 4.60 x 3.70 x 10^3 cfu/g (MIA), 5.30 and 6.00 x 10^3 cfu/g (TMA), 3.44 and 4.70 x 10^3 cfu/g while EMA and HUF counts of 3.10 and 3.00 x 10^3 cfu/g, respectively.The bacteria isolates were able to use crude oil at varied rates. The extensive use of petroleum products leads to contamination of almost all components of the environment (Chaillan *et al*., 2004).

On the other hand, increasing petroleum exploration, refining and other allied industrial activities in the Niger Delta have led to the wide scale contamination of most of its creeks, swamps, rivers and stream with hydrocarbons and dispersant products. The contamination of these habitats constitutes public health and socio-economic hazards (Okeretugba and Ezeronye, 2003).

The microorganisms in the oil polluted soil have efficient ability in utilizing the residual crude oil as a source of carbon and energy. This is in agreement with these previous researchers (Odokuma and Dickson, 2003). The microbial counts in this study were generally lower in density per kg of soil than those of Chikere and Okpokwasili (2002), but similar to those reported from various Niger Delta soil ecosystems.

In this study where the amount of colony-forming bacteria present was determined, the results show that total culturable heterotrophic bacterial counts were higher in the industrial area than the non-industrial area. The species of HUF identified were *Aspergillus, Mucor, Rhizopus* and *Cladisporium* while the HUB include *Bacillus, Pseudomonas, Escherichia, Micrococcus* and *Serratia;* which are common inhabitants of Niger Delta.

The increase in the bacteria population is attributed to the stimulatory effect of additional carbon and energy sources in the form of crude oil which leads to an enrichment of the oil degrading microbial population. More hydrocarbon utilizing bacteria (HUB) counts were recorded in the wet season than in the dry season. Statistically, there was significant difference (*P<0.05)* in seasonal variation of microbiological parameters (microbial diversity and abundance) for both dry and wet seasons in the industrial area and non industrial area, respectively during the study period.

Total petroleum hydrocarbon concentration in soil Fig. 5, shows the concentrations of total petroleum hydrocarbons (TPH) obtained during the dry and wet seasons. Results indicated that values of 51.50mg/kg, 55.01mg/kg, 89/87mg/kg, 58.94mg/kg and 103.85mg/kg were obtained from areas such as EMA, MIA, CBA, WLA and TMA, respectively in the dry season. The TPH results recorded during the wet season gave values for EMA, MIA, CBA, WLA and TMA, respectively as 46.6mg/kg, 50.01mg/kg, 83.87mg/kg, 55.94mg/kg and 94.85mg/kg, respectively.

It has been reported that biostimulation enhances the removal of total petroleum hydrocarbon from contaminated soil (Okpokwasili, 2006; Odokuma, 2012) had reported that the accelerating effect of amendment is stringer when nutrient availability is a limiting factor. This result was in tandem with the higher concentration of TPH recorded in the soils during the same study periods, confirming that these organisms require hydrocarbons as their sole source of energy. The microorganisms in the oil polluted soil have efficient ability in utilizing the residual crude oil as a source of carbon and energy. This is in agreement with those of previous researchers (Odokuma and Dickson, 2003).

The results of physiochemical analysis during the dry and wet seasons are as presented in Table 6. The CBA study are had 6.91 and 6.98 (PH), 1.619 and 1.616 (TOC), 4.0 and 3.7mg/kg (O/G), 3.9 and 3.5mg/kg (NO_3), 10.4 and 10.1mg/kg (PO_4), 21.0 and 20.2 mg/kg (SO_3), 1.346 and 1.344mg/kg (Ca), 0.064 and 0.063mg/kg (Na), in the dry and wet seasons. In the MIA study area, PH and TOC had values of 4.91 and 4.93 (pH), 0.429 and 0.419 (TOC), 4.5 and 5.2 mg/kg (O/G), 4.2 and 4.1 mg/kg (NO_3), 8.0 and 7.6 mg/kg (PO_4), 20.0 and 19.7 mg/kg (SO_4), 1.186 and 1.184 mg/kg (Ca), 0.081 and 0.080 mg/kg (Na) in the dry and wet season respectively.

Furthermore, in TMA and values of 4.35 and 4.38 (pH), 0.546 and 0.536 (TOC), 950 and 940 mg/kg (O/G), 5.0 and 4.5 mg/kg (NO_3), 13.0 and 12.8 mg/kg (PO_4), 25.0 and 24.5 mg/kg (SO_4, 0.986 and 0.977 mg/kg (Ca), 0.052 and 0.051 mg/kg (Na). Also, in WLA study area, pH was 6.71 and 6.72, while TOC values were 0.059 and 0.056. The O/G concentrations was 7.0 and 6.8mg/kg, 3.0 and 2.9 mg/kg (NO_3), 12.0 and 11.6 mg/kg (PO_4), 17.0 and 16 mg/kg (SO_3), 0.523 and 0.522 mg/kg (Ca), 0.038 and 0.037 mg/kg (Na), 1.509 and 1.507 mg/kg, in the dry and wet season.

Thus, in EMA, pH and TOC had values of 6.90 and 6.93 (pH), 0.215 and 0.214 mg/kg (TOC), 4.0 and 3.7 mg/kg (O/G), 2.7 and 2.8 mg/kg (NO_3) 10.5 and 9.7 mg/kg (PO_4), 12.9 and 11.9 mg/kg (SO_4), 0.847 and 0.38 mg/kg (Ca), 0.84 and 0.83 mg/kg (Na), respectively. Seasonal variations were observed n the concentrations of various soil parameters, including those whose concentrations were related or unrelated to petroleum effluent discharge.

While most of the parameters showed higher concentrations during dry season when water volume is decreased to its maximum, phosphate and nitrate which is components of agricultural fertilizers

commonly used by farmers in Port Harcourt area, showed reduced concentration in the wet season. Phosphate level in the soil samples has been associated with the oil operations because of the changes in concentration during the dry season which suggests an additional source of nutrients in the soil environment. The total heterotrophic bacteria and fungi of the soils revealed higher counts in the dry season than in the wet season.

This may be due to the more favourable physiochemical conditions such as nutrients (NO_3, PO_4^{2-} and SO_4^{2-}), pH and temperature contributed by allochthonous materials from the catchment areas of the soil samples during the period (Han and Gu, 2010; Gadd, 2008). Statistically, there was significant difference ($P<0.05$) in physicochemistry and microbial diversity for both dry and wet seasons, in the industrial area and non-industrial area, respectively during the study period.

The soil sample from the study area had no colour, odour and turbidity. The results of the chemical analysis of soil sample from these areas are presented in this study. The heavy metal concentration of soil samples obtained in the dry and wet season are as presented in Table 7. Study area CBA had 1.40 and 1.38 mg/kg (Cu), 5.0 and 4.0 mg/kg (Zn), 22.0 and 21.0 mg/kg (Fe), 4.6 and 4.5 mg/kg (Pb), 1.3 and 1.0 mg/kg (Cr), 1.9 and 1.7 mg/kg for Ni, respectively. Mile 1 (MIA) gave values of 14.0 and 13.0 mg/kg (Cu), 17.2 and 16.8 mg/kg (Zn), 980.0 and 978.0 mg/kg (Fe), 15.6 and 14.5 mg/kg (Pb), and 14.5 and 14.2 mg/kg (Cr) while Ni had 4.3 and 3.8 mg/kg during the dry and wet season, respectively.

The TMA area had values of 15.70 and 15.50 mg/kg (Cu), 6.6 and 6.45 mg/kg (Zn), 13,066 and 12,065 mg/kg (Fe), 35.7 and 34.6 mg/kg (Pb), 15.8 and 14.9 mg/kg (Cr), 9.2 and 9.1 mg/kg (Ni) respectively. Furthermore, WLA study area had 12.65 and 12.45 mg/kg for Cu, 5.9 and 5.7 mg/kg for Zn, 41.25 and 40.22 mg/kg for Fe, 50.0 and 48.3 mg/kg for Pb, 8.7 and 8.6 mg/kg for Cr and 11.5 and 10.6 mg/kg for Nickel (Ni).

EMA had values of 9.12 and 9.11 mg/kg (Cu), 2.8 and 2.6 mg/kg (Zn), 23.7 and 23.5 mg/kg (Fe), 2.3 and 2.2 mg/kg (Pb), 1.3 and 1.2 mg/kg (Cr), 0.9 and 0.7 mg/kg for Nickel (Ni) during the study season, respectively. Heavy metals receive particular concern considering their strong toxicity even at low concentrations (Bong *et al*., 2010: Appenroth, 2010). Soil with heavy metal can cause serious health effect with various symptoms depending on the nature and quantity of the metal ingested. They produce their toxicity by forming complexes with proteins, in which carboxylic acid (-COOH), amino ($-NH_2$), and thiol (-SH) group are involved (Abay *et al*., 2005; Apprenroth, 2010; Atlas and Bartha, 1973).

Statistically, there was a significant difference ($P<0.05$) in the seasonal variation of heavy metal concentrations for both dry and wet seasons, in the industrial area and non-industrial area, during the study period. The seasonal variation of bacteria population in the study sites during the dry and wet seasons are as represented in Table 4 and 5.

The percentage occurrence of all isolates during the dry season showed that *Citrobacter* had 2 (40%), *Escherichia coli* 3(60%), *Micrococcus* and *Pseudomonas* 1(20%), *Staphylococcus* 5(100%) while percentage occurrence of single isolate indicated 80% (TMA), 40% were obtained for WLA, MIA, CBA, and EMA. Values of 85.7% were obtained for TMA while 42.9% was recorded for WLA, MIA, CBA and EM in the wet season.

Again *Bacillus* sp. and *Escherichia coli* had 42.9%, *Citrobacter*, *Micrococcus, Pseudomonas* had 28.6%, *Serratia* had 14.3% while *Staphylococcus* recorded 71.4%. Results of fungal population of all isolates gave the following: *Aspergillus* 3(60%), *Candida, Pencillium* and *Saccharomycetes* had 1(20%) while *Mucor* sp. had 2(40%). Single isolate recorded 20% for TMA and EMA, 40% in WLA, MIA and CBA during the dry season. The wet season gave 3(42.9%) of *Aspergillus* and 2(28.6%) each for *Candida, Pencillum* and *Rhizopus* respectively. *Mucor, Saccharomyces* and *Trichosporon* had 1(14.3%), respectively. The percentage variation of single 4 isolate gave 42.9% for TMA, MIA and CBA while values of 28.6% and 14.3% were obtained for WLA and EMA, respectively.

5. Conclusion

The ability of microorganisms to absorb and transform pollutants is a promising aspect in respect of solving industrial pollution problems. The total petroleum hydrocarbon, oil and grease, and nitrate concentrations were higher in the highly industrialized areas than with others when compared with the control in both dry and wet seasons.

Heavy metals analyses indicated that chromium (Cr) and copper (Cu) were higher in the highly industrialized areas than with others with respect to the control. This study has shown that industrial effluents discharge indiscriminately resulted in the present of high concentrations of pollutants in the soil environment.

The contaminants have been shown to be present in concentrations which may be toxic individually to different soil organisms (Mbakwem-Aniebo *et al.,* 2014). Pollution of the soil ecosystem poses a serious threat of soil organisms and ultimately the entire ecosystem (Okpokwasili, 1998). The continued discharge of improperly treated effluent may further compound the worsening environmental problem of

soil and cause ecological imbalance of micro-flora and fauna in the soil.

Results were all statistically significant ($P<0.05$) that industrial activities affected microbial diversity and abundance and the physiochemical properties of soils. There was seasonal variation in the microbial diversity and abundance in the soils, indicating interplay between the soil micro-flora and physiochemistry. There was evidence of seasonal variation on microbial diversity and abundance because the microorganisms during the dry were higher than that of the wet (Atlas and Bartha, 1973).

Recommendations

It is recommended that mitigation actions such as bioremediation and proper waste management methods be put in place to ameliorate the negative impact of industrial activity on microbial diversity and abundance in soil environment around industrial areas in Port Harcourt. Primary treatment plants should be installed in all industrial areas for proper treatment of wastes which pose threat to human health and the entire ecosystem.

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