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Physicochemical and Microbiological Analyses of Crude Oil Impacted Soil of Okpare-Olomu Community of the Niger Delta

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Abstract
The study on the physicochemical and microbiological analyses of crude oil impactedsoil of Okpare-Olomu community of theNiger Delta was carried out. A total of twelve (12) different soil samples were collected from four (4) different locations in Okpare-Olomu Community and used for the purpose of this work. While the physicochemical parameters were analyzed according to standard methods, the microorganisms present in the soil samples were also identified using standard morphological, cultural and biochemical techniques. Results of the study showed that the crude oil pollution affected the edaphic physicochemical parameters significantly ($p \leq 0.05$). Specifically, the impact of the oil pollution was found to increase the acidity and electrical conductivity of the soil ($p > 0.05$), but lowered significantly the moisture content ($p < 0.05$). While the nitrate content of the polluted soil decreased (6.3 ± 0.29 to 3.7 ± 0.31 mg/kg), the phosphate (31 ± 1.00 to 38.3 ± 1.16 mg/kg), total organic carbon (1.17 ± 0.29 to 7.27 ± 0.68 mg/kg) and total petroleum hydrocarbon (49.7 ± 1.53 to 51724.67 ± 244.88 mg/kg) increased significantly ($p > 0.05$). The impact of the crude oil pollution also increased the microbial population (cfu/g) of the polluted soil significantly ($p > 0.05$).

Key words: *Physicochemical, soil, crude oil, hydrocarbon, pollution,microorganisms, Okpare.*

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I. Introduction

Soil is one of the major components of the ecosystem and it consists of both biotic and abiotic factors, and it has the ability to self-produce resources necessary for the development of living organisms (microorganisms) (Russell, 2005). It is made of physical, chemical and biological properties that are interrelated, making soil an effective medium for plant growth and other functions.Exploration and exploitation of oil has been on-going for several decades in the Niger Delta with its disastrous impacts on the environment, which has consequently affected the people of the region adversely (Kadafa, 2012). Hydrocarbons pollution of soils especially from petroleumhas constituted environmental issues over the years (Mmom and Deekor, 2010). No gainsaying that oil spillage in the Niger Delta region has led to very serious pollution and destruction of flora, fauna and resort centres, contaminated soils, forest destruction and biodiversity loss, pollution of drinkable waters (streams, rivers, underground water etc.) and lives along the Niger Delta region (Orimoogunje and Ajibola-James, 2013;Kadafa, 2012) in which Okpare-Olomu community belongs. In fact, the area in general is now seen as an ecological wasteland.This has affected the livelihood of the indigenous people who rely on the ecosystem services for survival, and thus resulting into increased poverty and displacement of people. Oil spillage has also caused regional crisis in the Niger Delta. Factors responsible for oil spillage in the zone are; corrosion of oil pipes and tanks, sabotage, port operations and inadequate care in oil production operations and engineering drills. The consequence is the massive oiling of the environment and destruction of vulnerable ecological units (Ajibola-James, 2011; Ebuehi et al., 2005; Wegwuet al., 2010). Petroleum hydrocarbons are naturally occurring chemicals, and as such, microorganisms which are capable of attenuating or degrading hydrocarbons exist in the environment. Moreso, microorganisms show tolerance limit for any particular environmental conditions, as well as optimal conditions for best performance (Udochukwuet al., 2014).

The effects of hydrocarbon pollution reflect the amount of toxic hydrocarbon in the environment and the different susceptibility of organisms, population and ecosystems, which are directly proportional to the amount released.However, the type of petroleum hydrocarbon released is important, as well as the susceptibility of the organisms due to the environmental processes acting on the released petroleum hydrocarbon(Kadafa, 2012). The toxicity to the organism depends on the available amount of petroleum available to an organism. When petroleum hydrocarbon is released into the environment, there are processes that alter the chemical composition of the petroleum hydrocarbon, which in turn affects the toxicity of that particular environment. The bioavailability and persistence of specific hydrocarbons, the ability of an organism to accumulate and metabolize, fate of the metabolized products, metabolites of the hydrocarbon interphase with the normal

metabolic process usually affect an organisms chances of survival and reproduction inthe environment(Briggs, et al., 1996). The indiscriminate and incessant oil activities that are often times illegal, resulting into spills in the area over time has necessitated the assessment into the physicochemical and microbiological study of the Okpare-Olomu soil, to ascertain the probable impact of the hydrocarbon pollution of the soil in relation to human health.

II. Materials and Methods

Study area description

The study area (Okpare-Olomu community) in the Niger Delta as shown in Figure 1, is located precisely in Ughelli South Local Government Area, Delta State and it lies within latitude 05^0, 27N and 05^0, 33N and longitude 005^0, 53 E and 006^0, 04 E of the Niger Delta, Nigeria.

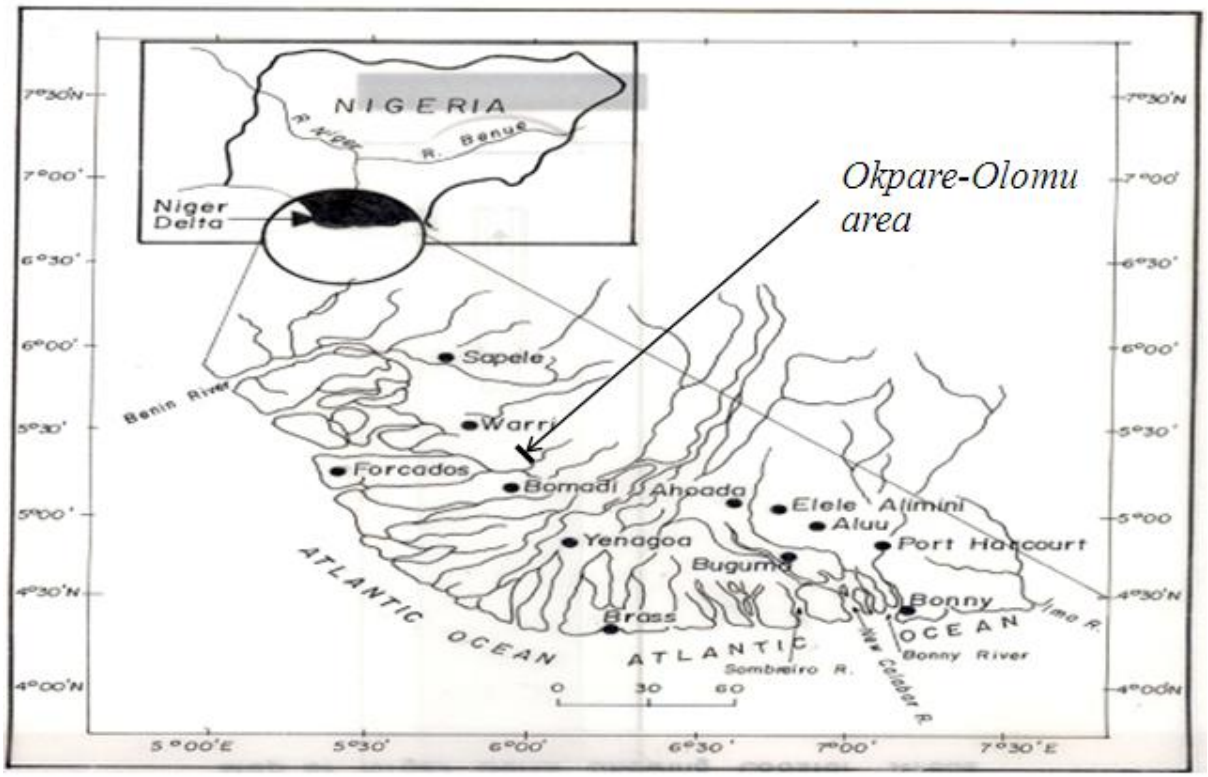

Figure 1: Physiographic map of the Niger Delta that harbours the Okpare-Olomu community (Mmom and Deekor, 2010)

Sample collection

The soil samples used for this work were randomly collected from four (4) different locations in Okpare-Olomu Community. The samples were labeled according to the sites of collection as: Site A – Control sample from fallow farm land at Okpare; Site B – soil polluted within a week from crude oil bunkering activities,; Site C – soil polluted within 2 months of crude oil bunkering activities , and Site D – soil polluted over a year from crude oil bunkering activities. The top soils were collected in triplicates, 40 meters apart from the four locations (axis) at a depth of 1cm - 8cm with the aid of a sterile soil auger. The samples were transported to the laboratory in sterile polyethylene bags. For samples that could not be processed immediately, they were however, stored at 4^oC but not longer than 18 to 24 hrs. Serial dilution of samples were carried out by measuring one gram of the soil samples and dissolving it in 9ml of sterile distilled water, and was tenfold serially diluted. In this process, the ten-fold serial dilution of the sample was achieved using normal saline as the diluent factor (Nduka, 2011 and Onyeagba, 2004).

Sterilization techniques

The polythene bags used for collecting the samples were cold-sterilized using UV-radiation box for about 12 hours (usually overnight), and glassware were treated in hot-air oven at 160^oC for 2 hours. Growth media and diluents (distilled water) on the other hand were autoclaved at 121^oC for 15 minutes, while the

working bench was surface sterilized by swabbing with 70% alcohol. The use of naked flame was applied during inoculation, serial dilution and sub-culturing in view of enhancing aseptic condition.

Determination of physiochemical parameters

The soil pH of the samples was determined using a pH meter (Jenway 3051, United Kingdom) in 1:1 soil solution in distilled water in accordance with the manufacturer's directions. While the determination of moisture content was carried out according to Nounamoet al.(2000), conductivity was determined using a conductivity meter (PW 9504 Philips, USA) with a cell constant of 1.2, where the electrical conductivity of the soil was recorded in microsiemens per centimeter (µS/cm). The organic carbons were determined by the chromic acid titration methodalso known as the reference method (Walkley and Black, 1934). While the determination of nitrate and phosphate contentswere carried out according to the brucine method (UNEP, 2004), the total petroleum hydrocarbon was carried out using the cold extraction method according to the ASTM - D-3694 (Ezekoye, 2015).

Determination of microbial parameters

Determinations of total heterotrophic bacteria and total hydrocarbon utilizing bacteria

These were achieved using the pour plate method on nutrient agar (NA). 1 g of soil sample was mixed with 9 ml of normal saline; where10-fold serial dilution was prepared. Following this, an aliquot (0.1 ml) was used to incubate the plate in triplicates on sterile nutrient agar; where10-fold serial dilution was prepared and used in the estimation of aerobic heterotrophic bacteria and fungi population by pour plate method.Aliquot of 1ml of the 10^{-4} dilutions of the samples were used to incubate the plates in a set of three (triplicate), and the plates were incubated for 18-48 hours at 37°Cthereafter; the colonies that formed on the medium were counted and mean counts were thus determined. Meanwhile, the use of vapour phase method was deployed for the enumeration of total hydrocarbon utilizing bacteria (THUB) (Hamamuraet al., 2006). The mineral salt medium used was a combination of $MgSO_4.7H_2O$ (0.42 g), KCl (0.297 g), KH_2PO_4 (0.85 g), $NaNO_3$ (0.424 g), K_2HPO_4 (1.27 g), NaCl (20.12 g), Amphotericin B (250 mg as Fungi zone), and Agar powder (20 g) (Ezekoye, 2015), that were weighed out appropriately into a conical flask and hydrated in 1000 mL distilled water. Using an autoclave, the medium was sterilized (121°C, 15 Psi) for 15 minutes and then removed into sterile Petri dishes. At this point, the gelled mineral salt agar (MSA) was then inoculated with appropriate dilutions of the soil sample. Filter papers (Whatman No. 1) that were saturated with Bonny light crude were placed aseptically onto the covers of Petri dishes and inverted. The plates were then incubated at 37°C for seven (7) days and thus colonies were counted.

Characterization and identification of the heterotrophic bacteria isolates from soil samples After counting and estimating the total bacterial load, morphologically distinct colonies were picked using a sterile inoculating needle and aseptically transferring it to the sterile nutrient agar slants for further characterization. The isolates were characterized up to genera (Cheesbrough, 2000) based on various staining and biochemical reactions such as Gram-staining, spore staining, motility test, oxidation fermentation (O/F) test, indole test, oxidase and catalase test. All bacterial isolates were characterized andidentified based on their cultural, morphological, andbiochemical characteristics.

Determination of total heterotrophic fungi

Aliquots of 1 ml of 10^{-4} dilutions of the sample were inoculated into potato dextrose agar (PDA) in a set of three (triplicate), and the plates were incubated at 28°C for 72 hours thereafter; the developing colonies that formed on the medium were counted and, the mean values were determined.

Cultural characteristics and morphological test

Each colony morphology such as size, shape, margin, colour, elevation, consistency, transparency was determined.Fungal growth on plate culture was observed; surface, spore and underside colour. Stained (lactophenol cotton blue) slide was examined using a microscope (x40) for structure of hyphae and details of sporulating structure.Gram staining and biochemical tests were however, done for fungi just as for bacteria above.

Statistical analysis

The statistical analyses were done using statistical package for social sciences (SPSS, Version 17.0). Analysis of variance (ANOVA), P - values test of significance, was carried out at 95% level of confidence. The P - values were used to ascertain the significance levels between various treatments and data obtained during the study.

III. Results and Discussion

Physico-chemical paramters

Results of the soil pH, moisture content and electrical conductivity are presented in Table 1. Soil pH value determines if the soil environment is acidic or alkaline. The reduced pH values obtained in this study in Table 4.1, shows that crude oil pollution made the soil to be acidic and thereby increased the acidity of the soil significantly ($p > 0.005$), as when the control sample (site A) exhibited a pH of 6.7 ± 0.20, which is an ideal pH for normal and unpolluted soil (Oyem, 2013), sitesB, C and D exhibited pH values of 4.9 ± 0.10, 5.5 ± 0.26 and 5.9 ± 0.21, respectively.Also, the result, however, indicated that pH increases with age of pollution, as the site polluted within a week (site B) had the lowest pH value (4.9 ± 0.10), site C which was polluted within 2 months had a middle value (5.5 ± 0.26), while site D of over a year pollution

Table 1: Soil physical parameter values in mean and standard deviation.

Site	pH value	Moisture content (%)	Electrical conductivity (µS/cm)
A	6.7 ± 0.20	40 ± 2.00	1023 ± 172.81
B	4.9 ± 0.10	30 ± 2.65	5868.7 ± 40.81
C	5.5 ± 0.26	24.7 ± 1.53	5118 ± 105.53
D	5.9 ± 0.21	34 ± 2.65	2981.3 ± 24.68

Site A – Control sample from fallow farm land at OkpareOlomu; Site B – soil polluted within a week from crude oil bunkering activities; Site C – soil polluted within 2 months of crude oil bunkering activities, and Site D – soil polluted over a year from crude oil bunkering activities.

had the highest value (5.9 ± 0.21). The increase in pH values with age of pollution could be associated with rainfall and perhaps, effect of bioremediation by soil microorganisms. The effect of low pH as revealed by the result due to the crude oil pollution will consequently manifest in poor growth and productivity of plants, and in most cases, in the death of plants. Study has shown that soil pH does not only affect the growth of plantsindirectly by affecting nutrients availability, but also the presence of toxins and the growth of soil microorganisms (Oyem, 2013).The result of the soil pH in this study is in agreement with previous work (Njokuet al., 2008; Njokuet al., 2009; Kumar et al., 2014 and Oyem, 2013), where crude oil pollution resulted into increasing the acidity of the soil environment. The soil moisture contentis generally described as the ratio of the mass of water held in the soil to that of the dry soil. The result of the moisture content presented in Table 4.1 shows that while the unpolluted and control sample as site A registered the highest moisture content (40 ± 2.00 %), the moisture contents for sites B (30 ± 2.65 %), C (24.7 ± 1.53 %) and D (34 ± 2.65 %) however decreased comparatively due to the effect of oil pollution. The presence of the oil in the polluted sites prevents or hinders the movement of water down the soil, by working against the gravitational force that pulls water down through the soil matrix. It also reduces the soil aeration by displacement of air in the oil-spilled soils.The result was also able to point out that site C experienced the lowest moisture content because of the prolong effect of time on water prevention and retention by the oil. The implication of this is that, less dissolved materials will be available for plants uptake and metabolism (Njoku et al., 2008). But for site D where pollution had spanned over a year, the moisture content increased from 24.7 ± 1.53 % (for site C) to 34 ± 2.65 % (for site D), respectively. This can be associated with reduced total petroleum hydrocarbon over time that thus promoted water down movement and aeration of the soil.Soil electrical conductivity (EC) is regarded as the measure of ionic concentration in soils, and thus, related to dissolved solutes. Result presented in Table 4.1 shows higher electrical conductivity of the polluted sites over the control site due to the crude oil pollution. While the control Site, A, recorded 1023 ± 172.81 (µS/cm), sites B, C and D recorded 5868.7 ± 40.81, 5118 ± 105.53 and 2981.3 ± 24.68 (µS/cm), respectively. The significantly higher electrical conductivity values ($p > 0.005$) could be attributed to high concentration of charged ions (cations and anions) in the oil impacted sites. The result also depicted reduction in electrical conductivity when the age of the polluted site was above a year, as site D recorded a significant reduction ($p < 0.005$) in EC (2981.3 ± 24.68 µS/cm) as against 5118 ± 105.53 µS/cm of site C. The reduction can be associated to effect of rainfall over time.

Results of the total organic carbon, nitrate, phosphate and TPH contents are presented in Table 2. The resultsshow that polluted soil samples were higher in total organic carbon (TOC) content than the control soil sample. While the control, site A had a TOC of 1.17 ± 0.29 %, sites B, C and D were 7.27 ± 0.68, 5.83 ± 0.58 and 3.50 ± 0.50 %, respectively, although most in site B; site polluted within a week. Result of this type should be expected due to the hydrocarbon presence in the crude oil, which has increased the soil TOC either through biodegradation or leaching process of the contaminant. Increase in organic carbon content in crude oil contaminated soil that was associated with biodegradation has been reported (Adenipekun and Fasidi, 2015). However, over time, the result showed decrease in organic carbon content with

Table 2: Soil chemical parametervalues in mean and standard deviation.

Site	Total organic carbon (%)	Nitrate content (mg/kg)	Phosphate content (mg/kg)	TPH content (mg/kg)
A	1.17 ± 0.29	6.3 ± 0.29	31 ± 1.00	49.7 ± 1.53
B	7.27 ± 0.68	3.7 ± 0.31	38.3 ± 1.16	51724.67 ± 244.88
C	5.83 ± 0.58	4.4 ± 0.36	34.7 ± 1.53	39277.33 ± 321.98
D	3.50 ± 0.50	4.8 ± 0.25	32.3 ± 0.59	14793.67 ± 157.72

TPH represents Total Petroleum Hydrocarbon; Site A – Control sample from fallow farm land at OkpareOlomu; Site B – soil polluted within a week from crude oil bunkering activities; Site C – soil polluted within 2 months of crude oil bunkering activities, and Site D – soil polluted over a year from crude oil bunkering activities.

soil polluted > 2 months. This can be attributed to its utilization by increased hydrocarbon utilizing microorganisms (Khanaferet al., 2017). Results for both nitrate and phosphate contents of the study area showed reductions in both contents in the polluted soil samples compared to the control. The nitrate values ranged between 3.7 ± 0.31 to 6.3 ± 0.29 mg/kg, where site B had the least nitrate value. This reduction can be associated with utilization of substrate in metabolism of organisms in building microbial biomass (Ezekoyeet al., 2015; Orji et al., 2012). The result also showed that the soil nitrate contents vary directly with the soil pH. Hence, the plausible reason why site B had the least nitrate content is because of its acidic nature as shown in Table 4.1, as soil pH below 5.5 reduces nitrification. Nitrification ceases at pH < 4.5, and the optimum pH for nitrification is reported to be between 6 and 8 (USDA, 2014). Conversely, the phosphate values ranged between 31 ± 1.00 to 38.3 ± 1.16 mg/kg, in which site B had the highest phosphate value while the control had the least. Contrary to the relationship that existed between the nitrate contents and the soil pH, phosphate contents rather exhibited an inverse relationship with the soil pH as site B with the lowest pH (Table 4.1) exhibited the highest phosphate content (38.3 ± 1.16 mg/kg) as against site A with the highest pH having the lowest phosphate value (31 ± 1.00 mg/kg). This is in agreement with previous study (Ojeet al., 2015). Total petroleum hydrocarbon (TPH) is used to describe a large group of chemical compounds that are obtainable from crude oil. Generally, there is array of different chemical compounds in crude oil and petroleum products. Practically, these compounds cannot be measured separately hence, they are determined as total petroleum hydrocarbon content (Khan and Kathi, 2014). Result showed TPH of the control site to be lowest (49.7 ± 1.53 mg/kg) as against the polluted sites which ranged between 14793.67 ± 157.77 mg/kg (site D) to 517224.67 ± 244.88 mg/kg (site B), due to the heavy oil spillage. This is in agreement with previous study (Oyem, 2013). The result presented, however, also indicated significant decrease in TPH over time ($p < 0.05$) among the polluted sites, as site D polluted over a year had the least, which can be associated with TPH utilization by microorganisms over time for growth and metabolic process (Udochukwu et al., 2014; Mmom and Deekor, 2010), whereas site B had the highest. In other words, the amount of hydrocarbon in the soil and age of contamination influence the rate of disappearance of the contaminant in the soil (Ijah and Anthai, 2003).

Soil microbiological parameters

The total heterotrophic bacteria counts (THBC) and the total heterotrophic fungi counts (THFC) of the study area were also determined and the results presented in Table 3. The results showed increase in THBC and THFC with age of pollution but displayed inverse variation with TPH with polluted sites. While the control (site A) exhibited the lowest microbial counts for both THBC ($1.87 \times 10^7 \pm 0.13$ cfu/g) and THFC (3.68 x 106 ± 0.09 cfu/g), the polluted sites ranged from 3.27 x 107 ± 0.12 and $5.73 \times 10^6 \pm 0.21$ cfu/g at TPH of 51724.64 ± 244.88 (site B) to $5.45 \times 10^7 \pm 0.10$cfu/g and $8.23 \times 10^6 \pm 0.14$ cfu/g at TPH of 14793.67 ± 157.72 (site D), respectively

Table 3: Soil microbiological parametervalues in mean and standard deviation.

Site	THBC (cfu/g)	THFC (cfu/g)	THUBC (cfu/g)	THUFC (cfu/g)
A	$1.87 \times 10^7 \pm 0.13$	$3.68 \times 10^6 \pm 0.09$	$3.45 \times 10^5 \pm 0.11$	$4.72 \times 10^5 \pm 0.17$
B	$3.27 \times 10^7 \pm 0.12$	$5.73 \times 10^6 \pm 0.21$	$4.97 \times 10^6 \pm 0.31$	$3.48 \times 10^6 \pm 0.20$
C	$3.93 \times 10^7 \pm 0.06$	$7.16 \times 10^6 \pm 0.17$	$8.53 \times 10^6 \pm 0.19$	$4.43 \times 10^6 \pm 0.17$
D	$5.45 \times 10^7 \pm 0.10$	$8.23 \times 10^6 \pm 0.14$	$5.85 \times 10^6 \pm 0.11$	$3.66 \times 10^6 \pm 0.29$

Key: THBC, THFC, THUBC, THUFC represent Total Heterotrophic Bacteria Counts, Total Heterotrophic Fungi Counts, Total Hydrocarbon Utilizing Bacteria Counts and Total Hydrocarbon Utilizing Fungi Counts, respectively. Site A – Control sample from fallow farm land at OkpareOlomu; Site B – soil polluted within a week from crude oil bunkering activities; Site C – soil polluted within 2 months of crude oil bunkering activities, and Site D – soil polluted over a year from crude oil bunkering activities.

For both THBC and THFC. The increase in the microbial counts could be attributed to the pollution from crude oil that supported their growth; however, the very high concentration of the TPH arising from the presence of high amount of the crude oil may have hindered their growth.This is in agreement with previous study (Ekhaise and Nkwelle, 2011). Results of the Total hydrocarbon utilizing bacteria counts (THBC) and total hydrocarbon utilizing fungi counts (THFC) obtained in this study showed that microorganisms which included bacteria and fungi utilized oil in contaminated soil as their sole sources of carbon and energy (Ekhaise and Nkwellem 2011). Though there were variations in both the total hydrocarbon utilizing bacteria and fungi, but maintained same trend with THBC and THFC above with respect to TPH. Results indicated that while both THUBC and THUFC were lowest in the control (3.45 x 105 ± 0.11 and 4.72 x 10^5 ± 0.17 cfu/g), they only increased between one week (4.97 x 10^6 ± 0.19 and 3.48 x 10^6 ± 0.20 cfu/g, site B) and two months of age of pollution (8.53 x 10^6 ± 0.19 and 4.43 x 10^6 ± 0.17 cfu/g, site C) and thereafter decreased after one year of pollution (5.85 x 10^6 ± 0.11 and 3.66 x 10^6 ± 0.29). The decrease in value could be associated with over depletion of the major source of hydrocarbon (crude oil) due to utilization by the microorganisms, which may have resulted in the death of some of the microorganisms due to survival of the fittest. Because as TPH decreased, THUBC and THUFC also correspondingly decreased.

Occurrence and frequency of microbial isolates from the different sites understudied

The result in all the sampling locations as presented in Table 4 indicated the presence of twelve genera of bacteria namely; *Micrococcus species, Pseudomonas species, Bacillus species, Staphylococcus aureus, Streptococcus species, Flavobacterium,Klebsiella species, Acinetobacter species, Arthrobacter species, Enterobacter species, Rhodococcus species* and *Streptobacillus species*,where the unpolluted site, A, had the least bacteria genera occurrence, followed by site D, while sites B and C had the highest occurrence. Table 6 showed that of all the bacteria isolated, *Pseudomonas species* was the most prevalent isolate occurring at all the sample collection sites witha frequency of 18.5 %, while *Streptobacillus species* was the least with an occurrence frequency of 2.2 %. On the other hand, nine genera of fungi were identified

Table 4: Occurrence of bacterial isolates from the different sites understudied.

Bacteria	Site A	Site B	Site C	Site D
Micrococcus spp.	+	+	+	+
Pseudomonas spp.	+	+	+	+
Bacillus spp.	+	+	+	+
Staphylococcus aureus	+	+	+	+
Streptococcus spp.	+	-	-	+
Flavobacterium	-	+	+	-
Klebsiellaspp	-	+	+	+
Acinetobacter spp.	-	+	+	+
Arthrobacter spp.	+	+	+	-
Enterobacter	-	-	+	+
Rhodococcus	-	+	+	+
Streptobacillus	+	+	-	-

Site A – Control sample from fallow farm land at OkpareOlomu. Site B – soil polluted within a week from crude oil bunkering activities; Site C – soil polluted within 2 months of crude oil bunkering activities, and Site D – soil polluted over a year from crude oil bunkering activities.

as shown in Table 5, and these include; *Aspergillusniger, Aspergillusflavus,Aspergillusversicolor,Trichodermal species, Penicillium species, Fusarium species, Mucor species, Mucor species* and*Rhizopus*, in which the unpolluted site, A, had the least fungi occurrence, while all the polluted sites had a uniform occurrence but with variation in genera. Table 6 showed that of all the fungi isolated, *Aspergillusniger*was the most prevalent isolate occurring at all the sample collection sites, having a frequency of 21.2 %, while *Rhizopus* had the least frequency of occurrence of 5.2 %.

Table 5: Occurrence of fugal isolates from the different sites understudied.

Fungi	Site A	Site B	Site C	Site D
Aspergillusniger	+	+	+	+
Aspergillusflavus	-	-	+	+
Aspergillusversicolor	-	+	+	-
Trichodermal spp.	-	+	+	+
Penicillium spp.	+	+	+	+
Fusarium spp.	-	+	-	+
Mucor spp.	+	+	+	+
Rhizopus	+	-	-	-

Site A – Control sample from fallow farm land at OkpareOlomu; Site B – soil polluted within a week from crude oil bunkering activities; Site C – soil polluted within 2 months of crude oil bunkering activities, and Site D – soil polluted over a year from crude oil bunkering activities.

Table 6: Identified bacterial and fungi isolates and their frequencies of occurrence in all the sites understudied.

Bacteria isolate	% frequency of occurrence	Fungi isolate	% frequency of occurrence
Micrococcus spp.	14.2	*Aspergillusniger*	21.2
Pseudomonas spp.	18.5	*Aspergillusflavus*	12.5
Bacillus spp.	13.4	*Aspergillusversicolor*	8.0
Staphylococcus aureus	10.9	*Trichodermal spp.*	10.1
Streptococcus spp.	3.3	*Penicillium spp.*	15.0
Flavobacterium	3.1	*Fusarium spp.*	9.3
Klebsiellaspp	9.0	*Mucor spp.*	18.7
Acinetobacter spp.	11.5	*Rhizopus*	5.2
Arthrobacter spp.	7.1		
Enterobacter	2.5		
Rhodococcus	4.3		
Streptobacillus	2.2		

IV. Conclusion

The results of study on the impact of crude oil pollution inOkpare-Olomu community showed that crude oil pollution affected both the physico-chemical and microbiological properties of the soil significantly. While it increased the acidity and electrical conductivity of the soil ($p > 0.05$), the moisture content decreased significantly ($p < 0.05$). Chemically, apart from the nitrate content of the polluted soil that showed decrease in value to the control, the phosphate, total organic carbon and total petroleum hydrocarbon increased significantly ($p > 0.05$). The impact of the crude oil pollution also increased the microbial population (cfu/g) of the polluted soil significantly ($p > 0.05$), as the total heterotrophic microbial counts of both bacterial and fungi, and total hydrocarbon utilizing bacteria and fungi counts increased over 3-fold, since the microorganisms used the crude oil as their source of carbon and energy for growth and metabolism. The study also found out that the pollution increased both the bacteria and fungi genera in the oil impacted soil. While *Pseudomonas species* was the most prevalent bacteria isolate occurring at all the sample collection sites, *Aspergillusniger*was the most prevalent fungi isolate that occurred at all the sample collection sites. This study has thus shown that oil activities (illegal) going on at the Okpare community impacts the environment negatively, especially polluting the soil ecosystem and leaving it a breeding ground for pathogens at the expense of human lives.

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