

# The Effects of Oil Spillage on the Properties of Soil and Environment around the Marketing Outlets of some Petroleum Marketing Companies in Calabar, Cross River State, Nigeria

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# The Effects of Oil Spillage on the Properties of Soil and Environment around the Marketing Outlets of some Petroleum Marketing Companies in Calabar, Cross River State, Nigeria

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# The Effects of Oil Spillage on the Properties of Soil and Environment around the Marketing Outlets of some Petroleum Marketing Companies in Calabar, Cross River State, Nigeria

# Abstract

Soil and environmental protection and sustainability are important aspects of natural resource management that have been neglected for too long. The objective of this study was to determine the damages caused by oil spill to the soil and environment. Soils samples were collected from the surroundings of five petroleum marketing companies - Mobil, Total, Conoil, NNPC and Oando petrol stations in Calabar, Cross River State. The samples were collected from the oil spill affected soils and non-oil affected soils to serve as control. Composite soil samples were collected from the top soil surface (0-15cm) using soil auger. Some physico-chemical properties that reflect soil nutrients content and fertility status (Ca<sup>2+</sup>, Mg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, C, N, P, pH, ECEC, particle size, electrical conductivity, and hydrocarbon content) and microbial flora populations were determined using standard physico-chemical and biological methods. Results from the oil spill affected and non-oil spill affected soils were compared. There was a significant decrease in Ca<sup>2+</sup>, Mg<sup>2+</sup>, K<sup>+</sup>, ECEC, silt fraction, clay fraction in oil affected soils as well as significant increase in the sand fraction content, Na<sup>+</sup> content, electrical conductivity, hydrocarbon content of the oil-spill affected soils when compared with the non-oil spill affected soils. The acidic nature of the soils could not be attributed entirely to the oil-spilled since the control sample soils were equally acidic. The results of microbial flora in areas with and without oil spill shows little effect on the microbial populations of the soils studied. The average microbial population of the soils with oil-spill was 2.78 x  $10^6$  cfu/g, while that without oil-spill was 3.6 x  $10^6$  cfu/g. for bacteria count. The fungi populations of oil affected soils was  $3.4 \times 10^4$  cfu/g while the non-oil affected soils was  $3.0 \times 10^4$  cfu/g. The bacteria and fungi microbes identified from oil affected soils were Ganobacterium, Micrococcus luteus, Pseudomonas maltophilia and Aspergilus flavin, Rhizopus spp, Mucor spp, Candida spp while that of nonoil affected soils were Nocardia, Bacillus substillis, Pseudomonas putidae and Candida spp, Mucor spp and Aspergillus spp. The significant variation of the results of physico-chemical and microbiological population observed were indicative of the effect of oil spillage on the soils. The effects of oil spillage on the environment around the petrol stations were also observed by the scanty and burnt nature of the vegetation due to contact with petroleum product.

**Keywords:** Oil spillage, Soil properties, Petroleum marketing outlets, Environment, Non-spillage affected soils.

# Introduction

Calabar, Cross River State is located in the rainforest agro-ecological area of the Niger Delta region of Nigeria (Human Rights Watch, 1999). Niger Delta is a region with abundant rivers/creeks including good weather and low level of agricultural land cultivation. The major occupation of the people are fishing and subsistence farming such as the cultivation of vegetables and corn production given the abundant water bodies endowment.

Fossil fuel appeared to offer a limitless source of energy to drive development. While oil and energy provide multiple benefits to human society, every stage in the life cycle from exploration to use has harmful effects on the environment - the soil physico-chemical and biological properties of the soil and the health of community members due to its spillage. The negative impacts of oil spillage include destruction of life, loss of fertile soil, pollution of air and water and damage to the ecosystem of the host communities (Aghalino, 2000). The ecological problems observed as a result of oil spillage include

brownish vegetation and soil erosion, diminishing resources of the natural ecosystem and adverse effect on the life, health and economy of the people (Abii & Nwosu 2009).

Oil spill is the release of liquid petroleum hydrocarbon into the natural environment as a result of human activities. Oil-spill pollution is hazardous and problematic worldwide (Okereke, Obiekezie & Obasi, 2007). Most people think of marine oil spills when they visualize an oil spill, but the escape of oil into the soil is a problem on the land as well. Since many humans rely heavily on petroleum products such as plastic, fuel, and lubricating oil, oil spillage is an unfortunate by-product of the human way of life (Bob, 2007). Therefore the objective of this study is to determine the kind of damages caused by oil spillage in Cross River State agro-ecological area.

Oil was discovered in Nigeria at Oloibiri in the Niger Delta after half a century of exploration. The discovery was made by Shell-BP, at the time the sole concessionaire. Nigeria joined ranks of oil producers in 1958 when its oil field came on stream producing 5,100 barrels per day. After 1960, exploration rights in onshore and offshore areas adjoining the Niger Delta were extended to other foreign companies.

In 1970, the end of the Biafran war coincided with the rise of world oil price, and Nigeria was able to reap instant riches from its oil production. Nigeria joined the Organization of Petroleum Exporting Countries (OPEC) in 1971 and established the Nigerian National Petroleum Company (NNPC) in 1977; a state owned and controlled company which is a major player in both the upstream and downstream sectors. By the late sixties and early seventies, Nigeria had attained a production level of 2 million barrels of crude oil per day. Although production figures dropped in the eighties due to economic slump, 2004 saw a total rejuvenation of oil exploration to a production record level of 2.5 million barrels per day.

All stages of oil exploitation impact negatively on the environment, and the greatest single intractable environmental problem caused by crude oil exploration in the Niger Delta region is oil spillage. According to Chindah and Braide, (2000), over 6000 spills had been recorded in the 40 years of oil exploitation in Nigeria, with an average of 150 spills per annum. In the period 1976 - 1996, 647 incidents occurred resulting in the spillage of 2,369,407.04 barrels of crude oil. With only 549,060.38 barrels recovered, 1,820,410.50 barrels of oil were lost to the ecosystem.

The environmental consequences of oil pollution on the inhabitants of Delta State are enormous. Oil spills have degraded most agricultural lands in the State and have turned hitherto productive areas into wastelands. With increasing soil infertility due to the destruction of soil micro-organisms, and dwindling agricultural productivity, farmers have been forced to abandon their land, to seek non-existent alternative means of livelihood. Aquatic life have also been destroyed with the pollution of traditional fishing aqua sources, exacerbating hunger and poverty in fishing communities.

Bob, (2007) in a study on the effect of oil spill on crop production in the Niger Delta, reported that oil spill causes great damage to plant community due to high retention time of oil occasioned by limited air flow. The oil hamper proper soil aeration as oil film on the soil surface acts as a physical barrier between air and the soil thus affecting the physico-chemical properties of the soil such as temperature, structure, nutrient status and pH. Oiled shoots of crops like pepper and tomatoes may wilt and die off due to blockage of stomata thereby inhibiting photosynthesis, transpiration and respiration. Germination, growth performance and yield of these crops are stifled by oil spillage (Aghalino 2000).

In a study of the socio-economic impact of oil pollution, (Onwurah, 1999) stated that crude oil exploitation has had adverse environmental effect on soils, forests and water bodies in host communities in the Niger Delta. Farmers have lost their lands, and are consequently forced to emigrate to other

communities in search of livelihood exerting additional pressures on natural resources in such areas. According Okereke, Obiekezie and Obasi (2007), 67.7 percent of 797 respondents interviewed on the socio-economic impact of oil pollution identified farmland degradation as a major problem.

# **Materials and Method**

The study area, Calabar, is the capital of Cross River State in the southern part of Nigeria. It is located within latitude  $8^{0}17^{1}$  East and longitude  $4^{0}58^{1}$  North. Meteorological data showed that annual temperatures and relative humidity values in Calabar ranged from 26-30<sup>o</sup>C and 51-93% respectively, while the annual rainfall values ranged between 3,000mm-3,500mm with no or very little rainfall in November/December.

Soil samples for this study were collected from five petroleum products marketing companies. These include: Mobil petrol station at Marian, Total petrol station at Marian, Conoil petrol station at Highway by Mobil junction, Oando petrol station at Murtala Muhammed Highway and NNPC megastation at Murtala Muhammed Highway, all within the Calabar metropolis. At each of the five petrol stations, two composite soil samples were collected from the sites and adjacent area from a depth of 0-15cm to give total samples of ten from the polluted site and a geographically similar unpolluted site located sixty meters (60m) adjacent to the polluted site; using soil auger. The samples were designated MSa and MSb, TSa and TSb, CSa and CSb, OSa and OSb, NSa and NSb, with the initial letter representing the company name, (Sa) representing oil contaminated soil and Sb representing uncontaminated soils respectively. The soil samples were put into poly ethylene bags, labeled accordingly and taken to the laboratory for subsequent analyses.

## Laboratory Analysis

*Soil Reaction pH:* pH was determined using a pH meter glass electrode. Twenty grams of the soil sample were weighed and suspended in 50ml of distilled water and properly stirred (1:2:5). The pH meter was calibrated using buffer solution at pH 7 pH 4 before taking measurements.

*Organic Cabon:* Organic carbon was analyzed by the wet oxidation method of Wakley and Black. One gram of the soil samples was suspended in 10ml of potassium dichromate, 20ml concentrated sulphuric acid (H<sub>2</sub>SO<sub>4</sub>), 200ml of distilled water, 5ml of orthophosphoric acid and was titrated against 0.5ml of ammonium ferrous sulphate using 1ml of Naphenylamine indicator.

*Determination of Particle Size:* The particle size distribution was determined by hydrometer method (Bouyoucous, 1962). 100g of the soil samples was suspended in 50ml and 200ml of calgon and distilled water respectively. The suspension was stirred vigorously and left to stand overnight. The next day, the mixture was stirred for about 10 minutes before emptying it into 1000ml volumetric measuring cylinder. Water was added and with the hydrometer to raise the volume of the suspension to 1000ml mark. The hydrometer was removed and the suspension shaken vigorously. First temperature and hydrometer reading were taken after 40 seconds and second temperature and hydrometer reading were taken after 2 hours.

*Available Phosphorus:* Available phosphorus (P) was determined by the ammonium molybdate in ascorbic acid blue colour method of Bray and Kurtz (1945). Three grams of soil samples was suspended in 15ml of bray -1 P solution, shake for 1 minute and leach using Whatman filter paper. 2ml of the leachate was taken into 50ml volumetric flask. Little quantity of distilled water was added, 8ml of ammonium molybdate in ascorbic acid, and more distilled water to raise the volume of the mixture to 50ml mark. Cover the flask, shake and allow to develop before taking the reading in spectrometer.

*Exchangeable Acidity:* Exchangeable acidity was determined by the titration method using phenolphthalein as indicator.

Five grams (5g) of soil samples was in 100ml of volumetric flask. Leached in 100ml of volumetric flask with potassium chloride solution. 50ml of the leachate was taken for samples whose pH values are 5.0 and above and 25ml of pH below 5. First titration was made against 0.1 molar solution of NaOH for H<sup>+</sup> and Al<sup>3+</sup> while the second titration against HCl is for Al<sup>3+</sup> only.

*Exchangeable Base:* Exchangeable Cations (EC) were first extracted by ammonium acetate extraction method (Jackson 1999). Then sodium (Na) and potassium (K) were determined using flame photometry while calcium (Ca) and Magnesium (Mg) were determined by titration method against EDTA using oriochrome black T indicator.

*Effective Cation Exchange Capacity:* The Effective Cation Exchange Capacity (ECEC) was determined by summation of exchangeable base and exchangeable acidity [12].

# Media Preparation for the Microbial Load Determination

*Soil Extract:* 1000g of top fertile soil was suspended in 1 litre (1000ml) of distilled water and stirred vigorously using stirring rod. The mixture was filtered with Whatman filter paper. The filtrate was autoclave at a temperature of  $121^{0}$ C and pressure of 1b/sq inch for 15 minutes to sterilize the extract.

*Medium Preparation for Bacteria:* 28g of agar was suspended in 1000ml of distilled water in a 1 litre conical flask and mixed properly. The mixture dissolved completely by boiling it over a Bunsen burner for 10-15 minutes and sterilized the mixture by autoclaving at temperature of 121<sup>o</sup>C under 1b/sq inch pressure for 15 minutes.

*Medium Preparation for Fungi:* Malt extract agar was used as medium for fungi. 26g of agar was suspended in 1000ml of distilled water and 20ml of malt extract was added. The mixture was mixed properly, and sterilized by autoclaving at a temperature of 121<sup>o</sup>C and 1b/sq inch pressure for 15 minutes.

Gram Staining Reagents: 70% alcohol. This was prepared by taking 70ml of ethanol into 30ml of distilled water. Gram iodine or crystal violet Solution: Crystal violet 10g Distilled water 1000ml The solution was made in bulk and filtered Iodine solution Iodine 10g Potassium iodide (KI) 20g Distilled water 1000ml 10g of iodine was piped into 1 liter conical flask and 20g of KI was added and mixed properly. Small amount of water was added to dissolve the mixture completely before making it up to 1 litre. Safranin Safranin (2.2% solution in 99% ethyl alcohol) 10ml distilled water

*Serial Dilution:* 9ml of distilled water was pipette into clean oven dried test tubes based on the number of samples involved and sterilized using the autoclave. 10g of the soil samples was suspended in 95ml of

sterile distilled water in a beaker. 70% alcohol was used to clean the stirrer rod and the mixture was stirred between 30-60 seconds and allowed to stand for 30 minutes.

1ml of the mixture was introduced into the test tube that contained 9ml of sterilized distilled water using 10ml sterilize pipette starting from the first according to their label in the test tube rack from  $10^{-1}$  to  $10^{-6}$ , and mixed properly. Another clean sterile pipette was used to transfer 1ml from test tube3 ( $10^{-1}$ ) to the second test tube ( $10^{-2}$ ). The same procedure was used for the remaining test tubes.

1ml was transferred from the  $6^{\text{th}}$  (10<sup>-1</sup>) test tube into a stirred Petri dish using a sterile pipette. Fifteen millimeters of soil extract agar was carefully poured into the Petri dish and stirred carefully to ensure homogeneous mixture in order to have discrete colony. The plate was allowed to gel and then inverted, sealed using masking tape to prevent the vapour falling back to contaminate the culture before incubating at temperature of 28-37°C for 24 hours. The same procedure was carried out on other samples. The colonies were counted using colony counter.

*Gram Staining:* Gram staining was used in the preliminary identification of the bacteria. The principle behind this lies in the strength of the microorganism to retain the basic dye colour after discolouration with 70% alcohol. Smears the glass slide by cleaning it with 70% alcohol using cotton wool to ensure clean, transparent and grease free slide. A sterilized wire loop in a burner was used to pick culture and stained on the slide. Add a drop of water and wave over a burner flame to make it thin film, then wave over the air to fix it and place the slide on a metal.

Add few drops of crystal violet for 20 seconds, wash for 2 seconds with sterile distilled water. Add grams iodine for 60 seconds, decolorized with 70% alcohol for 10-20 seconds. Wash with sterilized distilled water for 2 seconds. Add few drops of safranin for 20 seconds and wash with sterilized distilled water for 2 seconds and then blot dry. In gram staining you considered the colour, the shape and the arrangement. The slide was view microscopically under x40 magnification objective lens. Gram positive (+ve) organisms were seen as blue or violet colouration while the red colouration indicate gram negative (-ve). The violet colour is the primary while red is the secondary colour.

*Sub-culture:* Sub-culture involved the picking up of cells from a colony on a fresh media agar and incubated. The goal of sub-culturing was to maintain pure culture.

*Pure Culture:* Pure culture was obtained by picking up colony cell from the sub-culture using sterilized wire loop and streaked on a fresh agar media and incubated at  $37^{0}$ C.

# **Characterization and Identification of Isolates**

Various biochemical test were carried out for the characterization and identification of the isolates. *Catalase Test:* A little quantity of the organism from the pure culture was pick up using a sterile wire loop and placed on a clean glass slide. A drop of 3% hydrogen peroxide was added. The observation of bubbles indicated a positive result while a negative result indicated no bubbles  $H_2O - O_2$ . This was carried out to show if bacteria isolate has peroxidase enzyme. Which can breakdown hydrogen peroxide into water and oxygen.

*Oxidase Strips:* A little quantity of the organism from the pure culture was picked using a sterile wire loop and placed on a piece of oxidase strips. A change of colour to violet show positive result while a negative result show no colour change.

*Sugar Fermentation:* 3g of peptone water was suspended into 300ml distilled water in a beaker and was stirred to dissolve the mixture. 10ml was pipette from the mixture into a test tube, corked using cotton wool and was then sterilized with autoclave at a temperature of 121<sup>o</sup>C under pressure of 1b/sq inch for 15 minutes. 0.1g or 1% of glucose, sucrose, lactose and mannitol was suspended in the test tube. Four test tubes for each isolate and was inoculated with the test organisms and incubated at 37<sup>o</sup>C for 48 hours. Acid production was shown by colour change from yellow to whitish colouration which indicated positive result while negative result show no colour change.

Identification of fungi isolates was based on the colony and cell morphology. Cell morphology was viewed and described under microscope. A drop of distilled water was dropped at the center of a clean slide. The fungus growth was picked from the petri dish using sterilized wire loop and mix with the water. The slide was covered with cover slip and then viewed microscopically under X40 magnification objective lens.

#### **Results and Discussion**

#### Particle size distribution

*Silt Fraction:* Table1 shows that the values of the silt fraction ranged from 5.0 - 12.0% in oil affected soils and 8.0 - 15.0% in non-oil affected soils. The result shows that oil affected soils has lower silt fraction than the non-oil affected soils. The values shows that in oil affected soils, the silt fraction decreased by 3% in Total filling station, 1% in NNPC mega station, 3% in Oando, 5% in Conoil and 7% in Mobil filling station respectively. This could be attributed to the differences in the chemical component of the fuel in sales resulting from refining processes.

Sample	Sand (%)	Silt (%)	Clay (%)	Texture (%)				
TSa	94.3	5	0.7	Sandy				
TSb	89.3	8	2.7	Sandy				
NSa	78.3	8	13.7	Loamy sand				
NSb	77.3	9	13.7	Loamy sand				
OSa	82.3	12	5.7	Loamy sand				
OSb	76.3	15	8.7	Loamy sand				
CSa	79.3	9	11.7	Loamy sand				
CSb	89.3	15	4.7	Loamy sand				
MSa	94.3	5	0.7	Sandy				
MSb	79.3	12	8.9	Loamy sand				
Mean	83.3	9.8	7.12					
Range	76.3-94.3	5.0-15	0.7-13.7					
SD	6.91	3.62	4.93					
CV (%)	8.32	36.94	69.94					

Table 1: Particle Size Distribution

Note: Sa = Total oil-affected soils, TSb = Total non-oil affected soils, NSa = NNPC mega station oil affected soils, NSb = NNPC megastation non-oil affected soils, OSa = Oando oil affected soils, OSb = Oando non-oil affected soils, CSa = Conoil oil-affected soils, CSb = Con non-oil affected soils, MSa = Mobil oil affected soils and MSb = Mobil non-oil affected soils

*Clay Fraction:* The values of clay content ranged from 0.7% - 13.7% in oil affected soils and 2.7 - 13.7% in non-oil affected soils. The values shows that oil affected soils have lower clay content than the adjacent non-oil affected soils in 3 petrol stations (Total, Oando and Mobil). In NNPC, the values of both oil and non-oil affected were the same - 13.7%. While in Conoil, the clay content was higher in oil affected soil

than the adjacent non-oil affected soil by 7%. In total, clay content decreased by 2%, 3% in Oando and 8.2% in Mobil filling stations respectively.

Sand Content: The values of sand content ranged from 78 - 94.3% in oil affected soils and 76 - 89.3% in non-oil affected soils. The result shows higher sand content in soil affected oils than the non-oil affected soils. The values show a high sand fraction in both soils studied and this may be characterized by sand formed on unconsolidated coastal plain sand and sandstones. However, the oil affected soils showed a significant increase in sand content than the non-oil affected soils. Total has 5% increase in sand content, NNPC 1%, Oando 6%, and Mobil 5% respectively. In Conoil, sand content was higher in non-oil affected soils than the oil affected soil.

Since sandy soil is not fit for crop production, the presence of oil-spillage which significantly increased the sand percentage has adverse effect on the fertility of the soil. The high sand content may be as a result of probable high drainage of oil into the lower horizon of the soil, washing away nutrients, fine particles and causing aeration problems as the air pores are blocked by oil.

#### **Chemical Properties of the Soil**

Soil reaction (pH): From Table 2, there was a slight variation in the pH values of the soils, which ranged from 5.2 - 5.8 in oil-affected soils and 5.5 - 6.2 in non-oil affected soils. The soils were all slightly acidic and this acidity cannot be attributed entirely to the oil spillage since the non-oil affected soil was equally acidic. The acidity is typical of the soils of the south eastern part of Nigeria. However, the oil affected soils have lower pH values (more acidic) than the non-oil affected soils. The results show 0.4 increase in acidity of oil-affected soil in Total, 0.3 in NNPC mega-station, 0.3 in Oando, 0.4 in Conoil and 0.2 in Mobil filling station.

Sample	Soil reaction	Organic C (%)	Available P (%)	Total N (%)	Electrical conductivity	Hydrocarbon content	Ca2+ cmol/kg	Mg2+ cmol/kg	K+ cmol/kg	Na+ cmol/kg	H+ cmol/kg
-					(Mscm-1)	(PPM)	U	U	U	U	C
TSa	5.8	3.59	75.12	0.31	44	719	2	0.2	0.2	0.09	1.16
TSb	6.2	1.5	80.75	0.12	40	281	4.8	0.2	0.6	0.06	0.92
NSa	5.2	2.65	82.5	0.22	75	366	5.4	0.6	0.12	0.1	0.88
NSb	5.5	2.05	82.75	0.17	57	112	5.8	3.8	0.15	0.09	0.96
OSa	5.5	1.6	88.75	0.13	34	770	5	0.4	0.11	0.1	1
OSb	5.8	2.39	85.5	0.2	27	295	9.2	0.8	0.12	0.09	0.48
CSa	5.6	1.08	74.63	0.09	73	808	11	0.8	0.12	0.09	0.72
CSb	6	3.59	90.13	0.3	41	289	6	1.4	0.1	0.08	0.96
MSa	5.7	3.93	82.13	0.33	99	816	6	0.2	0.11	0.09	0.6
MSb	5.9	3.13	79.38	0.26	66	290	5.4	1	0.1	0.09	0.84
Mean	5.72	2.55	82.16	0.21	55.6	474.6	6.06	0.94	0.17	0.09	0.85
SD	0.29	0.99	5.10	0.09	22.48	270.03	2.46	1.08	0.15	0.01	0.20
CV (%)	4.9	0.39	0.06	0.40	0.40	0.57	0.41	1.15	0.88	0.13	0.24

#### Table 2: Chemical Properties of the Soil

Note: Sa = Oil affected soils, Sb = Non-oil affected soils

*Organic Carbon:* From Table 2, the values of the organic carbon ranged from 0.08 - 3.93 in oil affected soils and 1.5 - 3.5 in non-oil affected soils. The values were higher in oil affected soils than the adjacent non-oil affected soils in 3 petrol stations (Total, NNPC and Mobil) while reverse was the case with Oando and Conoil petrol stations.

*Available Phosphorus:* From Table 2, the values of available phosphorus ranged from 74.63 - 88.75 in oil affected soils 79.38 -90.63 in non-oil affected soils. The values were lower in oil affected soils of Total, NNPC and Conoil petrol stations than the adjacent non-oil affected soils; the reverse was the case for Oando and Mobil petrol stations. The values of the available phosphorus of both oil affected soils and non-oil affected soils were high as shown by their values above. These values are essential component of nucleic acid (DNA, RNA), phospholipids, co-enzymes, high energy phosphate bond (ADP, ATP) in crop production. This could be attributed to the effects of oil and its chemical composition on the vegetation of the surrounding environment.

*Electrical Conductivity:* From Table 2, the values of electrical conductivity ranged from 34.0 - 99.0 in oil affected soils and 27 - 66.0 in non-oil affected soils. The values were higher in oil affected soils than the adjacent non-oil affected soils. In Total petrol station, the value increased by 4.0, NNPC by 18.00, Oando by 7.00, Conoil by 32.00 and Mobil by 33.00. From the result, it can be inferred that oil spillage increases the redox properties of the soil in contrast to non-oil affected soil. This increase in electrical conductivity following oil spillage is indicative of high accumulation of heavy metals ionic concentration such as nickel, copper and lead. High conductivity is an indirect measurement of ionic concentration. Enhanced concentrations of nickel, copper and lead in the soils may result in their absorption by plants at a high level, which may bring about possible bioaccumulation by plants and animals and can lead to toxic reactions along food chain.

Excessive level of ionic concentration such as nickel in the soil is toxic to some soil fauna like earthworms, which are adjuncts to the microflora in organic matter decomposition and may also reduce heterotrophic activity of the microflora. Exposure to lead ions (Pb2+), even at low-level concentrations of 0.1mg/kg in soil is known to reduce heterotrophic activity of microflora. This may be one of the reason bacteria populations are low in oil affected soil than non-oil affected soil as observed in this research work.

*Hydrocarbon Content:* From Table 2, the values ranged from 366 ppm - 816 ppm in oil affected soils and 12 ppm - 295 ppm in non-oil affected soils. The value are higher in oil affected soils than the adjacent non-oil affected soils. In Total, NNPC, Oando, Conoil, Mobil petrol stations, the value increased by 438, 254, 475, 519 and 526 respectively. The results showed that Mobil petrol station at Marian had the highest hydrocarbon content of 816ppm while NNPC mega-station at Murtala Muhammed highway had the lowest hydrocarbon content of 366 ppm. Contamination caused by petroleum product contains a variety of hydrocarbons. As the hydrocarbons are very many, it was not useful to measure the individual amount of each hydrocarbon found together in a particular sample of soil. The total amount of hydrocarbons content found in sample is useful as a general indicator of petroleum contamination at any particular sites (ATSDR, 1999).

Hydrocarbons enter the body when air is breathe in, food is swallowed, through contaminated water or by touching it. Most components of hydrocarbon will enter the bloodstream rapidly when we breathe in, in the form of vapour or mist or when swallowed. The compounds in different petroleum hydrocarbon fractions affect the body in different ways. Some of the petroleum hydrocarbon compounds, particularly the smaller compounds such as benzene, touene, and xylene (which are present in gasoline) can affect the human central nervous system. One petroleum compound (benzene) has been shown to cause cancer (Leukemia) in human. The International Agency for Research on Cancer (IARC) has determined that benzene is carcinogenic to human (ATSDR, 1999). Petroleum hydrocarbon also affects soil fertility, it 'sterilize' the soil and prevent crop growth and yield for a long period of time (Onwurah, 1999). This may also ascertained why there were brownish and dead grasses in an oil spilled soil as observed during the course of this research work in all the petrol stations.

Table 3: Microbial Count of Soils Affected with Petroleum Spillage and Non-affected Soils							
Sample (sa)	Bacteria	Sample (sb)	Bacterial				
	Count (CFU/g)		Count (CFU/g)				
TSa	4.5 x 106	TSb	4.7 x 106				
NSa	1.8 x 106	NSb	1.7 x 106				
OSa	1.7 x 106	OSb	3.2 x 106				
CSa	1.6 x 106	CSb	3.9 x 106				
MSa	4.3 x 106	MSb	4.5 x 106				
Mean	2.78 x 106		3.6 x 106				

## Microbial Count of Soils Affected with Petroleum Spillage and Non-affected Soils

Note: Sa = Oil affected soils, Sb = Non-oil affected soils

*Calcium:* From Table 3, the values of calcium ranged from 2.0 - 11.0 in oil affected soils and 4.8 - 9.2 in non-oil affected soils. The values were lower in oil affected soils than the adjacent non-oil affected soils. This shows that oil spillage decreases the calcium content of the soil. In Total petrol station, the value decreased by 2.8, NNPC - 0.4, Oando - 4.2, in Conoil and Mobil, results show higher calcium in oil affected soils than non-oil affected soils by 5 and 0.6 respectively.

*Magnesium:* In Table 3, the values of magnesium ranged from 0.2 - 0.8 in oil affected soils and 0.2 - 3.8 in non-oil affected soils. The values were lower in oil affected soils than the adjacent non-oil affected soils. Though no decrease was noted in Total petrol station, the magnesium content around NNPC petrol station decreased by 3.2, Oando by 0.4, Conoil by 0.6 and Mobil by 0.8.

*Nitrogen:* From Table 3, the values of nitrogen ranged from 0.09 - 0.33 in oil affected soils and 0.12 - 0.3 in non-oil affected soils. The values were higher in oil affected soils of Total, NNPC and Mobil petrol stations than the adjacent non-oil affected soils. The reversed was the case with Oando and Conoil petrol stations. This could be attributed to the changes in the microbial component of both the oil-affected and non-oil affected soil.

*Potassium:* From Table 3, the values of potassium ranged from 0.09 - 0.12 in oil affected soils and 0.1 - 0.15 in non-oil affected soils. The values were lower in oil affected soils than the adjacent non-oil affected soils. In Total petrol station, the value decreased by 0.03, NNPC 0.03, Oando 0.01 respectively. The soil around Conoil and Mobil petrol stations did not show any decrease in potassium values.

*Sodium:* From Table 3, the value of sodium ranged from 0.09 - 0.1 in oil affected soils and 0.06 - 0.09 in non-oil affected soils. The values are higher in oil affected soils than the adjacent non-oil affected soils. This result shows that oil spillage increases the sodium content in the soil. In Total, the value increased by 0.03, NNPC 0.91, Oando 0.91, Conoil 0.01 and Mobil 0.0 respectively.

The values of Ca, Mg and K were lower in oil affected soils than the adjacent non-oil affected soils. Sodium shows a slight increase in oil affected soils than the adjacent non-oil affected soils. The reduction of the amount of Ca, Mg and K content by oil spillage is indicative of the negative effects on the soil fertility status. The general reduction in the cations content of the soil by oil spillage render the soil unsuitable for crop production.

*Effective Cation Exchange Capacity (ECEC):* The values ranged from 3.54 - 13.01 in oil affected soils and 6.1 - 10.89 in non-oil affected soils. The values were lower in oil affected soils than the non-oil

affected soils. This result shows that oil spillage reduces the ECEC of the soil which will have a significant negative effect on crop yield and land productivity.

*Base Saturation:* From Table 3, the value of base saturation ranged from 67.2 - 92.3% in oil affected soils and 84.9 - 93.8% in non-oil affected soils. The values are lower in oil affected soils than the adjacent non-oil affected soils.

#### Fungi identification, count and characteristics of probable isolates

Table 4: Fungi Cou	int		
Sample (sa)	Fungi count	Sample (sb)	Fungi count
	(CFU/g)		(CFU/g)
TSa	5.0 x 104	TSb	4.0 x 104
NSa	2.0 x 104	NSb	2.0 x 104
OSa	3.0 x 104	OSb	2.0 x 104
CSa	4.0 x 104	CSb	3.0 x 104
MSa	3.0 x 104	MSb	4.0 x 104
Mean	3.4 x 104		3.0 x 104

Note: Sa = Oil affected soils, Sb = Non-oil affected soils

#### Table 5: Characteristics and Identification of Probable Isolates

Sample	Cultural characteristics	Morphological gramstain	Catalase	Oxidase strips	Fermentation	Probable isolate
	Greenish colour, circular and waving	Gram +ve cocci chain	+ve	+ve	- A A A	Ganobacterium
	Whitish and circular	Gram +ve cocci	+ve	+ve	AAA-	Micrococcus luteus
Sa	Greenish colour, small, circular and waving	Gram –ve rod in chain	+ve	+ve	A	Pseudomonas matophillia
	Yellowish colour and circular	Gram +ve cocci	+ve	+ve	ААА.	Nocardia
Sb	Greenish colour, circular, flat and opaque	Gram +ve rod occurring in chain	+ve	+ve	- A A A	Bacillus substilis
	Greenish colour, small and waving	Gram –ve rod in chain	+ve	+ve	A A	Pseudomonas putidae

Note: Sa - Oil affected soils, Sb - Non-oil affected soils

Sample	Colonial morphology	Cell morp	Probable isolates				
Sa	morphology	Nature of hyphae	Colour of hyphae	Appearance	Arrangement	Spore	
	Small round, green colour with smooth surface	Septate	Green	Sporangiospore of conidiosphore	Single arrangement	Tiny spherical spore, scattered	Aspergillus flavin
	Yellowish green with smooth surface	Non- septate	White (Transparent)	Sporangiophore	Single arrangement	Ovoid spore	Rhizopus spp
	Whitish grey colour with velvet surface	Non septate	White (transparent)	Erect sporangiosphore	Single arrangement	Small ovoid spore	Mucor spp
	Brownish with smooth surface	Non septate	Brown	Filamentous yeast-like mold	Dusttered arrangement	Ovoid spore	Candida
	Yellowish green with smooth appearance	Septate	Yellow	Elongated conidiosphore	Inter wine arrangement	Small round spore	Aspergillus spp
Sb	Brownish with smooth surface	Non septate	Brown	Filamentous yeast-like mold	Clustered arrangement	Ovoid	Candida spp
	Whitish grey colour with velvet surface	Non septate	White (transparent)	Erect sporangiosphore	Single arrangement	Small ovoid spore	Mucor spp
	Yellowish with smooth	Septate	Yellow	Filamentous	Single arrangement	Small ovoid spore	Mucor spp

Table 6: Fungi Identification

Table 4, 5 and 6 shows the microbial populations of the soil samples collected from both oil and non-oil spilled affected soil. The average bacterial population of the different samples of soil from oil-spilled soils was 2.78 x  $10^{6}$ cfu/g while the average population from the non-oil spilled area was 3.6 x  $10^{6}$ cfu/g. The isolated bacteria genera in the oil-spilled soils were *Ganobacterium, Micrococcus luteus, Pseudomonas maltophilia*. The isolated bacteria genera in the non-oil spilled soil were *Nocadia, Bacillus substilis* and *Pseudomonas putidae*. Fungi were found in both oil affected and non-oil affected soil. The fungi populations in the oil-spilled soil averaged  $3.4 \times 10^{4}$ , while that of non-oil spilled soils is  $3.0 \times 10^{4}$ . The fungal species of oil affected soils were *Aspergillus flavin, Rhizopus spp, Mucor spp, Candida spp* and *Aspergillus spp*. The non-oil spilled soils are *Candida spp, Mucor spp* and *Aspergillus spp*.

Difference in microbial population is a reflection of many factors such as physical factors - pH, temperature, oxygen, moisture, hydrostatic pressure and osmotic pressure and nutritional factors - chemical requirement, trace elements and organic growth factor (Haris, 1962). The differences in both bacterial and fungal populations could then be attributed to possible change in the chemical constituents, nutrient and oxygen supply to the soil. Some of these factors were affected following every oil spillage, which finally become limiting over time. At initial oil spilled, some of the microbial are killed and only those that are tolerant to the products will be found in the soil spills with oil which later multiply. This may explain while the bacteria population in non-oil spilled soil is higher than oil spilled soils.

The slightly high fungi population in the oil affected soils could be a result of the increase in the nutrient level (Carbon level) and due to the fact that fungi tolerate acidic environment than bacteria following oil spill (Okereke et al., 2007). The population abundance may decrease with time since abundance in fungi due to oil spillage is followed by rapid decline in their number, which could be best explained by the depletion in nitrogen due to biodegradation. Nitrogen is used up during biodegradation of petroleum compounds (Haris, 1962). This reduces the amount of nitrogen available to the flora hence their population would decline. The slight difference in bacterial population of oil-spilled soil and non-oil spilled soil may be due to the effect of slight increase in the acidity of the soil spilled with petroleum oil.

Following every oil spillage, there is always slight increase in soil acidity of the affected environment mostly when the spilled petroleum compounds are of high Sulphur content (Haris, 1962).

The slight increase in the soil acidity of the soils studied could therefore be due to high Sulphur content of the oil-spilled compounds. This explains the slight decrease in bacterial population in the oil spilled soils. Also, the depletion in the oxygen level of the spilled soil may have contributed to the population differences.

### **Conclusion:**

As much as possible, oil spillage or soil contamination by petroleum products should be avoided because it has so much negative effects on the chemical and biological properties of soils vis a vis the crops or vegetation in the surrounding. The activities of microorganisms, especially beneficial microorganisms is so important to nutrient release to crops/vegetation grown on a particular soil hence the need for avoidance of pollution and contamination of soil is vital. Petroleum products are very toxic to living organisms in soils which indirectly control the chemical and biochemical activities in soils for plant nutrition.

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