

Effects of Oil Pollution on the Soil of Umuokpara in Abia State

*Ekwenye, U.N. and Aneke, N. C.

Department of Microbiology, Michael Okpara University of Agriculture Umudike, Nigeria.

Abstract

The effects of oil pollution on the soil of Umuokpara in Abia state was investigated. Soil samples were taken from three sites; from the mining spot, 100 meters away from the spot and 200 meters away from the spot. Soil samples collected were subjected to Laboratory analysis. The soil texture varied from loamy sand to sandy loam on the surface to sandy loamy in the sub-surface horizons. The soil from the oil exploration were very strongly acidic unlike that collected from 100m and 200m away from the mining spot. The organic carbon ranged from 3.53 – 5.70g/kg, phosphorus ranged from 13.70 – 16.30mg/kg. A survey of microflora were also carried out. Seven bacterial species were isolated namely; *Pseudomonas* spp., *Bacillus* spp, *Clostridium* spp, *Streptococcus* spp, *Staphylococcus* spp, *Micrococcus* spp and *Alcaligenes* spp. Four fungal species were also isolated namely; *Penicillium* spp, *Aspergillus* spp, *Fusarium* spp and *Actinomyces* spp. The microbial population was observed to increase at the distance away from the mining spot. The oil therefore influenced microbial population in the soil. The microorganisms were able to grow and degrade oil substrates into other metabolites less harmful to the environment. Results of the findings proved that soil quality of the area has been degraded

*Corresponding Author.

Introduction

The environmental impact of oil exploration is one of inevitable consequence of economic development and civilization in a technical age. In Nigeria, the oil industry is the most important sector of the economy and it has since experienced a tremendous increase as a major economic activity. Petroleum provides about 90% of the export earning and services as a primary raw material for chemical industries (Akachuku and Ekwenye)

The high demand for petroleum products in form of cooking gas, aviation fuel, gas oil, engine lubricating oil, asphalt and coal tar means increased production. The incidences of recorded environmental pollution due to high rate of petroleum related activities have been associated with frequent oil spillage especially through oil well blow outs, tanker accidents, pipelines breakage and sabotage. These mishap results in the release of crude oil and refined petroleum products into territorial and aquatic environment (Okpokwasili and Nnorom, 1990).

The effects of oil pollution on soil depends on the size and grade of oil spilled. The mobility of the oil in the polluted soil also depends on the quantity and viscosity of the oil in one hand the porosity of the soil concerned on the other. Following the gravitational forces, the oil moves through the upper part of the soil resulting in absorption, immobilization and chemical components of the soil giving rise to alterations of the properties (Nwangwu and Okey, 1981).

Crude oil pollution of soil and underground water causes physical changes in microbiological, chemical and physical properties of soil and the growth of vegetation (Ellis and Adams, 1997).

The contamination of soil normally results from a range of activities related to our industrialized society. Contaminated land is described as land that contains substance that when present in sufficient quantities or concentration can probably cause harm to human beings directly or indirectly and to the environment in general. The damages in soil ranges from destruction of soil organisms to change in microbial spectrum of the polluted area (Akomeo, 1981).

Petroleum has an average 85 percent carbon, 13 percent hydrocarbon and 2 percent sulphur, nitrogen and oxygen (Cenci and Caldine, 1971). Today's society is increasingly concerned about soil degradation, the sustainability of soil productivity, and maintenances of biodiversity. The environmental consequences of soil pollution include adverse effects on the soil microflora all of which assist in soil fertility (Odu, 1981; Torstensson *et al.*, 1998). Petroleum contamination of soil results on the soil remaining unsuitable for crop growth for months or several years (Odu, 1978). Oil pollution affects plants directly, and in the soil creates adverse conditions detrimental to plants growth (Odu, 1972; Akachuku and Ekwenye; 1995). The pipeline breakage necessitated this study.

This paper reports the microbial flora and physicochemical properties of soil at

Umuokpara after an incident of pipeline breakage.

Materials and methods

Study area

The soil samples used in this study were obtained from Umuokpara in Abia State, Nigeria. The oil polluted soil samples were collected from different spots namely.

At the spot of mining, 100m away from the spot of mining, 200m away from the spot of mining.

Method of samples collection

Soil samples were collected randomly at a depth of 15cm from an Agricultural farm in Umuokpara, Abia state. Samples were homogenized, dried, sieved through a 2mm mesh and stored in polythene bag at room temperature in the laboratory. Some samples were also taken to laboratory for determination of microbial population.

Physico-chemical laboratory analysis

pH determination:

It was done by weighing one gram of air-dry soil into a 50ml beaker. Then 25mls of distilled water was allowed to stand for 30minutes and stirred with a glass rod. The electrodes of the digital pH meter model 3505 were inserted partly into the settled suspension and pH reading was taken.

Determination of available Phosphorous

: One gram of soil was weighed and 20ml of Bray J Solution was poured into the sample (Bray J = HCl + NH₄F + distilled water). This was shaken for 1 minute on a mechanical shaker and the suspension centrifuged at 2,000rpm for 15 minutes. After centrifuging, the mixture, 5ml of the solution was pipetted into a plastic bottle and 43ml of distilled water was added to it (A). Two millilitres of the solution of ascorbic acid mixed with reagent A was also added to make it

$$\% \text{ total N in the soil} = \frac{N (T - B \times 14 \times 100)}{1000 \times W}$$

Determination of exchangeable bases

It was determined for Calcium, Magnesium, Potassium and Sodium. Determination of exchangeable acidity was done using the method of Chapman and Prott, (1985). The particle size was determined using the method of Pames, (1990). Separation of sand fraction was

up to 50ml. Then the reading was taken using a spectrophotometer 20 D Plus.

Determination of organic matter

This was determined using the method of Pames, 1990. Five grams of each soil sample was further ground to pass 50 mesh size sieves. One gram of the ground sample was weighed into a flat bottom flask, 200ml of Conc. H₂SO₄ was added and then 10ml of the potassium dichloride (K₂Cr₂O₇) solution was added. The suspension was allowed to stand for 30minutes after which 50ml of distilled water was added to increase bulk. The solution was titrated against standard ferrous ammonium sulphate solution using barium diphenyl sulphate as the indicator. The colour change at the end point was pale blue to purple red. % organic carbon in soil = $\frac{\text{Mek}_2\text{Cr}_2\text{O}_7}{\text{MeFeSO}_4} \times 0.003 \times 100 \times F$.

Where F = Correction Factor = 1.33,

Meq = Normality of solution x ml of solution used
% of organic matter in soil = % organic carbon x 1.724.

Determination of total Nitrogen

One gram of each soil sample was weighed into a digestion tube. 50ml of diluted H₂SO₄ and Selenium tablet was added. It was digested in Kjeldahl digester flask. The flask was heated at a low heat and was allowed to cool, after which 45ml of distilled water was added to the digested soil. The entire solution was transferred into 50ml of conical flask, 10mls of boric acid (indicator) was added which was then placed under the condenser of the distillation apparatus (Markham apparatus) and 10ml of digested solution got from the first 50ml was added into the distillation apparatus together with 10ml of NaOH. Two drops of mixed indicators was titrated with 0.2N H₂SO₄. The colour change at the end point was from green to pink.

carried out to determine the % clay, % silt and % sand.

Determination of Microbial Population

The stock samples were prepared by adding 1 gram of soil sample to 10ml of sterile water and was shaken properly. Test tubes containing 9ml of sterile water was prepared and 1ml of the stock sample was

added to 9ml sterile water in the test tube (10^{-2}). Subsequent dilutions were then made up to 10^{-6} . Dilution series were achieved for each soil sample using a separate sterile pipette in each case (Bezbaruah *et al.*, 1994). From each of the diluted samples ranging from 10^{-1} to 10^{-6} , 0.2ml was inoculated into each of the Nutrient agar and Sabouraud dextrose agar plates by the spread plate technique. The plates were incubated in an inverted position at 37°C for 24 – 27 h.

Determination of petroleum hydrocarbon utilizing microbes

Mineral salt was prepared according to Mills *et al.*, 1978. Each bacteria isolate was cultured using a nutrient agar containing a prepared mineral salt and 2ml of crude oil. The mineral salt used includes NaCl 10g, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.42g, KCl 0.29g, KH_2PO_4 0.83g, NaHPO_4 1.25g, NaNO_3 0.42g and 1 litre of distilled H_2O colonies that developed and showed a zone of clearance of oil were counted as oil degrading microbes.

Microbial isolation and identification

The mixed cultures observed on the plates were examined and subcultured into freshly prepared nutrient agar and Sabouraud dextrose agar plates to obtain pure cultures. The pure culture organisms were isolated into agar slants and incubated at 37°C for 24hours – 27hours, after which they were removed and stored in the refrigerator for further tests.

Morphological and biochemical characterization of microbial isolates

Morphological and biochemical tests were carried out to characterize the organisms isolated from various polluted soil samples. These tests include Gram staining, motility, citrate utilization, nitrate, capsule, sugar fermentation, catalase, indole, methyl red, hydrogen sulphide and Urease tests following the methods of Cruickshank *et al.*, 1982, Pelezar and Chan, 1977 ; Cheesbrough, 1991.

Determination of petroleum hydrocarbon utilizing microbes

Mineral salt was prepared according to Mills *et al.*, 1978. Each bacteria isolate was

Cultured using a nutrient agar containing a prepared mineral salt and 2ml of crude oil.

The mineral salt used include: NaCl 10g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.42g; KCl 0.29g; KH_2PO_4 0.83g; NaHPO_4 1.25g ; NaNO_3 0.42g; Distilled water 1 litre

Colonies that developed and showed zone of clearance of oil were counted as oil degrading microbes.

Results and discussion

The effect of crude oil pollution on soil particle size is shown in Table 1. Soil samples collected at the source of pollution had higher level of sand than those ones at 100m and 200m respectively from the source of pollution. The observed difference could be attributed to the physiographic position of the sampling points. The source of pollution was at a higher slope position than that of the lower positions from the upper slope due to erosion. Loss of finer particles will leave more coarse particle. This also explains the observed variations in clay content.

Exchangeable calcium in the soils studied ranged from 2.00 to 4.00 Cmol/kg (Table 2). The soil further away from the source of pollution had the highest level of Ca followed by the soil at source of pollution. The values of exchangeable Ca obtained in this study were higher than a range of 1.10 to 1.90 cmol/kg obtained for soils under some indigenous multipurpose tree species (Ano, 1997).

The total petroleum hydrocarbon from the source was 268.4, 100m away was 35.0 and 200m away was 21.0. This actually indicated the amount of crude oil found in that soil. The base saturation ranged from low to high (77.90 – 82.90). It was relatively higher in non-polluted site than the polluted site. The value decreased with increase in profile depth due to eluviations and illuviation as a result of leaching and deposition. The relatively high base saturation in non-oil exploration area (control) was attributed to the fact that the soil is not subjected to chemical soil degradation arising from oil spillage and gas flaring. Effective cation exchangeable capacity was gotten by calculation of all exchangeable acidity (Table 2).

The bacteria and fungal isolates from the polluted soil are shown in Tables 4 and 5.

Magnesium showed the same level at the surface and the sub soils and ranged from 1.20 to 1.60cmol/kg. Potassium and Sodium ranged from 0.072 – 0.250 and 0.087 to 0.183 cmol/kg respectively (Table 2). The low value found in the surface horizon decreased down the profile, this may be due to leaching.

Organic matter was found to be high on the top soil due to deposition of carbon compound from crude oil and gas flaring around the site (Table 3). Also, the soil pH values increased from 4.22 to 4.66 (Table 3). This shows that the soil is highly acidic. The difference in acidity level could be due to the activities of Oil Company such as gas flaring which enriched the soil with Al^{3+} and H^+ washed down in the acid rains. Toxic chemicals from burrow pit could be washed down to farmlands contributing to soil acidity. The available Phosphorus was found to be low due to the soil has been contaminated with oil spill (Table 3).

More so, the effect of oil pollution on microbial population is a depression of microbial population (Table 6, 7 and 8). This most likely account for the lesser microorganisms isolated from the spot. The bacterial and fungi (Table 4 and 5) were estimated in the soil of the area under study

to determine any alteration in microbial number, which could have resulted from gross pollution of the environment by the oil spillage. Oil pollution reduces oxygen and moisture contents of the soil as it causes soil particle to stick together, reducing soil interstices. The counts for heterotrophic bacteria in the control sample were high and moderate in the impacted samples likewise the fungi. Alexander (1977) reported that a fertile soil contains billions of bacteria per gram and millions of fungi per gram of soil. That is to say that bacteria pollutions in natural soils are far higher than fungi pollutions. High concentration of crude oil in the soil environment inhibits/suppresses microbial growth. The microbial counts obtained in this study, particularly those samples that had lower counts, showed that contamination of pollutant perhaps was recent crude oil spillage in the area.

In conclusion, microorganisms can be used extensively to correct the havoc oil pollution poses to the ecosystem as they have been observed to be able to grow and degrade oil substrates into other metabolites such as ketone, aldehyde, microbial protein and organic acids which are less harmful to the environment.

Table 1: Effect of distance from source of crude oil pollution on soil particle size distribution.

	Sand %	Silt %	Clay %	Texture
Source of Pollution	86.7	5.1	8.2	Loamy Sandy
100 metres away from the source of pollution	78.7	7.1	14.2	Sandy Loamy
200 metres away from the source of pollution (control)	74.7	5.1	20.2	Sandy Loamy

Table 2: Exchangeable bases as a function of distance from source of crude oil pollution

	Ca	Mg	K	Na	EA	ECEC	BS
					(Cmo/kg)	(Cmo/kg)	(%)
Source of pollution	2.40	1.20	0.250	1.100	1.12	5.07	77.90
100 meters away from the source of pollution	2.00	1.60	0.092	0.087	1.04	4.82	78.40
200 meters away from the source of pollution	4.00	1.20	0.072	0.183	1.12	6.58	82.90(control)

Key: EA=Exchangeable acidity, ECEC=Effective cation exchangeable capacity, BS=Base saturation

Table3: Effect of distance from source of crude oil pollution on levels of organic matter, total nitrogen and available phosphorus in the soil

Distance	Organic Carbon (g/kg)	Organic matter(g/Kg)	pH	Total Nitrogen(%)	Available Phosphorus (mg/kg)
Source of pollution	5.75	9.92	4.22	0.13	11.70
100 meters away from the source of pollution	5.61	9.68	4.49	0.14	13.30
200 meters away From the source of Pollution(control)	3.53	6.08	4.66	0.09	18.40

Table 4 Morphological and Biochemical Characteristics of Bacteria from the examined polluted soil

Media	Nature of colony formation on plate	Gram reaction and microscopy	Motility	Catalase	Oxidase	V.P	Methyl	Indole	Citrate	NO ₃ reductase	urease	Lactose	Glucose	Sucrose	Maltose	Xylose	H ₂ S	Most probable micro organisms
Nutrient agar	Cream colour spread in colonies with wavy edges dull in appearance	Gram positive rod, in short chains central spores	+	+	-	-	-	-	-	+	+	-	+	+	+	±	+	<i>Bacillus</i> spp
"	Colony spreading a little greenish yellow pigments	Gram negative	+	-	-	+	+	-	-	+	+	-	+	-	+	-	+	<i>Pseudomonas</i> spp
"	Cream coloured small raised colonies sometimes slightly red but none diffusing.	Small gram positive mostly in single	+	+	±	-	+	-	±	-	-	-	-	-	-	-	-	<i>Micrococcus</i> spp
"	Cream slightly raised colony with entire edges later turned brown	Grams rods mostly in singles	+	-	-	+	+	-	+	+	-	-	-	-	-	-	-	<i>Alcaligenus</i> spp
"	orange in colour	Gram positive cocci spherical cells	+				+	-	+	-	+	+		+	+			<i>Staphylococcus</i> spp
"	Circular and slightly raised	Cocci in chains gram negative	-	-	+	-	+	-			+	+	+			+		<i>Streptococcus</i> spp
"	Creamy colony	Short rod in chains gram negative	-	+	+	+		-			+	+	+	+	-	+		<i>Acinetobacter</i> spp

Table 5: Characteristic and identification of fungal isolates

Media used	Nature of colony Formation on plates	Microscopic morphological characteristics	Most probable microbes
Sabouraud Dextrose agar	white fluffy growth, later the central part turned Pink.Reverse side creamy	Septate hyphae goat shape called microconidia	<i>Fusarium</i> spp
"	white, later turned greenish velvety and creamy and reverse.	Spores in chain and single conidiophores tip swollen	<i>Aspergillus</i> spp
"	small smooth colonies	Asexual spores or conidia	<i>Actinomyces</i> spp

Table 6: Bacteria Isolated from oil polluted and non oil polluted soil and their frequencies

Organisms	<i>Pseudomonas spp</i>	<i>Bacillus spp</i>	<i>Micrococcus spp</i>	<i>Alcaligenes spp</i>	<i>Staphylococcus spp</i>	<i>Streptococcus spp</i>	<i>Clostridium spp</i>
Source of pollution	+ve	+ve	+ve	+ve	-ve	-ve	-ve
100m away from source of pollution	+ve	+ve	-	+ve	+ve	+ve	+ve
200m away from source of pollution	+ve	+ve	-	+ve	+ve	-ve	+ve
% of frequency	100%	100%	25%	100%	67%	67%	100%

Table 7: Fungal Isolated from oil Polluted and non-polluted Soil and their Frequencies

Organisms	<i>Penicillium</i>	<i>Aspergillus</i>	<i>Fusarium</i>	<i>Actinomyces</i>
Source	+ve	+ve	+ve	-ve
100	+ve	+ve	-ve	+ve
200	+ve	+ve	-ve	+ve
% of frequency occurrence	100%	100%	67%	67%

Table 8: Microbial Population of Soil Sample

Sample	Total heterotrophic bacteria (cfu/g)	Total heterotrophic fungi (cfu/g)	Total degrading bacteria (cfu/g)	Total degrading fungi (cfu/g)
Source of population	1.37×10^3	1.03×10^5	1.35×10^5	1.0×10^5
100m away from source of pollution	3.0×10^5	2.7×10^5	3.1×10^5	1.6×10^5
200m away (control) from source of pollution	4.6×10^6	3.1×10^5	4.3×10^5	2.0×10^6

Key cfu/g: Colony forming unit per gram

References

Akachuku, C.O. and Ekwenye, U.N.; (1995). Impact of Petroleum Industry on Nigeria Ecosystems, Paper presented at the 4th Annual Scientific

Conference of the Nigerian Society for Biological Conservation. Uyo Akwa Ibom State, Nigeria.

Akemeo, U.O. (1981). A Study of the Effects of oil Spills on Groundwater.

- The Petroleum Industry and Nigeria Environment. Proceedings of 1981 International Seminar, NNPC.
- Alexander, M. (1977). Introduction to Soil Microbiology. 2nd Edition John Willey and Sons, New York, U.S.A.
- Ano, A.O. (1997). Effectiveness of Selected Indigenous and Exotic Multipurpose Trees in Soil Fertility Regeneration of Highly Weathered and Soil of Eastern Nigeria. Proceedings of 25th Annual Conference of the Forestry Association of Nigeria, Held in Ibadan, Oyo State, Nigeria. Pp: 220 – 225.
- Awuah, R.T. and Kpodo, K.A.. (1996) High incidence of *Aspergillus flavus* and aflatoxins in stored groundnuts in Ghana and use of microbial assay to assess the inhibitory effects of plant extracts on aflatoxin synthesis. *Journal of Mycopathology* 134: 109-224
- Bababunmi, E.A, Uwaifo, A.O and Bassir, O. (1978) Hepatocarcinogenes in Nigerian foodstuff. *Journal of Nutrition and Dietetics* 28:188-209
- Bezbaruah, R.H., Gogei, B.K. and Pillai, K.R. (1994). Isolation and Identification of Microorganisms involved in Microbial Desulphurization of Petroleum Crude. *Applied Environmental Microbiology* 34: 120 – 125.
- Cenci, G. and Caldemi, G. (1971). Catechol dioxygenase Expressed in a *Pseudomonas* Fluorescent rain exposed to different aromatic compound. *Applied Microbiology and Biotechnology* 47: 306 – 308.
- Cheesbrough, M. (1991), *Microbial Butter* Worth – Heinemann Ltd. Oxford Pp: 448.
- Cruickshank, R. Duguid, J.P., Manmion, B.P., and Swain, R.H. (1982). *Medical Microbiology* 12th Edition, New York Churchill living Stone. Pp: 274 – 278.
- Ellis R.J. and Adams, R.S. (1997). Communication of Soils by Petroleum Hydrocarbons. In A.G. Norman Edition. *Advances in Agronomy*. Academic Press. New York. Pp: 300 – 350.
- Hendrickse, R.R. (1983) Aflatoxin and Kwasiokor epidemiology and clinical studies in Sudanese children and findings in autopsy liver samples from Nigerian and South Africa. *Journal of Medicine and Pathology* 76:559-566
- Hendrickse, R.R. (1984) the influence of aflatoxin on child health in the tropics with Particular references to kwasiokor. *Journal of Medicine and Hygiene* 78: 435
- Li, F.Q. Yoshizawa, T., Luo, S.Y. and Li, Y.W. (2001) Aflatoxins and fumonisines in Corn from the high incidence area for human Hepatocellular carcinoma in Guangxi China. *Journal of Agriculture and Food* 49: 4122-4121.
- Mills, A.L.; Brevil, C. and Colwell, R.R. (1978). Enumeration of Petroleum Degrading Marine and Esfucrine Microorganisms by most probable Number (MPN METHOD). *Canadian Journal of Microbial*. 24: 552 – 557
- Nwangwu, U. and Okey, N. (1981). Environmental Pollution in Nigeria Oil Industry. The Role of Research and Development. The Nigerian Petroleum Industry and Environment Proceedings of 1981 International Seminar. NNPC Akwa Ibom State, Nigeria.
- Odu, C.I.I. (1981). Degradation and Weathering of Crude Oil Under Tropical Conditions in Petroleum Industry and the Nigeria. Environmental Proceedings of the 1981 International Seminar pp: 143 – 153.
- Odu, C.I.I. (1978) Microbiology of Soil Contaminated with Petroleum Hydrocarbon. National Rehabilitation and Reclamation of Soils Affected. Institute of Petroleum Technology Publication. Pp: 77 – 102.
- Odu, C.I.I. (1972). Oil Pollution and Environment. Bulletin of the Science Association of Nigeria. 3: 10 – 12.
- Okpokwasili, G.C. and Nnorom, E.E. (1990) Microbial Degradation of Petroleum

- Hydrocarbons by Braikish water Isolates. In T.V.I. Akpata and D.U.U. Okali (eds), Nigerian Wetland, Proceedings of Man and Biosphere State-of-Knowledge Workshop Port Harcourt, Nigeria MAB-5 National Committee, Ibadan.
- Pames, R. (1990) Fertile Soil: A grower's Guide to Organic and Inorganic Fertilizers.
- Doavis C.A. Fertile Ground Books 37: 15 – 20.
- Pelezar, M. and Land Chan S.E. (1977) Laboratory Exercise in Microbiology. Black Dot Inc., U.S.A. Pp: 297 – 309.
- Thomas, H.A.B., Oker, O.A., Odukoga, G.K, Isameh, A. and Adepoju, A. (2003) Dietary aflatoxin exposure and demo prevention of cancer. *Journal of Environmental Contamination and Toxicology*, 71: 142-145.
- Torstenon, L., Mikael, P. and Stenberg, B.O. (1998) Need of a strategy for Evaluation of Arabic Soil Quantity. *AMBIO*. 27: 4 – 7.
- Uriah, N., Ibeh, I.N. and Olufemi, F. (2001) A study of the impact of aflatoxin on human reproduction, *Journal of Reproduction and Health*, 5:106-110
- Wild, C.P. (1996) Summary of data on aflatoxin exposure in West Africa. Proceeding of the workshop on Mycotoxin in food in Africa 1995 at Cotonu in Benin. International Institute of Tropical Agriculture P:2