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Short Communication

Microbial flora of oil-spilled sites in Egbema, Imo State, Nigeria

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The microbial flora of areas with and without oil spillage within the Egbema oil field in Ohaji/Egbema of Imo State was determined by standard microbiological methods. Preliminary results show moderate biological activities in both environments studied. The average microbial population of the area with oil spillage was 3.08 x 10^7 cfu/g, while that of the adjoining environment was 3.14 x 10^7 cfu/g for bacteria. Fungi population was of the order 10^6. The microorganisms isolated were identified to species level. Majority of the microorganisms were true bacteria. Actinomycete species were also found. In general, species difference between the two environments tended to be considerably significant. Since species and microbial population differences in soils are directly proportional to the availability of carbon source (nutrient), soil acidity, oxygen level and other factors, the significant variation in species and slight difference in microbial population observed are indicative of the effects of oil spillage on microbial flora of a given area.

Key words: Biodegradation, bioremediation, environment, microflora, oil-spill, pollution.

INTRODUCTION

Many oil-producing communities have been suffering from the after effects of oil spillage. The accidental discharge of petroleum products on soil or water surfaces is termed oil spill. Oil-spill pollution has been hazardous and problematic World-wide (Vincent, 1980). The Nigerian National Petroleum Coporation (NNPC) in 1986 reported that a total of about 5,000 barrels of crude oil was spilled from Nigerian Agip Oil Company (NAOC) pipeline near Oshika in Rivers State in August, 1983 (IPS, RUST, 1986). Analysis of the oil samples from the affected environment showed that the organic content of the soil in polluted area was slightly higher and there was also slight increase in soil acidity.

Recorded incidences of oil spill show that a lot of problems have arisen as a result of it. A lot is being spent by affected countries in the cleaning of the spills. United states of America for example, spends millions of dollars in the control and cleaning of oil spills, (API, 1975). Lives have been lost as a result of diseases caused by oil spills (Poly, 1992).

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When there is oil spill, certain microorganisms, which degrade it, grow on it, degrading the crude to different components. The disappearance of spilled crude from the environment is attributable to the activities of the microflora of the soil (Brinton and Warren, 1976). Doestch (1973) reported that *Pseudomonas, Flavobacterium, Alcaligen, Achromobacter, Arthrobacter, Bacillus* and *Micrococus* species are among the hydrocarbon-oxidizing microorganisms. The discovery of the activities of microorganisms in the breakdown of crude to less harmful products, gave rise to bioremediation. Bioremediation involves the use of microorganisms to accelerate the natural breakdown of oil into less harmful products (Poly, 1992). Man-made bioremediation technologies are intended to improve the effectiveness of natural biodegradation (Sandra, 1988).

Egbema, an oil-producing community in Imo State has had several incidences of oil spill. Property worth of millions of naira has been lost as a result of these spillages. This research therefore was aimed at determining the microbial flora of the oil-spilled sites in Egbema, compared with other adjoining environment with the hope of determining possible differences and the effect of such differences on the environment.

Table 1. Total microbial population of soil samples from Egbema.

Sample	Oil – spilled Area		Non Oil- spilled Area	
	Bacteria (cfu/g)	Fungi (cfu/g)	Bacteria (cfu/g)	Fungi (cfu/g)
1	8.4×10^7	12×10^6	4.8×10^7	2.0×10^6
2	1.8×10^7	6×10^6	1.6×10^7	8.0×10^6
3	1.7×10^7	-	6.3×10^7	-
4	1.4×10^7	-	1.1×10^7	-
5	2.1×10^7	-	1.9×10^7	-
Average	**3.08×10^7**	**9.0×10^6**	**3.14×10^7**	**5.0×10^6**

Table 2. Bacteria most frequently isolated from soil samples from Egbema.

Oil-spilled area	Non oil-spilled area
Pseudomonas spp.	*Pseudomonas spp.*
Bacillus spp.	*Arthrobacter spp.*
Corynebacteriaum spp.	*Actinomycetes spp.*
Staphylococcus spp.	
Actinomycetes spp.	

Table 3. Fungi most frequently isolated from soil samples from Egbema.

Sample	Morphology	Type of organism	Species
Oil-spilled Area	Ovoid Spheres	Yeast-like	*Candida spp*
	Filamentous	Mold	*Mucor spp*
	Filamentous	Mold	*Rhizopus spp*
	Filamentous	Mold	*Aspergillus spp*
Non oil-spilled Area	Ovoid Spheres (ii) Filamentous	Yeast-like Mold	*Candida spp Mucor spp*

MATERIALS AND METHODS

Soil samples used for this study were collected using polythen bags, from two different locations in Egbema in Ohaji/Egbema local government area of Imo State. Five different soil samples were collected from where there was oil spillage. Another five soil samples were collected from the adjoining environment where there was no oil spillage. The samples were labeled accordingly (SA1 - SA5 and SB1 – SB5). Standard microbiological procedures were employed in collection and handling of the soil samples and they were analyzed within 24 h of collection.

In the laboratory, 10 g of each of the soil samples was weighed and transferred into 250 ml flask containing 90 ml of sterile distilled water. The suspensions were shaken intermittently for about 30 min. Each solution was allowed to stand for about 1 h after which the suspension was decanted into another 250 ml flask. Serial dilution of each suspension was made and 1 ml of the required dilution added to sterile petri dishes.

The total plate count was done by the pour plate method using nutrient agar (oxoid). The plates were incubated at 37^0C for 48 h. Total plate count (APC) was carried out using a colony counter (Scientific-Cock Limited) model M.E. 16. Plates for culturing fungi were made of Potato Dextrose Agar (PDA) and were incubated at 30°C for three days. Identification of fungi isolates was based on the colony and cell morphology while characterization and identification of bacterial genera was done according to Bergey's Manual of Determinative Bacteriology (John, 1993).

RESULTS AND DISCUSSION

Table 1 shows the microbial population of soil samples collected from both locations in Egbema. The average bacterial population of the different samples of soil from oil-spilled area was 3.08^7 cfu/g while the average population of the soils from the non-oil spilled area was 3.14×10^7 cfu/g. The most commonly isolated bacteria genera in the oil-spilled area were *Pseudomonas, Bacillus, Corynebacterium, Staphylococcus* and *Actinomycetes.* The most frequently isolated bacteria from the non-oil spilled area were *Pseudomonas, Arthrobacter* and *Actinomycetes* (Table 2).

Fungi were found in both areas. The fungi population in the soils from the oil-spilled area averaged 9.0×10^6, while that of the surrounding area is 5.0×10^6 cfu/g. The most predominant fungal species from oil-spilled site were *Candida, Mucor, Rhizopus* and *Aspergillus.* The non-oil spilled area had only *Candida* and *Mucor* species (Table 3).

The results show that the two sites studied had some microbiological characteristics in common. The microbial biomass of the environment were of the order 10^7 for bac-

teria and 10^6 for fungi (Table 1), showing some differences when compared with the normal microbial population. Normal microbial population is in the order of 10^6 to 10^9 bacteria per gram of soil. However, Doetsch (1973) reported that the total number of bacteria per gram of soil fluctuates between 10^5 and 10^9 depending on the composition of the soil and the determinative method used for analysis. The bacterial populations of the two areas fall within this range since they were of the order 10^7. Difference in microbial population is a reflection of many factors such as nutrient and oxygen levels, temperature and availability of minerals (Haris, 1962). The differences in both bacterial and fungal populations could then be attributed to possible change in nutrient and oxygen supply to the soils.

Carbon (nutrient) level of a given soil increases following every oil spillage, which also affects nitrogen level in the soil and other mineral elements, which finally become limiting with time. The high fungal population of the spilled area could be as a result of the increase in the carbon level of this area following the spillage. The population 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 biodegraded. Nitrogen is used up during biodegradation of petroleum compounds (Haris, 1962); this reduces the amount of nitrogen available to bacteria hence their population.

The slight difference in bacterial population of the two areas might be due to the effect of slight increase in the acidity of the soil spilled with crude 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 increase in the soil acidity of the area studied could therefore be due to high sulphur content of Egbema crude. This explains the slight decrease in bacterial population in the oil-spilled area. The fungal population may have increased due to the fact that fungi tolerate acidic environment than bacteria. Also the depletion in the oxygen level of the spilled area contributed to the population differences.

There were no living grasses around the area spilled with crude oil as at the time this study was carried out; which is quite different from the other environment where there were much green grasses growing. This could be due to oxygen depletion brought about by the waxy petroleum compounds that covered the area, and also because of the increase in the soil acidity following the spillage of high sulphur content crude oil.

The microbial content of the soil samples collected from the oil-spilled area included *Pseudomonas, Bacillus, Corynebacteruim, Staphylococcus, Ctinomyces, Candida, Aspergillus, Mucor* and *Rhizopus*, which resemble the same microorganisms isolated from other oil-spilled areas studied and reported (Haris, 1962; Doetsch, 1973). The differences in microbial populations and strain of microorganisms respectively could majorly be attributed to the volume and time of the spillage. The Egbema oil spillage was about a year old at the time this study was carried out and was a minor spillage and hence the slight differences in microbial population and content recorded.

REFERENCES

American Petroleum Institute (API) (1975). The influence of microorganism in the quality of petroleum products, API, USA.

Brinton MM, Warren L (1976). Petroleum Microbiology. *Industrial Microbiology* Elsevier press, New York. pp. 17-23.

Doetsch RN (1973). Bacteria as environmental determinants. Introduction to bacteria and their Ecobiology Elsevier press, New York. pp. 326-328.

Haris, J.O (1962). Microbiological studies of oil and gas pipelines. *Technology bulletin*. pp 135.

Institute of pollution studies, Rivers State University of Science and Technology (IPS, RSUT), (1986). Oshika Oil Spill Environmental Impact Assessment, 1986.

John G Holt (1993). Bergy's Manual of Determinative Bacteriology (9th edition). Lippin cott W.Willams, USA. p. 816.

POLY FS (1992). Attack of the oil-eating Microbes Well Servicing Vol. 32 No. 2. pp. 20–28.

Sandra M (1988). Pollution goes to sea. Development Forum, Vol. 12(2):15–20.