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RESPONSE OF FUNGI TO DIESEL OIL CONTAMINATION OF A SOIL IN NIGERIA

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ABSTRACT: *Since petroleum production began, pollution of natural environments by crude oil and its products had been devastitating; exposure of microorganisms to the crude oil contamination could have some measurable effects on soil microbial community and in turn alter soil fertility. This study therefore focused on the response of fungi to diesel oil contamination in a soil. Soil samples (3kg weight) were contaminated with 90ml, 180ml, and 270ml volumes of diesel oil; uncontaminated soil (0ml volume) served as control. Microbiological analysis of the soil samples was carryout on saboraud dextrose agar and mineral salts oil ager at days 1, 7, 14 and 21 intervals after addition of diesel oil to the soils. Mean counts of heterotrophic fungi ($X10^3$CFU G^{-1} soil) were: 0ml, 7.0, 90ml, 4.5, 180ml, 4.5, and 270ml, 4.0. Mean densities of hydrocarbon-utilizing fungi ($X10^2$CFU G^{-1} soil) for 0ml, 90ml, 180ml, and 270ml soil options were: 5.0, 3.5, 6.8 and 3.0 respectively. Fungal organisms isolated include Aspergillus niger, Aspergillus species, Fusarium species, Mucor species, Rhizopus species and Saccharomyces species, which occurred in control soil and polluted soils but Mucor species did not occur in 90ml soil option. The study showed that heterotrophic fungi responded negatively to addition of diesel oil to soil while hydrocarbon-utilizing fungi showed both positive and negative response depending on the volume of diesel added to soil. Occurrence of fungal organisms in polluted soils explained the fact that fungi are capable of utilizing diesel oil and can be used in cleanup operations in crude oil spillage sites.*

KEYWORDS: Response**,** Diesel Oil, Contamination, Fungi, Soil

INTRODUCTION

Petroleum production began in 1958 and since then, cases of petroleum and refined petroleum products' spills onto agricultural lands through petroleum production operations have been reported (Odu, 1977; Awobanjo, 1981; El Hanafy *et al*., 2015; De *et al*., 2000; Obire and Anyanwu, 2009; Al-Nasrawi, 2012). The incidences of recorded environmental pollution, due to high rate of petroleum related activities have been associated with frequent oil spills especially through oil well blowouts, tanker accidents, rupture of pipelines, and sabotage. These mishaps result in the release of crude oil and refined petroleum products into the terrestrial and aquatic environment (Okpokwasili and Amanchukwu, 1988; Al-Nasrawi, 2012).

Diesel fuel is a hydrocarbon product boiling between approximately 150^{o}C and 400^{o}C with carbon chain length of C_{15} – C_{22}. Classification of diesel differs from country to country; some classes containing selected cracked distillates such as light cycle oils (Campos *et al*; 1974). A variety of additives maybe used to improve the stability of the fuel; these include aliphatic amines, chelating agents, detergents, and corrosion inhibitors (Mukhopadhyay and

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Chakraborty, 2015; Ali *et al*., 2015). Some of which can act as nutrient force for microorganisms.

The soil environment is the most dynamic site of interactions in nature and it is also the region in which many biochemical reactions concerned in the decomposition of organic matter and nutrition of plants particularly agricultural crops occur (Marquez–Rocha *et al*., 2001). Soil pollution with petroleum and its derivatives is one of the causes of degradation of natural environment (Riis *et al*., 1995; Ojumu *et al*., 2004). The use of microorganisms and their activities in tests for effects of a specific chemical substance in soil, as well as in studies of soil pollution, has often been recommended (Riis *et al*., 1995; Ojumu *et al*., 2004). Diesel oil pollution could affect soil microbial flora such as the fungal community also known as "mycoflora of the soil". This could reduce numbers of microbes in polluted soil below those of non-polluted soil. Soil microorganisms are responsible for the breakdown of organic matter including hydrocarbons, conversion of inorganic components from one form to another (Ojumu *et al*., 2004).

Many fungal species inhabit the soil, and at high level of water activity can cause spoilage. Some members of the genera are however, known to have relevance in man's daily life. *Aspergillus niger* is responsible for the spoilage of many foods (Ikediugwu and Ejale, 1980; Kuku, 1985). Also, the metabolic process of *A. niger* has been harnessed into very useful products (Nishio *et al*., 1981; Kuku, 1985; Dinchev, 1981; Sauer and Borroughts, 1980). *Penicillium* species has the ability to produce antibiotics (Okafor, 1987).

The effect of oil pollution in the soil environment is largely determined by both biotic and abiotic factors of the soil. These biotic and abiotic properties of soil determine the persistence of oil pollutants in the environment, although this can be dependent to some extent on the quality and mixture of the hydrocarbon. In fact, there are measurable effects of oil on ecologically important microbial populations caused by exposure to crude and refined oils (La-Rue, 1977). Once crude oil is discharged into the soil, it rapidly sinks into soil and volatile fraction escapes leaving the less volatile fraction for microbial degradation (Davis and Huges, 1968; Ojumu *et al*., 2004). This is due to a major complex mixture of hydrocarbons containing paraffins, olefins, kerosene and octane (Atlas, 1981). The sensitivity of soil microflora to petroleum hydrocarbons is a factor of the quantity and quality of oil spilled and previous exposure of natural soil microbes to oil (Bossert and Bartha, 1984).

The aim of this study therefore, was to investigate the response of fungi to the presence of diesel oil in soil; the objectives being to enumerate fungi in polluted and diesel-polluted soils as to ascertain the effect of the diesel at population level of fungi. Furthermore, to isolate fungi in the soils as to know the type fungi associated with diesel-contaminated soil and the species capable of utilizing diesel oil.

MATERIALS AND METHOD

Study Area

The study site was a fallow patch of land wound Nkpolu-Oroworukwo community in Diobu area of Port Harcourt metropolis. The area is plan land within the Niger Delta Area of Southern Nigeria. The study site has no presence of crude oil production activity.

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Collection of Soil and Diesel oil Samples

Soil samples were collected from the study area in four replicates which were measured in 3kg weight into fresh unused black polythene bags each. Surface soil (0 - 15cm depth) was collected using a clean auger borer. The soil samples were taken to the green house for treatment application.

Collection of Diesel

Diesel oil was obtained from Conoil filling station located along Ikwerre Road in Mile 3 Area of Port Harcourt. This was used for polluting the soil.

Preparation and Treatment of Soil Samples

Treatment of the soil samples involved addition of diesel oil to the soil samples to simulate polluted condition in the soils. The soil samples were packaged in 3kg weight into fresh unused polythene bags perforate at the bottom to allow excess leachate drain off during incubation period. Four sets of samples were labeled A, B, C and D and left for 2 days before being watered/moistened with 600ml sterile tap water. Thereafter, the soil samples were contaminated by the addition of various volumes of diesel oil in concentrations of 90ml, 180ml and 270ml onto soils of B, C, and D set-ups respectively. Soil sample A was left unpolluted (0ml diesel oil) and served as control. Microbiological analysis of the soil samples was done at weekly intervals after treatment application.

Microbiological Analysis of Soil Samples

Microbiological analysis was done at days 1, 7, 14 and 21 intervals after addition of diesel oil. For the purpose of enumeration and isolation of heterotrotrophic fungi and hydrocarbon-utilizing fungi, ten-fold serial dilution, as described by Obire and Wemedo (1996), was employed to obtain appropriate dilutions used to inoculate agar plates by spread-plating: sabuoraud agar for heterotrotrophic fungi and mineral-oil agar for hydrocarbon utilizing fungi. The inoculated plates were incubated at 28 ± 2^0C for 2 – 5 days. After incubation, plates were examined and colonies that developed were counted and recorded; and taken as total heterotrophic fungal counts and hydrocarbon-utilizing fungal counts enumerated. The difference in counts between the control soil and diesel-contaminated soils was taken as the effect of diesel contamination on the fungal population of the study soil.

Identification of fungal isolates was done by macroscopy to observed colonial morphology colour of colony, texture, shape and surface appearance; and microscopy by wet preparation and slide culture to reveal the nature of the filaments and reproductive structures such as conidiospores and sporangiospores. All identification of pure isolates was made on the basis of their cultural and morphological characteristics and by reference to Alexopoulos and Sun, 1962; Barnett and Hunter, 1972; Abbey, 1995; Winn *et al*., 2006).

Results

Results of this study was based on the enumeration of heterotrophic fungi and hydrocarbon-utilizing fungi to obtain their populations at the various concentrations of diesel-contaminated soils and control soil; and to know the types of fungi associated with the diesel-contaminated soils. Fungal populations for heterotrophic and hydrocarbon-utilizers are shown in tables 1 and 2 respectively. Types of fungi isolated from the soils are shown in table 3.0

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Table 1.0: Counts of heterotrophic fungi in control and diesel – polluted soils

Days of Analysis	Number of colony forming units per gram soil ($X10^3CFU\ G^{-1}$)			
	Control soil (0ml)	Concentration of diesel in polluted soils		
		90ml	180ml	270ml
1	6.9	5.0	3.0	5.0
7	7.0	3.0	6.2	3.8
14	8.2	4.9	4.0	3.0
21	6.0	5.2	4.8	4.2
Mean	7.0	4.5	4.5	4.0

Ranges of counts of heterotrophic fungi in control and diesel-polluted soils are: 0ml, 6.0 to $8.2X10^3CFU\ G^{-1}$; 90ml, 3.0 to $5.0X10^3CFU\ G^{-1}$; 180ml, 3.0 to $6.2X10^3CFU\ G^{-1}$ and 270ml, 3.0 to $5.0X10^3CFU\ G^{-1}$. Ranges of hydrocarbon-utilizers in the soils are 0ml, 4.9 to $5.0X10^2CFU\ G^{-1}$; 90ml, 3.0 to $4.0X10^2CFU\ G^{-1}$; 180ml, 5.9 to $8.1X10^2CFU\ G^{-1}$ and 270ml, 2.2 to $4.0X10^2CFU\ G^{-1}$. Fungal types isolated during this study include *Aspergillus niger*, *Aspergillus species*, *Fusaruim species*, *Mucor species*, *Penicillium species*, *Rhizopus species* and *Saccharomyces species.* Of the seven fungi isolated, all occurred in both uncontaminated and contaminated soils except *Mucor species* which did not occur in 180ml concentration.

Table 2.0: Counts of hydrocarbon-utilizing fungi in control and diesel-polluted soils

Days of Analysis	Number of colony forming units per gram soil ($X10^2CFU\ G^{-1}$)			
	Control soil (0ml)	Concentration of diesel in polluted soils		
		90ml	180ml	270ml
1	5.1	4.0	7.0	3.3
7	4.9	3.8	6.3	3.0
14	5.3	3.0	5.9	4.0
21	5.0	3.2	8.1	2.2
Mean	5.1	3.5	6.8	3.1

DISCUSSION

Response of fungi (heterotrophic and hydrocarbon-utilizers) to diesel oil contamination of a soil was investigated in this study. Counts of heterotrophic fungi fluctuated between the control (uncontaminated) soil and diesel oil-contaminated soils; and within the days of analysis. Densities of fungi were highest in control (0ml, unpolluted) soil throughout the period of analysis when compared to the polluted soils. At day 1 of addition of diesel, fungal counts were highest in 0ml concentration, decreased at 90ml and 180ml concentrations and increased again at 270ml treatment. At day 7 after addition of diesel oil, counts of fungi were highest in 0ml, decreased in 90ml, increased in 180ml and decreased again in 270ml diesel treatment soil. At day 14 and day 21 of diesel treatment, fungal counts almost had similar pattern of fluctuations with control soil having highest counts, which decreased gradually with increasing concentrations of diesel oil from 90ml to 270ml. Mean densities of fungi were highest in control soil, decreased and remained the same in 90ml and 180ml soils but decreased slightly in 270ml diesel polluted soil. The results showed that fungal populations

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decreased as the pollutant concentration increased; and that diesel oil generally depressed fungal densities of the study soil. Statistically there was significant difference ($P<0.005$) between fungal counts of control soil and counts of those of diesel-polluted soil but no significant difference between fungal counts of polluted soils. The statistical difference observed in the fungal populations of control soil and diesel-polluted soil showed real pollutant effect which caused decrease in fungal densities of polluted soils far below the counts in control soils.

Table 3.0: Fungal types isolated from control and polluted soils

Fungal types	Concentration of diesel in polluted soils			
	Control(0ml)	0ml	180ml	270ml
Aspergillus niger	+	+	+	+
Aspergillus species	+	+	+	+
Fusarium species	+	+	+	+
Mucor species	+	-	+	+
Penicillium species	+	+	+	+
Rhizopus species	+	+	+	+
Saccharomyces species	+	+	+	+

KEY: + = bacterial species isolated, - = bacterial species not isolated

Counts of hydrocarbon utilizing bacteria of control soil and diesel-polluted soils showed similar pattern of fluctuations throughout the period of the experiment. Control soil (unpolluted soil) had somewhat high counts which decreased in 90ml concentration became highest in 180ml and decreased again in 270ml concentrations but lower than in 180ml concentration which had the highest mean counts. Presence of hydrocarbon utilizing fungi in high numbers in unpolluted soil, without any history of oil spillage, showed that microorganisms occur in nature in the environments particularly in areas where oil exploration activity takes place. In polluted soils, pollutant effect was expressed differently; diesel oil depressed microbial growth/activity in 90ml and 270ml concentrations. In 180ml concentration, counts of hydrocarbon utilizing bacteria were highest which could be taken as the optimum concentration of diesel oil capable of stimulating growth/activity of hydrocarbon-utilizing fungi in soil. Statistically there was significant difference ($P<0.005$) between counts of hydrocarbon-utilizing fungi of control soil and diesel-polluted soil as well as within counts of polluted soils.

Of the six fungal organisms isolated, all occurred in control soil and polluted soils except *Mucor species* which did not occur in 90ml concentration of diesel oil. Occurrence of fungal organisms in control and polluted soils explained the fact that fungi have the ability to utilize and grow in diesel oil contaminated soil.

In conclusion, response of fungi to diesel oil contamination of soil was observed in this study. The difference in fungal populations of uncontaminated and diesel oil contaminated soils for both heterotrophic and hydrocarbon-utilizing fungi was taken as the effect of addition of diesel oil on the fungal populations of soil. The fungal counts observed in this study were somewhat significant to predict the effect of the pollutant (addition of diesel oil) to the soil. There was general decrease in heterotrophic fungal counts in polluted soils when compared with those of control soils. Heterotrophic fungi responded negatively to presence of diesel oil

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in the soil which showed that diesel oil had depressive effect on soil fungi. In contrast, hydrocarbon-utilizing fungi responded differently to the various concentrations of diesel oil in the soil. Diesel oil reduced numbers of hydrocarbon-utilizing fungi in 90ml and 270ml concentrations but stimulated their growth in 180ml concentration. The study also showed that fungi possess the ability to utilize diesel oil as sources of carbon and energy which can be isolated and used in remediation study.

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