JASEM ISSN 1119 - 8362

J. Appl. Sci. Environ. Mgt. 2002
Vol. 6 (1) 14 - 17

Myco-Flora of a Kerosene-Polluted Soil in Nigeria

WEMEDO, S. A*, OBIRE, O. and DOGUBO, D. A.

Department of Biological Sciences, Rivers State University of Science and Technology, Nkpolu-Oroworukwu, P. M. B. 5080, Port Harcourt

ABSTRACT: The myco-flora of a Kerosene-polluted soil was investigated. Soil samples collected from a fallow patch of land were polluted with 90 ml, 180 ml, and 270 ml concentrations of kerosene. The 0 ml (untreated soil) served as control. Cultivation of the organisms was done on potato dextrose agar (PDA) after 2 days, 7 days and 14 days of soil contamination. The study revealed that various fungal genera were associated with kerosene-polluted soil. The fungal genera isolated include *Penicillium, Aspergillus, Fusarium, Rhizopus, Saccharomyces* and *Mucor species.* The growth of fungi observed showed delayed growth in polluted samples whereas growth was rapid in control samples for all the concentrations. The fungal counts were generally high in the control when compared to those of the polluted samples. This revealed that kerosene had depressing effect on the growth and activity of the soil fungi @ *JASEM*

The environmental impact of oil exploration and exploitation is one of the inevitable consequences 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. 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).

The increase in demand for crude oil as a source of energy and as a primary raw material for industries has resulted in an increase in its production, transportation and refining which in turn has resulted in gross pollution of the environment (Gutnick and Rosenberg, 1977). Major petroleum products are formed in the course of refining operations – distillation, cracking, solvent refining and chemical treatment (Garner *et al.* 1947). Crude oil is an extremely complex mixture of aliphatic and aromatic hydrocarbons, including volatile components of the gasoline or petrol fraction, the higher boiling point kerosene and lubricant fraction, and the largely solid alsphaltene residue. The water insoluble kerosene fraction poses the greatest pollution problem, since the petrol fraction evaporates rapidly and the insoluble "tar balls" of the alsphaltene residue either sink in mid-ocean or can be removed from beaches without great difficulty (Grant and Longs, 1947)

Kerosene is a component of crude oil. It occurs as carbon 9 – carbon 14 fraction with boiling point range of 320°F – 465°F (Bell, 1976). It is used as a source of energy. Kerosene is used as fuels in some jets, illuminating oil for wicks and lamp (Garner, *et al.* 1947). Pollution affects soil microbial flora and one of such is the fungal community also known as the mycoflora of soil.

Jones (1977) observed that kerosene – polluted soil has reduced numbers of microbes when compared to non-pulluted soil. The levels of activity in the remaining microbes, however, were not affected by the pollution (Jones, 1977). Various microorganisms have been reported as possessing the capability for utilizing hydrocarbon as their sole source of carbon and energy (Zobell, 1946; Obire and Wemedo, 1996)

In Nigeria, not much information exists on the pollution status of kerosene on the environment and its effect on the macro-and micro-flora of soils in particular.The objectives of this study therefore, were to investigate the effect of kerosene on soil fungi and to enumerate, isolate and identify the fungi associated with kerosene – polluted soil.

MATERIALS AND METHODS

Sample Collection

Soil samples were collected from fallow patch of land around the Department of Biological Sciences at the Rivers State University of Science and Technology. Surface soil (0 to 20 cm depth) was collected using auger borer.

Kerosene used for contaminating the soil samples was obtained from Unipetrol Filling station in Port Harcourt.

Preparation and Incubation of Soil Samples

The soil samples obtained for analysis were bulked, air dried and passed through a 2 mm sieve to remove the coarse fragments. Three kilogrammes (3 kg) of the fine earth was transferred into 5-liter size plastic planting buckets perforated at the bottom. There were four buckets each labeled A, B, C and D. All the buckets were watered for two days with sterilized

*Corresponding author

distilled water and then each (A, B, C and D) was polluted with 0 ml, 90 ml, 180 ml and 270 ml of kerosene respectively. The first bucket that was unpolluted (0 ml) served as control. The buckets placed in the greenhouse were incubated for two weeks or 14 days.

Microbiological Analysis of Unpolluted and Polluted Soil Samples

Microbiological analysis of the soil samples was made 2 days, 7 days and 14 days intervals after addition of kerosene. One gramme of soil was weighed out from each of the buckets and shaken in 10 ml of sterile distilled water in test tubes. 1 ml of each sample was transferred into 9 ml of distilled water in next test tubes and serial dilution was made up to 10^{-3}. Pure cultures of fungi were isolated by plating out 0.1 ml aliquots of the 10^{-3} dilution onto freshly prepared potato dextrose agar medium (PDA) in petri dishes. 0.1 ml of Streptomycin was incorporated into the medium to suppress bacteria growth. All plates were incubated at 28^0 C for 5 – 7 days and discrete colonies that developed were counted and recorded. Color pigmentation, diameter and colonial characteristics of the colonies were observed and recorded. The spread plate technique using a sterile bent glass rod was used for inoculations.

Microscopic Examination and Identification of Fungal Isolates

Microscopic Examination of mould growth was done by observing the colonial morphology – colour of colony, texture, shape and surface appearance; and cultural characteristics – asexual and sexual reproductive structures like sporangia, conidial head, arthrospores, the vegetative mycelia, septate or non-septate (Alexopoulos and Sun, 1962; Barnett and Hunter, 1972). Microscopic examination of the moulds was done by using needle mount method. A small portion of each colony was picked with sterile needle and teased out in a drop of clean water on a clean microscopic slide. Slides were also prepared likewise, using methylene blue in place of water. These were also covered with clean cover slips and examined under the microscope, starting with a low power objective (x10), then the high power (x40) objective for a better field view and magnification.

All identifications of pure isolates were made on the basis of their cultural and morphological characteristics and by reference to Alexopoulos and Sun (1962; Barnett and Hunter, (1972).

RESULTS AND DISCUSSION

The fungal types isolated from the soils are shown in Table 1.0. Seven isolates were encountered and identified to generic level and/or specific level.

Table 1.0: Fungal types isolated from soils

Fungal Types	Kerosene Concentrations			
	Control 0%	90 ml 3%	180 ml 6%	270 ml 9%
Aspergillus niger	+	+	+	+
Aspergillus Fumigatus	+	+	+	-
Fusarium sp.	+	+	+	+
Penicillium sp.	+	+	+	+
Rhizopus sp.	+	+	+	+
Saccharomyces sp.	+	+	+	+
Mucor sp.	+	+	-	+

Key: + = Fungi species isolated
- = Fungi species not isolated

The fungi isolated in this study include *Aspergillus niger, Aspergillus fumigatus, Penicillium species, Rhizopus species, Fusarium species, Saccharomyces species* and *Mucor species.* The different genera occurred in all soil samples with the different concentrations of kerosene. The volumes of kerosene giving the different concentrations were 0 ml (Control), 90 ml, 180 ml and 270 ml.

All the organisms occurred in the control samples. *Aspergillus, Penicillium, Rhizopus, and Saccharomyces species* occurred in the control samples as well as in the samples containing the other concentrations. *Fusarium species* did not occur in soil samples containing 270 ml of kerosene but grew in other concentrations. *Mucor species* did not grow in soil samples with 190 ml of kerosene but grew in other concentrations.

The response of fungi to kerosene in the soil was observed. This study revealed that the incubation period of isolates in control samples (0 ml) was shorter than in the other concentrations. *Rhizopus and Aspergillus species* were observed to have grown rapidly in all the three concentrations (90 ml, 180 ml, and 270 ml). Whereas *Penicillium, Fusarium, Mucor*

and Saccharomyces species showed delayed growth in the samples containing the three concentrations. The delayed growth observed in this investigation may be due to the fact that the organisms had to adapt in the new substrate they found themselves, so as to utilize the carbon and energy sources available. In general, this investigation revealed that fungi organisms are associated with kerosene-polluted soil. The mean densities of fungi in soil samples are shown in Table 2.0. The counts observed were generally higher in the control than in the polluted soils with different concentrations of kerosene.

Table 2. 0: Mean densities of fungi in control and polluted soil samples

Days of Analysis (after addition of kerosene to soils)	Number of cfu/g soil ($x10^3$) Control	Polluted Samples		
	0 ml	90 ml	180 ml	270 ml
2	14.0	9.0	9.5	8.0
7	13.5	9.0	11.0	9.0
14	14.0	9.5	8.0	8.0

Key: cfu/g = Colony forming units per gram of soil.
90 ml, 180 ml and 270 ml are the volumes of kerosene used to pollute the soils.

There was also variations in plate counts of the different genera with some having higher frequency and others having lower frequency. In all the three days of analysis after addition of kerosene, the fungal counts were high at 0 ml (control), decreased at 90 ml and increased again at 180 ml concentration, and decline at 270 ml concentration except at 14 days where the counts for 180 ml and 270 ml were similar. Odu (1972) found that contamination of soil by oil and its products resulted initially in a depression of microbial numbers and activity even in cases of relatively light contamination. This was followed by a stimulation of microbial activity. This was observed in this study. However, the final decline in fungal numbers at 9% contamination may be due to accumulation of acidic metabolites as the organisms utilized the kerosene (Jones *et al*, 1970), perhaps at higher concentrations.

The effect of kerosene on soil fungi was noted in this study. The difference in the fungal counts of the unpolluted soil (0 ml) and that of the polluted soils (90 ml, 180 ml, and 270 ml) was taken as the effect of the addition of kerosene on the fungal populations of the soil.

The fungal counts observed in this study is somewhat significant to predict the effect of kerosene addition to the soils. There was general decrease in the fungal counts between the control samples and that of the polluted samples. This suggested that the pollutant had adverse effect on the fungal populations of the soil.

In conclusion, the present investigation revealed that kerosene-polluted soil could still harbour viable fungi. Fungi possess the ability to utilize kerosene as sources of carbon and energy. A comparative analysis of the fungal counts between control samples and those of the polluted samples showed a depression in the growth and activity of fungi in the soil. Kerosene had toxic effects on the soil fungi

REFERENCES

ALEXOPOULOS, C. J., SUN, S. S. (1962): Introductory Mycology, 2nd Edition, John Wiley and Sons, New York, U. S. A.

BARNETT, H. L., HUNTER, B. B. (1972): Illustrated Genera of Imperfect Fungi. 3rd Edition. Burgess Publishing Company, U. S. A.

BELL, H. S. (1976): Hydrocarbon Processing. Refining Process Handbook. pp. 160 – 164.

GARNER, F. H., EVANS, E. B., GEORGE, S. (1947): Review of Petroleum Technology. Published by the Institute of Petroleum, Manson House, London.

GIBSON, D. T., (1971): The Microbial oxidation of aromatic hydrocarbons. Rev. Microbial. 1: 199 – 223.

GRANT, W. D., LONGS, PE. (1981): Environmental Microbiology. Blackie and Sons Limited Publishers, Glasgow, London.

GUTNICK, D. L., ROSENBERG, E. (1977): Oil tankers and pollution: A Microbiological Approach. Annu. Rev. Microbial. 31: 379 – 396.

JONES, J. G. (1977): The Long-term effects of kerosene pollution on the Micro-flora of a Moorland Soil. J. Appl. Bacterial. 43: 123 – 128.

JONES, J. G., KNIGHT, M., BYROM, J. (1970): Effect of gross pollution of kerosene hydrocarbon on the Micro-flora of a Moorland Soil. Nature 227: 1166.

OBIRE, O., WEMEDO, S. A. (1996): The Effect of Oilfield Wasterwater on the Microbial Population of a Soil in Nigeria. Niger Delta Biologia. 1(1): 77 – 85.

ODU, C. T. I. (1972): Microbiology of Soils Contaminated with Petroleum Hydrocarbons. I Myco-Flora of a Kerosene-Polluted Soil in Nigeria n: Extent of Contamination and some soil and microbial properties after contamination. J. Inst. Petrol. 58: 201 – 208.

OKPOKWASILI, G. C., AMANCHUKWU, S. C. (1988): Petroleum Hydrocarbon Degradation by *Candida species.* Environment International. 14: 243 – 247.

ZOBELL, C. E. (1946): Action of Microorganisms on hydrocarbons. Becteriol. Rev. 10: 1 – 49.