THE EFFECT OF POULTRY MANURE ON OIL CONTAMINATED SOIL

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ABSTRACT

Bio-remediation of polluted soil, using poultry manure was investigated in the laboratory. Diesel oil was added to soil to a mean contamination level of 1.6% for laboratory experiment. From the contaminated soil, mean bacterial count was 5.22×10^7 while that of fungi was 4.02×10^6 cfu/g. *Micrococcus* (16.6%), *Acinetobacter* (21%), *Bacillus* (25.0%), *Pseudomonas* (18.3%), *Aspergillus* sp. (54.1%) and *Penicillium* (20.8%) were among the microbial isolates from the oil contaminated soil. *Zea mays* could not grow on oil-contaminated soil until after treatment with poultry manure. There was no significant difference in the dry weight of *Zea mays* biomass grown on contaminated soil fertilized with poultry manure and the uncontaminated soil at 5% probability level. Complete emulsification of oil was observed in Basal medium containing 2% of diesel oil by *Bacillus* sp. following 10 to 21 days of incubation.

Keywords: oil contamination of soil, poultry manure, Microbial emulsification of oil

INTRODUCTION

Bio-remediation is a rapidly developing field of environmental restoration, utilizing microbial activity to reduce the concentration or toxicity of various chemical substances such as petroleum products or aliphatic and aromatic hydrocarbons (Alexander, 1994). Oil spill bioremediation methods aim at providing favourable conditions of oxygen, temperature and nutrients to maximize biological breakdown (Alexander, 1994). The optimization of process for biological remediation of contaminated soil is of practical importance to diminish time and to save treatment cost (Aislabie *et al.*, 1998).

Several organisms are known to degrade a few petroleum products (Pierzynski *et al.*, 1994). Bio-remediation by these organisms is expected to proceed at increased rate after the nutrient addition and with enriched microbial culture (Anonymous, 1994).

Extensive use of land farming has been by oil industry to treat petroleum waste (Bartha and Rossert, 1984).

In the oil producing areas of Nigeria, farming operations are faced with problems of oil contamination of the environment. Several works have investigated the role of microorganisms in the treatment of such contaminated farmlands. Hashem (2000) investigated the influence of crude oil contamination on the chemical and microbiological aspects of Saudi-Arabia soils, and reported that the numbers of bacteria and fungi per gram of soil were higher in uncontaminated than petroleum contaminated soils. It was also indicated that bacterial genera belonging to *Artrobacter, Bacillus, Micrococcus, Pseudomonas* and *Staphylococcus* were isolated along with *Penicillium, Aspergillus* and *Cladosporium*. Similarly, De and Bello (2002) reported the isolation of *Penicillium* sp *Aspergillus* sp Fusarium sp, Trichoderma sp and Moiterella sp from oil contaminated soil.

Other work on bioremediation of contaminated soil and water include those of Radwan *et al.,* 1995, Korda *et al.,* 1997, Fuentes *et al.,* 1998, De and Bello, 2002.

This study was aimed at examining the enhancement of productivity of oil-contaminated soil using poultry manure.

MATERIALS AND METHODS

Collection and Contamination of Soil Sample

The soil samples (7.0kg) were collected from a farmland at a depth of 1 – 20cm at the University of Ado-Ekiti, Nigeria. The soil was divided into two equal portions. Half of the soil samples (3.5kg) were contaminated with 500ml of diesel oil. The contaminated soil was weighted into seven plastic bowls, such that each contained 438g of oil contaminated soil. The bowls were allowed to acclimatized for seven days at 27 ± 1°C. The oil-contaminated soil was moisturized using a watering can. The uncontaminated soil was treated similarly.

Estimation of Microbial Population in Oil Contaminated Soil

Estimation of microbial populations was done after 7 days of acclimation using standard plate counts techniques (Olutiola et al., 1999). Ten grammes of contaminated soil were suspended in 100ml of sterile distilled water. The suspension was diluted to 10^{-7}. One milliliter of dilution 10^{-5} was inoculated in nutrient agar at 45°C for bacterial counts. Similarly 1ml of the dilution was added to Potato Dextrose Agar (PDA) at 45°C. The mixtures were separately poured into sterile petri dishes and allowed to set. The plates for fungal growth were incubated at 28°C ± 1°C while those for bacterial counts were incubated at 37°C for 72h. Four plastic bowls of oil-contaminated soil were selected randomly for this experiment. Microbial population of uncontaminated soil was also determined using the same procedures.

Determination of Oil Concentration and pH of the Soil

Ten grammes of contaminated soil were weighed and exhaustively extracted using N-hexane. The extracts were filtered using Whatman filter paper and allowed to

evaporate in pre-weighed crucibles over a period of five days when constant weights were obtained. The quantity of oil in the contaminated soil was determined in terms of percentage in soil samples. The pH of the contaminated and uncontaminated soil was determined using a pH meter model calibrated with buffer solutions. Soil samples (5g) were suspended in 50ml of distilled water and agitated for 30mins before dipping the pH electrode. The pH values were observed and recorded.

Isolation and Characterization of Microorganisms from the soil samples

As earlier described 10g of contaminated or uncontaminated were suspended in sterile distilled water respectively. The suspensions were diluted to 10^{-6}. Dilutions 10^{-3} and 10^{-5} were used to inoculate Nutrient Agar and Potato Dextrose Agar (PDA) at 45°C. Nutrient agar plates were incubated for the isolation of bacteria at 37°C. The PDA plates were also incubated at 29°C for 72h for isolation of fungi. Discrete colonies on the Nutrient Agar and PDA plates were purified by sub culturing several times. The pure cultures of microbial isolates were kept on slants for morphological and biochemical studies. Cultural morphological studies, Gram-reaction, motility and spore tests were carried out according to standard techniques. Biochemical test on the isolates included catalase and oxidase production, citrate utilization, indole production and fermentation of sugars such as glucose, maltose, mannitol, lactose and sucrose. Probable identification was done using Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons, 1974).

Growth of *Zea mays* on Poultry manure treated oil contaminated soil:

Three sets of experiments were carried out. Maize (*Zea mays* hybrid white 8321-21) seeds were collect from International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. The oil contaminated soil sample was weighed into plastic bowls. To a set of seven bowls containing oil contaminated soil samples was each added in a ratio 1:10w/w poultry manure collected from poultry farms in Ado Ekiti. The mixtures were left to moisturize and allowed to acclamatise over a period of six days. A second set of seven bowls containing uncontaminated soil was treated similarly. Three grains of maize were planted in each bowl. The growth of maize was observed for six weeks with

occasional wetting and then harvested. Whole plants from each bowl were carefully uprooted and thoroughly washed with water to remove stones and debris. The whole plant materials were allowed to dry in an oven at 60°C to a constant weight. This was taken as dry weight of biomass of the maize plants. Differences in the maize yield in terms biomass of the whole plants on oil contaminated soil treated with poultry manure and uncontaminated soil was compared using t-test.

Determination of oil emulsification activities of the microbial isolates:

A modified mineral salt medium (MSM) described by Cane et al.(1983) and Ajisebutu (1987) was used, to study degradation and emulsification of diesel oil. The MSM contained disodium hydrogen phosphate (3.5g/L), potassium dehygrogen phosphate (1.5g/L), sodium nitrate (0.5g/L), magnesium sulphate (1.0g/L), and was separately sterilized at 1.2kg cm^{-2} for 15mins. The diesel oil sterilized using membrane filter was added to the MSM to a concentration of 0.2%v/v. Each MSM-oil was inoculated with 0.2ml of an overnight broth culture 0f microbial isolate to be tested. Two sets of control were set up. The first set consisted of MSM-oil without inoculation to distinguish biogradation from biotic loses. The second set of control experiment contained 0.2%w/v glucose to replace oil as carbon and energy source for the test organisms. All the cultures and controls were incubated at 37°C for 21 days with weekly monitoring. The growth of fungal isolates were monitored at 25°C + 1°C.

RESULTS AND DISCUSSION

The mean bacterial count in oil-contaminated soil was 1.50×10^7 cfu/g while the fungal count was 4.02×10^6. The mean oil concentration in contaminated soil was estimated to be 1.6%w/w while the pH was determined to an average of 6.0. Bacteria were predominant in oil-contaminated soil (Table 1)

Table 1: Effect of diesel oil on microbial population of soil

Soil sample	Quantity of oil (%)	Microbial counts cfu/g		pH of soil
P Sol A	1.6	1.41×10^7	3.8×10^6	6.8
P Sol A	0.8	1.20×10^7	4.1×10^6	6.5
P Sol A	2.4	1.20×10^7	2.5×10^6	7.3
P Sol A	1.6	9.0×10^7	5.7×10^6	6.6
Means (n=4)	1.6	5.22×10^7	6.0×10^6	6.8
UP Sol (control)	0.0	1.55×10^7	4.02×10^6	6.0

P Sol = Polluted soil
Up Sol = Unpolluted soil

Determination of the effect of poultry manure on the growth of *Zea mays* on oil-contaminated soil revealed that the presence of oil inhibited the germination of maize (*Zea mays*) grain. The addition of poultry manure at the ratio of 3:1 to the contaminated soil resulted in the growth of the plant with the amount of yield comparable to that of uncontaminated soil. However, there was no significant variation between the mean dry weights of the maize biomass harvested from uncontaminated soil and those grew on contaminated soil treated with poultry manure at probability level of 5% (Table 2).

Table 2: **Analysis of variance of growth of *Zea mays* (Hybrid White 8321-21) on poultry manure treated oil polluted and unpolluted soil.**

Source of variation	Sum of squares	Df	Mean square	F	Sig	Mean±SD dry wt o biomass (N=6)
PSM	2.912	1	2.912	36.825	0.004	7.31±0.80
UNPS	0.316	4	7.908			9.03±1.275
Total	3.228	5				

Table 3 shows the frequencies of microbial isolates from the different soil samples.

Table 3: **Incidence of bacterial and fungal isolates from oil contaminated soil.**

Bacterial isolates	**Incidence (%)**
Bacillus *sp*	15(25.0)
Pseudomonas sp	11(18.3)
Micrococcus sp	10(16.6)
Acinetobacter sp	16(21.0)
Fungal isolates	
Aspergillus *sp*	13(54.1)
Penicillium sp	5(20.8)

Figures in parenthesis of occurrences of the isolates

The genera of bacteria commonly encountered from oil-contaminated soil were *Bacillus* (25.0%), *Pseudomonas* (18.3%), *Micrococcus* (16.6%) and *Acinetobacter* (21.0%) while *Apergillus*(54.1%) and *Penicillum*(20.8%) were the fungi encountered. All the isolates encountered from contaminated soil demonstrated the ability to emulsify oil in minim salt medium containing 0.2% diesel oil (Table 4).

	Emulsification activity		
Microbial Isolates	**1-6 days**	**7-14 days**	**15-21 days**
Bacillus sp	++	+++	+++
Micrococcus sp	+	+	++
Pseudomonas sp	+	+	+++
Pseudomonas aeruginosa	+	++	++++
Bacillus subtilis	+	+	++
Acinetobacter sp	+	+	++
Penicillium sp	+	++	++++
Aspergillus sp	+	++	++++

\+ Slight emulsification
++ Moderate emulsification
+++ Complete emulsification

Pseudomonas aeruginosa, *Penicillium* and *Aspergillus* demonstrated pronounced emulsification of diesel oil within 21 days of incubation *Bacillus* sp was also effective in emulsification of the oil (Table 4). The results obtained reveal the effectiveness of organic matter in treating oil contaminated soils for crop production. The rhizosphere soil has been described as the zone of soil under the direct influence of plant roots and usually extends a few millimeters from the root surface (Curl and Truelove, 1986). Microbial activity is generally higher in the rhizospere due to readily biodegradable Substrates exuded by plants (Paul and Clatk, 1996). Radwan *et al.* (1998) investigated the rhizospheric hydrocarbon-utilizing microorganism as potential contributors to phytoremediation for the Kuwaiti desert and indicated that rhizospere soils of all plants

contained more hydrocarbon utilizers than the soil apart. According to Mayensin and Shinner (1999), the type and number of microbial species involved in bioremediation may influence the rate and extent of process. Furthermore, the factors that influence microbial growth can also influence the rate of bioremediation. Among the factors are temperature, oxygen, moisture and availability of organic nutrients in the environment (Zhon and Crawford, 1995; Alexander, 1994; Leahy and Colwell, 1999; Mohn and Stewart, 2000). Biological treatment of oil-contaminated soil has been considered to be more cost effective than incineration, if properly optimized (Cookson, 1995).

Despite a relatively long history of research on oil spill bioremediation, it remains an empirical technology. Essentially bacteria and fungi dominate adequately well-aerated soils except that bacteria alone can account for most of the biological and chemical changes in the environment containing little or no oxygen. Bathermann *et al.* (1994) indicated that major bacterial groups commonly encountered in oil-polluted soils are *Alcaligenes* sp., *Micrococcus* sp., *Actinomycetes, Clostridium* sp., *Bacillus* sp. and *Pseudomonas* sp. These authors also highlighted that the fungal groups found in soil after oil application are *Aspergillus* sp., *Penicillium* sp., *Mucor* sp., *Cladosporium* sp. as well as yeast.

Isolation of *Pseudomonas* sp., *Aspergillus, Penicillium* sp., *Micrococcus* sp., and *Bacillus* sp. from oil-contaminated soils was in agreement with the report of Bathermann, *et al.* (1994) and Hashem (2000). Similarly, Boyle and Shann (1995) observed that after the application of oil to soil, there was a significant increase in bacterial numbers which occurred in all group of bacteria except anaerobic spore formers. However, from several studies on oil degradation, the most important species of oil degrading bacteria belong to the genera *Pseudomonas* and *Arthrobacter*.

Soils in which there is hydrocarbon contamination show a district change in pH compared to normal soils. In such heavily contaminated soils, plants are affected and commercial agriculture may not be possible (Dave *et al.*, 1994). In this study effectiveness of poultry manure in the treatment of oil contaminated soil is in agreement with the work of Williams et al (1999). The authors highlighted that the remediation of soil contaminated with petroleum compounds was significantly enhanced when supplemented with poultry litter at the concentrations of 10% soil volume.

In conclusion, our results showed that the application of poultry manure might be a reliable method of solving the problem oil contamination of agricultural soils.

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