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European Journal of Experimental Biology, 2016, 6(4):62-68

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Effect of Chicken droppings amendment on bioremediation of crude oil-polluted soil

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ABSTRACT

The effect of poultry droppings on bioremediation of crude oil-polluted soil was evaluated. Various concentrations of the poultry droppings (10%, 30%, and 50%) were also studied. The physicochemical and microbiological properties of the soil were monitored for a period of 6 months. The poultry droppings had total heterotrophic bacterial and fungal counts of 4.2 × 10^4cfu/g and 1.8 × 10^4cfu/g respectively. The total hydrocarbon utilizers increased progressively from month 2 to month 3, after which a decline from month 4 down occurred. The total heterotrophic microbial counts also increased from month 2 to month 4 followed by a decline from month 5 down. The control showed slight increase in microbial growth. The microbial growth rate increased as the concentration of the poultry droppings increased. Statistical analyses showed a significant difference at ($P<0.05$), level between the amended options and control. The total hydrocarbon content of the oil-polluted soil decreased from 6609.83 to 2951.37ml/g. Bacillusspp, Pseudomonas spp, Flavobacterium spp, Fusarium spp and Aspergillus spp were isolated. Alkaline pH condition was observed in the poultry droppings as well as in the amended soils at 50 % and lower at the control. Ecotoxicity assay, measured in terms of germination index was used to evaluate the extent of contaminant removal. Using seeds of Viciafaba, germination index of 95 % was observed in the 50 % amended option only. The study therefore showed that poultry droppings can serve as a good remediation material in the reclamation of a crude oil-polluted lithosphere.

Keywords: Bioremediation, Crude oil, Polluted soil, Chicken Droppings, Biostimulation.

INTRODUCTION

Bioremediation is the use of naturally occurring microorganisms or genetically engineered microorganisms by man to detoxify man-made pollutants [1]; [2]. Since bioremediation is a microbial process, it requires the provision of nutrients among other limiting factors. The addition of organic waste materials such as poultry litter (PL) and Coir pith (CP) to the soil facilitates aeration through small pores and increases the water holding capacity of the soil, thus enhancing bioremediation [3];[4]. It allows natural processes to clean up harmful chemicals in the environment. Microscopic “bugs” or microbes that live in soil and groundwater use certain harmful chemicals such as those found in gasoline and oil spills.

Crude oil is a complex mixture of diverse hydrocarbons including alkanes, aromatics, alicyclics, branched hydrocarbons, and non-hydrocarbon compounds including polar fractions containing hetero-atoms of nitrogen, sulfur and oxygen (NSO fraction), and asphaltens [5];[6].

The high demand for petroleum products in the form of cooking gas, aviation fuel, gas oil, engine lubricating oil, asphalt and coal tar results in increased production and this eventually leads to oil spills and hydrocarbon contamination of the environment especially through oil well blow out, tanker accidents, accidental rupture of pipelines and routine clean-up operations [7]. Current technologies for cleaning hydrocarbon contaminated soil include soil washing, solvent extraction, thermal treatment, composting, chemical oxidation (Fenton's reagent, permanganate, ozone etc) and bioremediation (bioaugmentation, biostimulation and phytoremediation) [1]; [8]. Physical and chemical approaches are expensive and products may cause secondary contamination of soil and water resulting in the need for additional post-treatment. As such, there is a wide-spread interest in bioremediation for the complete mineralization of hydrocarbons to carbon dioxide and water which are environment friendly.

Therefore, attempts were made in the present study to determine the effectiveness of the use of poultry droppings in bioremediation of crude oil contaminated soil. Ecotoxicity assay expressed in terms of germination index using a selected agricultural seeds bean seeds (*Viciafaba*) was also evaluated.

MATERIALS AND METHODS

Collection of Samples

Soil sample was collected from four different locations for composite sample preparation in Aguleri and Nkwelle Ezunaka, both in Anambra State, Nigeria. Samples were stored in polythene bags and transported to the laboratory. The soil samples were air dried, sieved through 2mm mesh and stored in polythene bags at room temperature.

The crude oil was collected from Eleme oil field, Rivers state, Nigeria. The soil amendment material (poultry droppings) was collected from Aroma farms in Awka, and from Anambra Integrated Poultry farms, Nkwelle Ezunaka, both in Anambra State, Nigeria. The poultry droppings were air dried ground and stored in the laboratory at room temperature ($28\pm2^{o}C$).

Incorporation of Amendment Material into the Soil Sample

320 g of soil was moistened and kept at room temperature in the Microbiology laboratory for one week. The soil sample was polluted with the crude oil in the ratio of 5:1 i.e. 80g of soil was mixed with 16ml of crude oil and kept for 2 weeks. The poultry dropping was applied at 10%, 30% and 50% respectively to the oil polluted soils. The experimental samples were set up as shown in Table 1. Both the amended soil and the control (polluted soil without amendment) were incubated at room temperature and observed after every two weeks for 24 weeks after pollution and the effect of the amendment on the samples studied.

Table 1: Experimental design

S/No	Condition	Description
GCI	Chicken dropping added at 10%	80g of polluted soil + 8g of chicken droppings
GC2	Chicken dropping added at 30%	80g of polluted soil + 24g of chicken droppings
GC3	Chicken dropping added at 50%	80g of polluted soil + 40g of chicken droppings
GC4	No nutrient added	80g of polluted soil only (control)

**GC= Glass Container*

Bioremediation study

This was carried out ex situ in the Microbiology laboratory in situ in the field located at Nkwelle Ezunaka, Anambra State by making Mounds of the sample. 80g of the soil samples was mixed with 16ml of crude oil and was prepared in four places using a glass crucible and was left in the microbiology laboratory for 2 weeks. The poultry droppings were then added to the crude oil polluted soil at the various concentrations for 10%, 30%, 50% and the control was

left without amendment. The set up was then left for a period of 24 weeks. The total petroleum hydrocarbon (TPH) was determined using Gas chromatographic methods. After 24 weeks of remediation, ecological status of the pollution was tested using seeds of *Viciafaba*

Physicochemical analyses of soil sample and amendment material

Physicochemical analyses were carried out on both the poultry droppings before amendment and on the polluted soil after amendment with poultry droppings and the analysis was carried out at the start of the research, repeated after 12^{th} weeks and then concluded after the 24^{th} week. pH, Total nitrogen, phosphorus, and calcium were analyzed.

Microbial enumeration

The vapour phase transfer method [9] was used. Mineral salts medium was sterilized by autoclaving at 121^0C for 15 minutes and dispensed into Petri dishes. The plates were inoculated in duplicates with 0.1 ml aliquots of the 10^{-4} ten-fold serially diluted samples using spread plate technique. The plates were inverted over the dish covers containing 9cm Whatman No.1 filter papers earlier impregnated with crude oil. 0.5ml of streptomycin was added to the mineral salt agar to suppress bacterial growth on fungal plate and nystatin used on bacterial plates to suppress fungal growth.

The ability of the bacterial isolates to utilize crude oil as the only source of carbon and energy was determined by the method of [10]. 0.1ml of 24 hours old nutrient broth culture was inoculated onto each test tube containing 10ml of sterile mineral salt medium (MSM) of Bushnell and Haas (1941) and 1% crude oil. Control test tubes were set up containing 10ml of MSM with 1% crude oil without bacterial seeding. The tubes were incubated at 28^oC for 10 days. At the end of the incubation period, the growth of the isolates was determined by visual observation of the oil medium for turbidity, as compared to the control tubes [10].The extent of degradation of the crude oil by the bacterial isolates was gas chromatographically determined [11]. The amount of crude oil left after the incubation time was determined by extracting the residual oil with 50ml of toluene from the 100ml culture. The mixture was separated using a separator/ funnel and then filtered off with Whatman No 1 filter paper. The optical density was read on a spectrophotometer at 550nm wavelength. Using a previously prepared standard curve, the weight of the crude oil was determined. The amount of crude oil degraded was calculated by subtracting the weight of residual crude oil from weight of the added (initial) crude oil, divided by the weight of the initial crude oil and then multiplied by 100.

$$\text{Amount degraded} = \frac{\text{Weight of initial crude oil - Weight of residual of crude oil}}{\text{Weight of initial crude oil}} \times \frac{100}{1}$$

Crude oil utilization test was carried out for the confirmatory identification of actual petroleum-utilizing fungi using isolates obtained from the oil agar preliminary isolation medium. The vapour phase transfer method was used [12].

Putative petroleum-utilizing fungi isolates were streaked on plates of agar medium (one isolate per plate). In the inside of the Fein-dish cover was placed a sterile filter paper (Whatman No 1) saturated with filter-sterilized crude oil used in the study. This was aimed at supplying hydrocarbons as sole sources of carbon and energy for the growth of the micro-organisms on the mineral salts agar medium surface through vapour phase transfer. All the plates were inverted and incubated at 28^oC for 7 to 14 days [9]. Uninoculated plates served as control. Colonies which appeared on the mineral salts agar medium plates were picked and purified on plates of potatoes dextrose agar transferred onto Sabouraud dextrose agar slants. These were then considered confirmed petroleum-utilizing fungi

Total petroleum hydrocarbon (TPH) estimation

Total petroleum hydrocarbon content of the polluted soil samples were determined using Gas Chromatographic methods according to the toluene extraction method [1] and Sonication water bath methods. Fifteen grams (15g) of each of the sample was weighed into 50ml conical flasks, and then 1ml of 60ug/ml of 1-chlorooctadecane surrogate standard was added. Then 30 milliliters of dichloromethane (extraction solvent) was added to extract oil in the soil. After shaking vigorously in water bath for 5hrs, the mixture was allowed to stand for 60 minutes and then filtered through Whatman No.1 filter paper fitted with cotton wool and sodium sulphate into a clean beaker washed with methylene chloride. The residue was then washed with 20ml extracting solvent and then filtered through funnel. The extracted oil was transferred to vial and placed on a gas chromatographic chamber for analysis. The amount of

crude oil degraded was calculated by subtracting the weight of residual crude oil from weight of the initial crude oil, divided by the weight of the initial crude oil and then multiplied by 100 [13].

$$\text{TPH for Soil(mg/kg)} = \frac{\text{Instrument reading x Total weight of extract}}{\text{Weight of sample}}$$

Crude oil plant toxicity assay

Ecotoxicity is the subject of study in the field of ecotoxicology, which refers to the potential for biological, chemical or physical stressors to affect ecosystem. Aside the normal laboratory test, the soil was further subjected to ecotoxicity test to show the success of the remediation process and to determine the relationship between the growth rate of plants and treated soil at different amendment concentrations. Bean seed (*Viciafaba*) was first cultivated in the soil sample without pollution to determine its suitability for germination purposes. When this was ascertained, seeds were planted in both In-situ and Ex-situ environments of the crude oil polluted soil amended with the poultry droppings at varying concentrations.

Method of [14] was used. The Germination index was determined as follows:

$$\text{Germination Index, GI(\%)} = \frac{\text{(\% Seed Germination, SG) X(\% Growth of root, GR)}}{100}$$

$$\text{Where \% Seed Germination, SG=} \quad \frac{\text{(\%Germination on contaminated soil, EG) X 100}}{\text{(\% Germination on control soil, CG)}}$$

$$\text{And \% Growth of the root, GR=} \quad \frac{\text{(Elongation of root on contaminated soil, GERm) X 100}}{\text{(Elongation of root on control soil, GERCm)}}$$

Statistical Analysis

Analysis of results was performed using one way ANOVA

RESULTS AND DISCUSSION

Tabel 2: Shows physicochemical properties of the Poultry Dropping
Table 3: Shows total heterotrophic microbial counts of the poultry dropping and the soil before amendment
Table 4: Shows residual TPH at different amendment concentration between week1 and week 24
Table 5: Shows Hydrocarbonoclastic Bacterial Count for Polluted Soil Amended with Poultry Droppings (CFU/g)
Table 6: Shows Hydrocarbonoclastic fungal Count for Polluted Soil Amended with Poultry Droppings (CFU/g)
Table 7: Shows germination index (%) obtained from ecotoxicity assessment

Table 2: Physicochemical properties of the Poultry Dropping

Parameter	Values
pH	8.1
Total Nitrogen %	1.8
Total Phosphorus %	0.9
Total Calcium %	1.8

Table 3: Total heterotrophic microbial counts of the poultry dropping and the soil before amendment

Poultry droppings (10^4cfu/g)	Soil (10^4cfu/g)
Heterotrophic Bacterial count	Heterotrophic Bacterial count
3.40	2.58
Fungal count	Fungal count
1.77	1.74

Table 4: Residual TPH at different amendment concentration between week1 and week 24

Samples	InitialTPH(ml/g)	TPH(24 weeks)ml/g	total removed(ml/g)	% removal
10%	6609.83	3788.03	2821.80	42%
30%	6609.83	3144.06	3465.77	52%
50%	6609.83	2951.37	3658.46	55%
Ctrl	6609.83	4192.35	2417.48	36%

Table 5: Hydrocarbonoclastic Bacterial Count for Polluted Soil Amended with Poultry Droppings (CFU/g)

S/No	Chicken dropping	Months							Mean
		0 (start)	1 (4wk)	2 (8wk)	3 (12wk)	4 (16wk)	5 (20wk)	6 (24wk)	
1	10%	1.2	1.3	1.8	1.7	1.6	0.9	0.5	1.29
2	30%	1.4	1.7	2.0	2.3	2.0	0.7	0.5	1.51
3	50%	1.7	1.9	1.9	2.0	1.9	0.9	0.7	1.57
4	Non-amended	0.8	0.4	0.2	0.2	0.3	0.1	0.1	0.20

Table 6: Hydrocarbonoclastic fungal Count for Polluted Soil Amended with Poultry Droppings (CFU/g)

S/No	Chikcken dropping	Months							Mean
		0	1	2	3	4	5	6	
1	10%	0.8	0.8	1.2	1.4	1.1	0.3	0.1	0.59
2	30%	0.5	0.6	0.9	1.2	0.8	0.2	0.1	0.61
3	50%	0.6	0.6	1.1	1.3	0.5	0.4	0.3	0.69
4	Non-amended	0.5	0.2	0.3	0.2	0.2	0.1	0.1	0.22

Table 7: Germination index of *Viciafaba*

Amendment Conc. (%)	*Insitu* (%)	*Ex situ* (%)
Control	0.00	0.00
10	0.00	0.00
30	0.00	0.00
50	95.00	95.00
SWP	1 00.00	100.00

DISCUSSION

The design of the present study is presented in Table 1. The physicochemical parameters of the poultry droppings revealed an alkaline pH of 8.1 and a low value of 0.5% magnesium content (Table 2).

The isolation of diverse genera and species of bacteria and fungi from the poultry droppings in this work was in agreement with earlier report by [15];[16].The microbiological assessment (Table 3) of the poultry droppings in this research revealed that Poultry manure contains rich organic matter in which large number of soil diversity can thrive. When chicken manure was added to soil contaminated with 10% volume to weight of crude oil, it was reported that 75% of the oil was broken down after about two weeks; whereas additive-free soil was naturally remediated to just 50%. The microbial population of the poultry droppings before being used for amendment shows the mean count of 3.4 $x10^4$Cfu/g and 1.77 $x10^4$ Cfu/g for bacteria and fungi respectively.

The Gas chromatographic technique carried out on the polluted soil amended with poultry droppings showed that the total petroleum hydrocarbon (TPH) content was greatest in the control system with a value of 5327.84 mg/kg, while the least TPH value was obtained in the sample with 50% amendment with a value of 2951.37 mg/kg (Table 4). This however depicted a significant reduction of crude oil contaminant.

The hydrocarbon utilizing microorganisms isolated in the present study included: *Bacillus* spp, *Pseudomonas* spp, *Flavobacterium* spp, *Fusarium* spp, and *Aspergillus* spp. The mean hydrocarbon utilizing microbial counts of the crude oil polluted soil after 24 weeks of amendment showed the highest count of hydrocarbon utilizing bacteria at 50% amendment with a value of 1.57 $x10^4$ Cfu/g, while the least hydrocarbon utilizing bacteria count was obtained in the control sample with a value of 2.0 $x10^3$Cfu/g.

The highest count of hydrocarbon utilizing fungi was obtained at 50% amendment with a value of 6.9 $x10^3$Cfu/g, while the least hydrocarbon utilizing fungi count was obtained in the control sample without amendment with a value of 2.2 $x10^3$Cfu/g (Tables 5 and 6).

Ecotoxicity test carried out on the crude oil polluted soil amended with poultry droppings (Table 7) revealed growth of the *Viciafaba* after 5 days of incubation in the 50% chicken dropping amended system. There was no growth in the other treated options thus indicated that the added amendment concentrations (10 % and 30 %) were not enough to cause immediate restoration of the lost soil biological activities (seed germination).

CONCLUSION

The results in this study showed that 50% chicken droppings supported high crude oil remediation in the polluted soil**.** Poultry droppings is a potential source of nutrients for microbial activity and it habours microorganisms capable of utilizing hydrocarbons as source of carbon and energy thus, potentially useful in soil hydrocarbon pollution response action.

Acknowledgements
Authors immensely thank International Energy Services (IESL)ltd, Port-Harcourt, Nigeria for chemical and gas chromatographic analyses.

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