Comparative Studies of Bioremediation of soil Contaminated with Crude oil using Agricultural waste

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Abstract— In recent time crude oil spillage has greatly impeded and crashed the activities of commercial agricultural investors, peasant farmers and the indigenous people in oil producing areas of the country whose livelihood have been massively dependent on agriculture. There is therefore the need to find solution to the problem of oil spillage in these areas. This research project is aimed at determining the degradation profile of the petroleum hydrocarbons as a function of remediation period for the hydro carbon polluted soil. This implies the response study of crude oil polluted soil to purification. The polluted soil was obtained by mixing a bulk soil sample from fertile land within LASU Epe campus with crude oil to achieve a polluted soil. The polluted soil is subjected to bio stimulation to achieve biodegradation of the hydrocarbon polluted soil through ex-situ biore-mediation which was carried out at pilot level in the laboratory. The bioremediation process involves the intro-duction of purifying materials to the polluted soil. The materials used were cow dung, poultry droppings and NPK 15:15:15 fertilizer. The total hydrocarbon content of the soil both before and introduction of the biode-grading materials were determined using gravimetric analysis at intervals of seven days over a period of 31 days. The results obtained from the study showed that the polluted soil was degraded in its hydro carbon content and increased in its microbial population. It was observed that the poultry droppings performed most effectively of the three materials considered. Also, blending of poultry droppings with NPK 15:15:15 yielded appreciable biodegradation of the hydrocarbons. It could be concluded that the utilization of poultry droppings for bioreme-diation of soil polluted with crude oil can be employed for recovery of the fertility of the polluted soil in oil producing areas of Nigeria. It is recommended that the oil industry and environmental agencies in the country give consideration to usage of poultry droppings which are agricultural waste products for bioremediation of hydrocarbon polluted soil in Nigeria.

Keywords: Bioremediation, crude oil contaminated soil, total petroleum hydrocarbon, poultry droppings, cow dung

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1 INTRODUCTION

THE problems associated with contaminated sites now assume increasing prominence in many countries. Contaminated lands generally result from past industrial activities when awareness of the health and environmental effects connected with the production, use, and disposal of hazardous sub-stances were less well recognized than today. The problem is worldwide, and the estimated number of contaminated sites is significant. It is now widely recognized that contaminated land is a potential threat to human health, and its continual discovery over recent years has led to international efforts to remedy many of these sites, either as a response to the risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use. The global production of crude oil which is an extremely complex mixture of ali-phatic and aromatic hydrocarbons, including vola-tile components of gasoline, petrol, kerosene, lubri-cating oil, and solid asphaltene residues, is estimat-ed at more than four billion metric tons per year as at 2017 [3], [22]. It was reported that about 1.7 to 8.8 million metric tons of petroleum hydrocarbons escapes into the soil and water body every year [3]. Crude oil also contains compounds of oxygen, sul-phur, nitrogen and trace amounts of metals. Com-position of oil varies in different producing regions

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and even in different unconnected zones of the same formation. Some compounds are readily degraded (e.g n-alkane, isoprenoids, mono, cyclic aromates) while others stubbornly resist degradation (e.g asphaltenes) and still others are virtually non-biodegradable.

Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result. Therefore, attempts have been made to increase the efficiency of the process through various enhanced techniques. These techniques, also referred to as bio restoration in some old publications include land farming, composting, use of bioreactors , bioventing, biosparging, pump and treat strategies, bioslurping, biostimulation, and bioaugmentation [8], [7], [9], [21], [20], [17], [14], [18, [13], [19], [16], [2], [23], [1]. Many authors consider bioremediation to be a cost-effective (which involves the use of ubiquitous oleophilic microbes) and eco-friendly (which breaks down crude oil into non-toxic products and intermediates) clean-up method compared to other oil spill control techniques [6], [16], [25], [5], [13], [4], [10], [17], [24], [2]. The availability of nutrient has been observed to be one of the major factors that inhibit biodegradation. This release of organic pollutants into an environment produces an excessively high ratio of carbon/nitrogen or carbon/phosphorus ratio, which is unfavorable for microbial growth. It is therefore, pertinent to determine the threshold limit for the variable i.e. nitrogen, phosphorus, microbial organism that will produce optimum biodegradation [15].

Bacteria, particularly mixed populations rapidly degrade paraffins or aromatics as the sole carbon source or in whole crude oil. Branched alkanes are usually more resistant to biodegradation than normal alkanes but less resistant than naphthalenes. Rates of oil or hydrocarbon decomposition in soil appear to depend on the amount of oil present, provided the amount does not exceed the ability of the soil nutrient and oxygen. Decrease in activity at high oil loading concentrations was ascribed to inhibition of microbial activity by toxic components of the oil sludge. Terrestrial oil spills are characterized primarily by vertical movement of the oil into the soil unlike the horizontal spreading associated with slick formation. Infiltration of oil into the soil prevents evaporative losses of volatile hydrocarbon which can be toxic to microorganisms. Since oil bacteria and fungi do not normally occupy more than 1% of the total soil, pore space, movement of organisms or substrates is essential to decomposition. No one species of microorganism is capable of degrading all the components of a given oil. Some of the microorganism involves in biodegradtion of oil included bacteria (Acinetobacter, Aeromonas, Bacillus, Lactobacillus, Pseudomonas species) and fungi (Aspergillus, Candida, Fusarium species) as reported by [12]. This study was developed to investigate the relative effects of utilizing poultry droppings, cow dung and inorganic fertilizer in bioremediation of soil contaminated with crude oil.

2 Materials and Method

2.1 Material collection

The crude oil used in this study was gotten from the Niger Delta region of Nigeria, soil (25 kg) was obtained from agricultural farm of Lagos State University Epe. Cow dung was obtained from a cow farm while the Poultry dropping was obtained from a local poultry farm in Lagos State, Nigeria. The NPK (15:15:15) fertilizer was purchased from an agro-chemical store. All the reagents used for the study were analytical grade product of BDH Chemical Ltd, Poole England. Other materials used include 18 small plastic cylindrical bowl containers of 1.6 litre and 16 cm height. The soil was sundried for 14days to remove moisture and finally sieved using a 2 mm sieve to remove coarse, rough and unwanted particles. The cow dung and poultry droppings were also sundried for14 days, crushed and sieved using a 2 mm mesh sieve to homogenise to fine and soil absorbable size. The three treatment agents (cow dung, poultry droppings and NPK 15-15-15) were stored in the laboratory at ambient temperature.

2.2 Soil treatment

The experimental design involved spiking soil with petroleum hydrocarbon mixtures rather than the use of aged contaminated soil. Thus, there will be an increased bioavailability of the hydrocarbon mixtures to the intrinsic microorganisms. This is done in 1%, 3%, and 5% contamination parts.1kg of each of the three contaminated parts were then stimulated with 40g cow dung, 40g poultry dropping, 2g of NPK 15:15:15, 20g cow dung + 1g NPK 15:15:15, 20g poultry dropping + NPK 15:15:15 in five different perforated and well aerated cylindrical bowl respectively. Furthermore, 1 kg of each contamination parts were set aside as control portions (without any treatment agent) in another perforated and well aerated cylindrical bowl of same dimension with 15cm height and 1.6 litre volume giving a total of 18 portion samples.

The summary of the experimental design is shown in Table 1.

Table 1 Experimental design; Treatment structure of the each crude oil contaminated soil

Portion	Treatment
1	1000g of polluted soil + 40g Cow dung
2	1000g of polluted soil + 40g Poultry Dropping
3	1000g of polluted soil + 2g NPK 15:15:15
4	1000g of polluted soil +20g Cow Dung +1g NPK 15:15:15
5	1000g of polluted soil +2 0g Poultry Droping +1g NPK 15:15:15
6	1000g of polluted soil (Control)

After 14 days the soil samples were tested for the total residual petroleum hydrocarbon content (TPH) to determine the degradation profile of the petroleum hydrocarbons as a function of remediation period for the hydro carbon mixed soil and the control soil. Subsequently, after every 3 days the estimation of the TPH was carried out on all the soil samples. The residual total petroleum hydrocarbon (TPH) concentration of the petroleum control and treated soil samples in percentage (w/w) was monitored in 31 days of remediation and the petroleum hydrocarbons reduction in percentage was determined. The bioremediation effect of the treatment agents on the mixed and control soil sample for the total effect of biostimulating treatment ability to enhance petroleum hydrocarbon microbial degradations were evaluated.

2.3 Estimation of residual Total Petroleum Hydrocarbon (TPH)

The general gravimetric method was used [11] for the quantification of bitumoids extracted from contaminated soils, 50g of each polluted soil samples were weighed into a conical flask and extracted using 35 ml of chloroform. The mixture was vigorously shaken to enhance hydrocarbon extraction. The extract was collected in a test tube through a filter paper and a crucible full of anhydrous sodium sulphate solution was added to dehydrate the extract. An additional purification of extract was frequently performed using silica gel to remove organic matter content before weighing to obtain the total petroleum hydrocarbon (TPH).

3 Results

The results obtained showed that the treatment agents recorded different levels of hydro carbon degradation because they contained hydrocarbon degrading microorganisms and nutrients at different quantities which stimulate hydrocarbon degradation (Figure 1). Thus, the hydro carbon degrading microbes present in the treated soil were active at different degradation rate constants which vary for the different agents. This is in agreement with the findings of Agarry *et al.*, [4]. The degradation agents varies by characterisation in the amount of hydrocarbon degrading microbes they contain in the following decreasing order as shown in Figures 1, 2 and 3 :

Poultry Droppings > Cow Dung > Chemical Fertilizer

The control soil which was not treated with any agent experienced the least degradation because only intrinsic hydro carbon degradation microbes present in the control soil degraded the hydro carbon content of the control soil (Figures 1 to 3). No additional helping microbes or stimulating nutrients from agents were introduced, hence the least performance as shown in all the figures. At 1% contamination, the chemical fertilizer exhibited the least degradation of soil hydrocarbon among other agent as shown in Figure 1. This is hypothe-

sized to be due to the fact that the chemical fertilizer contained the least amount of hydrocarbon degrading microbes compared to other agents by physicochemical analysis. The chemical nutrient they contained was helpful by stimulating the intrinsic hydrocarbon degradation microbes present in the polluted soil. Hence it achieved better degradation than the control soil (Figure 1). The poultry dropping exhibited the highest degradation as it contained highest amount of hydrocarbon degradation microbes and nutrients over other treatment agents. The poultry droppings might have added extra extrinsic hydrocarbon degradation microbes and nutrients to the intrinsic hydrocarbon degradation microbes in the polluted soil than every other treatment agent.

On the average, from the result obtained as shown in Figure 4, there was a high and smooth gradient of degradation up to 14 days .This is explained by the high presence of hydrocarbon compound molecule which act as substrate fed on by the hydrocarbon degradation microbes for energy and carbon.
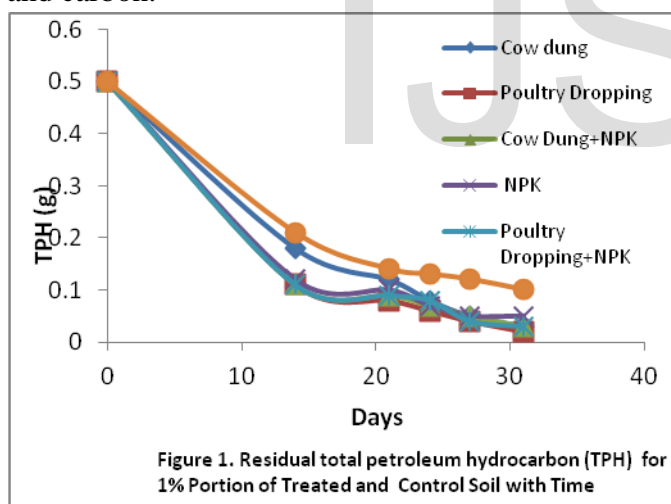

Figure 1. Residual total petroleum hydrocarbon (TPH) for 1% Portion of Treated and Control Soil with Time

The degradation slope gradient decreased towards the end of the experiment due to reduction in the hydrocarbon substrate concentration available to the microbes. Furthermore, the percentage reduction in the hydrocarbon content of the contaminated soil treated with poultry dropping was as high as 92% and higher than the values obtained for other treatment agents as shown in Table 2.
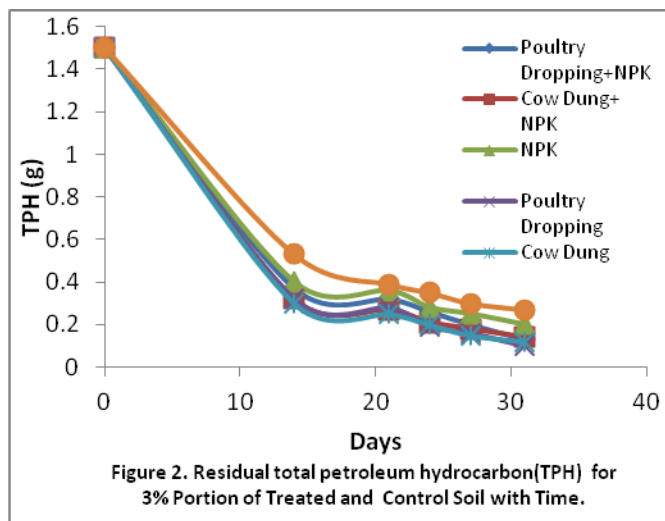

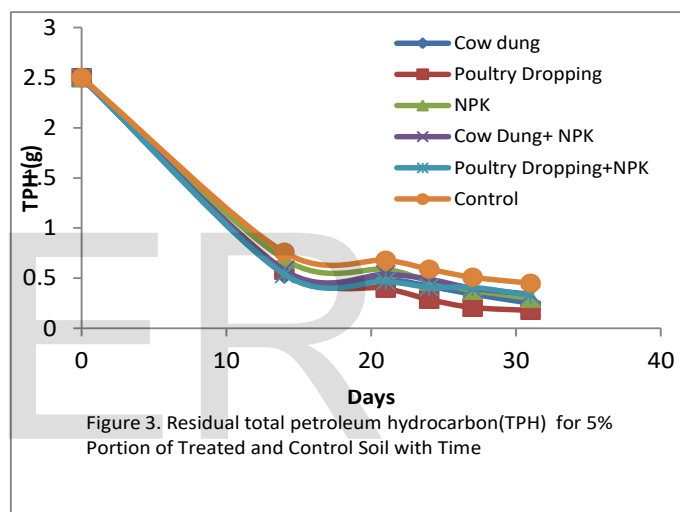Figure 2. Residual total petroleum hydrocarbon(TPH) for 3% Portion of Treated and Control Soil with Time.

Figure 3. Residual total petroleum hydrocarbon(TPH) for 5% Portion of Treated and Control Soil with Time

Table 2 Average total petroleum hydrocarbon for each treatment agent soil portion

No of Days	40g Cow dung (g/kg)	40g Poultry Dropping (g/kg)	NPK 15:15:15 (g/kg)	20g Cow Dung + 1g NPK 15:15:15 (g/kg)	20g Poultry Dropping + 1g NPK 15:15:15 (g/kg)	Control (g/kg)
0	1.50	1.50	1.50	1.50	1.50	1.50
14	0.36	0.37	0.40	0.34	0.40	0.50
21	0.31	0.25	0.35	0.31	0.28	0.40
24	0.25	0.19	0.27	0.25	0.23	0.36
27	0.20	0.14	0.23	0.20	0.20	0.31
31	0.14	0.11	0.18	0.16	0.16	0.27
% Reduction	90.7%	92.7%	88%	89.3%	89.3%	82%

4 Conclusion

The 40 g cow dung, 40 g poultry dropping, 2 g NPK 15:15:15, 20 g cow dung+1 g NPK 15:15:15, 20 g poultry dropping + 1 g NPK 15:15:15 achieved 90.7%, 92.7%, 88%, 89.3%, 89.3%, degradation of soil hydrocarbon respectively while the control soil experienced 82% hydro carbon degradation which is the least recorded. Therefore from the result recorded the bioremediation agents are presented in an order of decreasing effectiveness as follows:

Poultry Droppings > Cow Dung > Chemical Fertilizer

This has a direct variation by characterization to the amount of hydrocarbon degrading microbes they contain and mineral nutrient for biostimulation. Furthermore, it may be concluded that organic materials in general are very good well dependable agents of bioremediation of soil hydrocarbon as they house good amount of useful microorganisms. Hence other vast organic material sources (e.g. sewages, production wastes, etc) can find useful applications in bioremediation and become sustainable.

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