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Bioremediation of Crude Oil Polluted Soil Using Animal Waste

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Abstract Crude oil contaminated soil from Isaka mangrove in Okirika local government area of Rivers state was treated with three different organic wastes (goat manure, poultry droppings and cow dung), for a period of 28 days. The four treatment samples were tilled twice a week and watered with 50 ml of distilled water weekly. There was a general increase in microbial count for all the treatments with the amended samples having a higher microbial count. The total heterotrophic bacterial count for the A, B, C and D treatment options increased from $2.85x10^5$-$1.95x10^6$cfu/g, $3.02x10^5$-$3.09x10^6$cfu/g, $2.75x10^5$-$2.69x10^6$cfu/g and $2.88x10^5$-$2.51x10^6$cfu/g respectively. The hydrocarbon utilizing bacterial count for the A, B, C, and D treatment options increased from $2.51x10^5$-$1.74x10^6$cfu/g, $2.85x10^5$-$2.95x10^6$cfu/g, $2.63x10^5$-$2.51x10^6$cfu/g and $2.51x10^5$-$2.29x10^6$cfu/g respectively. There was a progressive increase in total heterotrophic fungal count, with B treatment option showing the highest increase at $3.02x10^6$cfu/g. The hydrocarbon utilizing fungal count for the A, B, C and D treatment options increased from $1.05x10^5$-$1.26x10^6$cfu/g, $1.10x10^5$-$2.24x10^6$cfu/g, $1.12x10^5$-$2.09x10^6$cfu/g, and $1.10x10^5$-$1.99x10^6$cfu/g respectively. By day 28, the percentage loss of biodegradation of total petroleum hydrocarbon for the B, C, D and A treatment options as measured with GC-FID were 87.1%, 76.6%, 70.7%, and 32.1%, respectively. Ten hydrocarbon utilizing bacterial isolates identified were *Escherichiacoli* ., *Citrobacter* sp., *Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., *Flavobacterium* sp., *Alicagenes* sp., *Corynebacterium* sp., *Arthrobacter* sp., *Aeromonas* sp., and seven hydrocarbon utilizing fungal isolates obtained were *Aspergillus* sp., *Candida* sp., *Fusarium* sp., *Mucor* sp., *Penicillum* sp., *Rhodotorula* sp., and *Rhizopus* sp. The results of this study indicated that nutrient amendment can enhance the rate of biodegradation of crude oil polluted soil.

Keywords: *bioremediation, biodegradation, crude oil contaminated, amendment*

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1. Introduction

Large amounts of organic and inorganic compounds are released into the environment yearly; as a result of human activities. These releases could be deliberate and well regulated (e.g. industrial emission) or accidental (e.g. oil spills).

In Nigeria, the incidence of recorded environmental pollution due to high rate of petroleum related activities has been associated with frequent oil spills, especially through oil well blow outs, tanker accidents and accidental or vandalization of oil pipelines. These mishaps result in the release of crude oil and refined petroleum products into terrestrial and aquatic environments [25].

Decontamination and clean-up of hydrocarbon polluted sites is of paramount importance because of the environmental degradation and health risk associated with this form of pollution. Soil contamination with crude oil causes organic pollution of groundwater which limits its use, as well as economic loss, environmental problems and decreases in the agricultural productivity of soil [35]. Hydrocarbon contamination of soil and freshwater especially polyaromatic hydrocarbon (PAHs) attract public attention because PAHs are toxic, mutagenic and carcinogenic [6]. Since it is widely recognized that contaminated land poses threat to human health, there is need to remediate many of these sites, either as a response to risk of adverse health or environmental effects caused by contamination or to enable the site to be redeveloped for use.

Bioremediation is an option that offers the possibilities to destroy or renders harmless various contaminants using natural biological activity [34]. Bioremediation involves three principal approaches namely, natural attenuation (reliance on natural biodegradation activities and rates), which is sometimes called intrinsic bioremediation; biostimulation (stimulation of natural activities by environmental modifications such as fertilizer addition to increase rates of biodegradation); and bioaugumentation (addition of exogenous microorganisms to the hydrocarbon-impacted ecosystem to supplement the existing microbial population). These three principles for in-situ biodegradation have been applied several times at pilot and field scale levels with varying degree of success [5,8,10,12,15].

Biostimulation is a method of biodegradation that is geared towards enhancing and speeding the process [18].

Most laboratory studies have shown that the addition of limiting nutrients like nitrogen and phosphorous has enhanced the rate of oil biodegradation [7,13]. Therefore, poultry droppings , cow dung and goat manure due to their high nitrogen content can be used to enhance biodegradation of crude oil in soil [3,28], increase soil fertility and at the same time solve the problem of waste management with regards to the animal waste.

The objectives of this study are to monitor the effectiveness of goat manure, cow dung and poultry droppings for enhanced biodegradation of crude oil polluted soil and to isolate and characterize hydrocarbon utilizing microorganisms present in the soil undergoing bioremediation.

2. Materials & Methods

2.1. Study Area

The polluted soil sample for this research was collected from Isaka Mangrove, in Okirika Local Government Area of Rivers State, Nigeria. The site has been polluted due to pipeline vandalization and local refinery activity going on in the area.

2.2. Sample Collection

Polluted soil sample was collected using a clean shovel at a sample depth of 0-15cm and from four different points. The four soils were mixed in a polythene bag and transported to Environmental Microbiology Research Laboratory of University of Port-Harcourt for bioremediation.

2.3. Source of Material

The cow dung was collected from cattle abattoir Alakahia, Port-Harcourt, whereas the poultry droppings and goat manure were collected from poultry farm and goat farm in Elioparanwo Port-Harcourt. The cow dung was sun dried for five days to drive off moisture. The goat manure was air-dried for two weeks and ground with a local mortar and pestle to a fine particle size. Crude oil black in colour was collected from Nigerian Agip Oil Company.

2.4. Preparation and Treatment of Soil Sample

The four samples were mixed in one polythene to get a composite mixture. One thousand gramsof polluted soil sample was placed in each of the four containers labelled A, B, C, &D. Soil sample in container Aserved as a control while B, C, & D were amended with 100g of goat manure, poultry droppings and cow dung respectively. The different treatment options were watered with 50ml sterile water weekly after treatment to moisten the soil. The soil options were mixed individually twice a week for aeration.

Table 1. Experimental Design

Experimental Set	Test Experiment
Set A	1000g of polluted soil sample
Set B	1000g of polluted soil sample + 100g of goat manure
Set C	1000g of polluted soil sample + 100g of poultry dropping
Set D	1000g of polluted soil sample + 100g of cow dung.

2.5. Physicochemical Analysis

The physicochemical analyses of the samples were carried out at intervals of two weeks. The following parameters were analyzed: pH, nitrate, phosphate, total organic carbon and total petroleum hydrocarbon.

2.5.1. Enumeration of Total Heterotrophic Bacteria

Soil samples were prepared by adding 1 gram soil to 9ml sterile distilled water. Serial dilution of the sample were carried out up to 10^{-3} and 10^{-4} dilutions. An aliquot (0.1ml) of the dilution was inoculated into a Petri dish containing plate count agar (PCA) in duplicate for total culturable heterotrophic bacteria. The inoculum was spread plated using sterile bent glass wood [29]. The plates were incubated at 28°C for 18-48hrs. Colonies that formed during this incubation period were counted using this formula

No of colonies x dilution factor

No of gram of soil used

Values were expressed as cfu/g (colony forming unit per gram).

2.5.2. Enumeration of Total Heterotrophic Fungi

The medium used for enumeration of total heterotrophic fungi was Sabouraud's dextrose agar. A 0.1ml aliquot of appropriate dilutions of sample was inoculated into two replicate Petri dishes containing Sabouraud's dextrose agar. The plates were incubated for 2-3days at room temperature and colonies formed were counted, the mean was taken and expressed as cfu/g.

2.5.3. Enumeration of Total Hydrocarbon Utilizing Bacteria

The mineral salt medium of Mills *et al.* [21] was used for the enumeration of hydrocarbon utilizing bacteria and fungi. The mineral salt agar used for enumeration of hydrocarbon utilizing bacteria was amended with 250mg of Amphotericin B sold as fungizone [28]. The medium was sterilized by autoclaving at 121°C, 15psi for 15mins before dispensing into sterile Petri dishes. The gelled mineral salts agar (MSA) was inoculated in duplicate with appropriate dilutions of the sample. Sterile filter paper (Whatman no 1) saturated with crude oil was placed inside the cover of the Petri dish, the Petri dish was closed, inverted and incubated at 28°C for 5-7days. The filter paper saturated with crude oil served as a sole source of carbon [1]. Colonies formed in the duplicate plates were counted and the mean values were recorded in cfu/g. Bacterial isolates growing on MSA were sub cultured into a nutrient agar plate. Pure cultures were subjected to Gram staining and different biochemical tests.

2.5.4. Enumeration of Total Hydrocarbon Utilizing Fungi

Enumeration of total culturable hydrocarbon utilizing fungi was done using mineral salts agar (MSA). The compounded medium for hydrocarbon utilizing fungi was

amended with 250mg of tetracycline to inhibit the growth of hydrocarbon utilizing bacteria [23]. Two replicate plates were inoculated by spread plating, and inverted over sterile filter papers moistened with crude oil, which were placed on the cover of the Petri dishes. The plates were inverted and incubated at 28^{o}C for 7-10 days [25].

2.6. Statistical Analysis

Statistical analyses were carried out using statistical package for social sciences (SPSS Version 16.0). Two way analysis of variance (ANOVA) was used where applicable for detection of significant differences among sample values. The Turkey's test was used in separating significant means.

3. Results and Discussion

Bioremediation of crude oil polluted soil was investigated using different animal wastes (goat manure, poultry droppings, and cow dung) to stimulate the indigenous microbial population.

The microbial counts and physicochemical parameters in the polluted soil samples are represented in Table 2. The bacterial counts for the heterotrophic and hydrocarbon utilizing bacteria were within the same range of 10^5cfu/g which indicates that most bacterial communities making up the total heterotrophic bacteria were capable of utilizing petroleum hydrocarbon. This phenomenon occurs in an environment that is chronically exposed to hydrocarbon from anthropogenic sources [12,32,36]. The baseline pH for the polluted sample was 7.34, this is still within the pH range that favours bioremediation level [20].

Table 2. Baseline characteristics of the polluted soil sample

Parameter	Values
Total heterotrophic bacteria (cfu/g)	2.85 x 10^5
Total heterotrophic fungi (cfu/g)	2.57 x 10^5
Hydrocarbon utilizing bacteria (cfu/g)	2.51 x 10^5
Hydrocarbon utilizing fungi (cfu/g)	1.05 x 10^5
Total petroleum hydrocarbon (mg/kg)	53,966.60
pH	7.34
Nitrate (mg/kg)	0.4
Phospahate (mg/kg)	20.39
TOC (%)	15.015

Changes in total heterotrophic bacteria count and total heterotrophic fungal count during the 28th day bioremediation study are represented in Figure 1 and Figure 2 respectively, with sample amended with goat manure showing the highest population of total heterotrophic bacteria at 3.09x10^6cfu/g. The total hydrocarbon utilizing bacteria increased progressively during the bioremediation period (Figure 3). Statistical analysis showed that there was significant difference at the $p<0.05$ for the four conditions. The total heterotrophic bacterial count and hydrocarbon utilizing bacteria counts in all soil samples amended with various waste were higher compared to counts for unamended soil. This could be ascribed to the presence of appreciable quantities of nitrogen and phosphorous in animal waste, two necessary nutrients for bacterial biodegradation activities [2,13,22]. Also the presence of indegineous microorganisms in the animal waste could also be responsible for the higher total heterotrophic and hydrocarbon utilizing fungal counts in amended samples. It was observed that the total heterotrophic bacteria, total hydrocarbon utilizing bacteria, total heterotrophic fungi and total hydrocarbon utilizing fungi increased with time during the study. This resulted in a corresponding removal of hydrocarbon with time particularly in the nutrient amended samples.

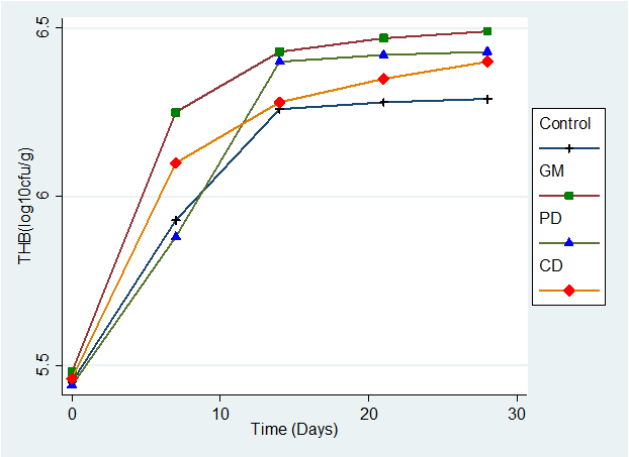

Figure 1. Changes in total heterotrophic bacterial count of crude oil polluted soil

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung

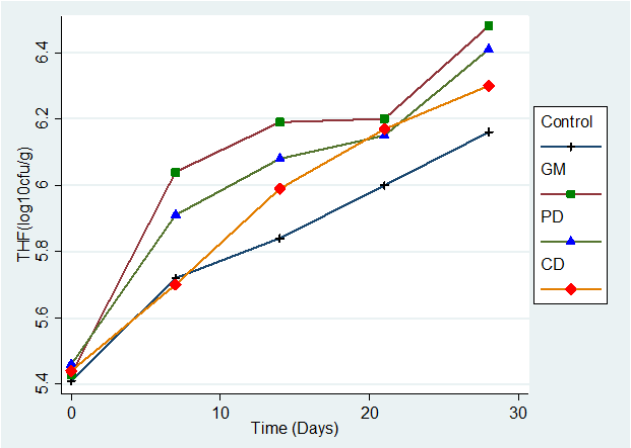

Figure 2. Changes in total heterotrophic fungal count in crude oil polluted soil

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung

Increase in the microbial counts for total heterotrophic bacteria, hydrocarbon utilizing bacteria, total heterotrophic fungi and hydrocarbon utilizing fungi in crude oil polluted soils/sediments amended with organic and inorganic nutrient sources have been reported by other researchers [7,10,28]. Roling *et. al*. [31] examined bacterial dynamics and crude oil degradation after nutrient ammendment and found out that the nutrient enhancement increased bacterial counts which impacted significantly with hydrocarbon attenuation. This has been reported by several researchers [25,26,27,30,33]. In the present study, the population of heterotrophic bacteria, hydrocarbon utilizing bacteria, total heterotrophic fungi and hydrocarbon utilizing fungi were significantly different at $p<0.05$ for the four treatment options. Soil amended with goat manure had the highest total heterotrophic bacterial count of 3.09x10^6cfu/g, followed by poultry droppings which had a count of 2.69x10^6cfu/g on the 28th day. Amendment of the crude

oil polluted soil with the various organic waste stimulated higher microbial proliferation in soil.

The hydrocarbon utilizing bacteria isolated from this study were *Escherichia coli, Citrobacter, Bacillus, Micrococcus, Pseudomonas, Flavobacterium, Alcaligenes, Corynebacterium, Arthrobacter* and *Aeromonas species.* Eziuzor & Okpokwasilli [10] also isolated *Arthrobacter, Bacillus, Citrobacter, Alcaligenes, Flavobacterium, Pseudomonas* and *Corynebacterium* in a study of bioremediation of crude oil polluted mangrove soil in Port-Harcourt using NPK fertilizer and organic waste as sources of limiting nutrient. Studies by Leahy and Colwel [17] reported that the following bacterial genera contain well known species of hydrocarbon degraders in marine sediments; *Acinetobacter, Alcaligenes, Arthrobacter, Staphylococcus, Bacillus, Flavobacterium, Norcardiaand Pseudomonas*. The bacteria isolated from this study were mainly gram negative bacteria (Table 3). This supports the findings of some researchers that have shown that oil polluted soils are dominated by gram negative bacteria [15,19].

Table 3. Microscopic and Biochemical Characteristics of Hydrocarbon Utilizing Bacteria isolated during the study

Isolate No	Shape	Gram Rxn	Motility	Citrate	Catalase	Indole	MR	VP	Starch hydrolysi	H_2S producti	Oxidase	Glucose	Lactose	Mannitol	Sucrose	Probable organism
HUB1	Rod	-ve	+	-	-	+	-	-	-	-	-	A/G	A	A	-	*E.coli* sp.
HUB2	Rod	-ve	+	+	+	-	+	-	+	+	-	A/G	A	A	A	*Citrobacter* sp.
HUB3	Rod	-ve	+	+	+	-	-	+	+	-	-	A	A	A	-	*Bacilli* sp.
HUB4	Cocci	+ve	-	-	+	-	+	-	-	-	+	A	A	A	A	*Micrococcus* sp.
HUB5	Rod	-ve	+	+	+	-	+	-	+	+	-	A/G	A	A	A	*Citrobacter* sp.
HUB6	Rod	-ve	+	-	+	-	-	-	-	-	+	AG	A	V	A	*Pseudomonas* sp
HUB7	Rod	-ve	+	+	+	-	-	+	+	-	-	A	A	A	-	*Bacillus* sp.
HUB8	Rod	-ve	-	+	+	-	+	-	-	-	+	A	-	-	A	*Flavobacterium* sp.
HUB9	Rod	+ve	+	+	+	-	V+	+	+	-	-	A	A	A	-	*Bacillus* sp.
HUB10	Rod	-ve	+	-	+	-	-	+	-	-	+	A/G	-	A	A	*Alicagenes* sp.
HUB11	Cocci	+ve	-	-	+	-	+	-	-	-	+	A	A	A	A	*Micrococcus* sp.
HUB12	Rod	-ve	+	-	+	-	-	-	-	-	V+	AG	A	V+	A	*Pseudomonas* sp.
HUB13	Rod	+ve	-	-	+	-	-	+	-	-	-	-	-	-	A	*Corynebacterium* sp.
HUB14	Rod	+ve	-	+	+	-	-	+	-	-	-	AG	A	A	A	*Arthrobacter* sp.
HUB15	Rod	-ve	+	+	+	-	-	+	+	-	-	A	A	A	-	*Bacillus* sp.
HUB16	Rod	-ve	+	+	+	-	-	-	+	-	+	A/G	A/G	A/G	A/G	*Aeromonas* sp.

The hydrocarbon utilizing fungi isolated from this study were *Aspergillus, Candida, Rhizopus, Fusarium, Mucor, Penicillium, Rhodotorula*and*Aspergillus* species (Table 4). Ahern et al [4] also isolated strains of *Candida* and *Rhodotorula* which are capable of oil degradation. In addition they isolated *Rhodosporium, Saccharomyces, Sporobolomyces* and *Trichosporon* which were not isolated in this study. Fungi reported as oil degraders in aquatic environments of petroleum producing area in Nigeria were *Candida, Rhodotorula, Saccharomyces, Sparobolomyces, Aspergillusniger, A.terreus, Blastomyces* sp., *Botryodipodiatheobromas, Fusarium* sp., *Nigrospora* sp., *Penicilliumchrysogenum, P.glabrum, Pleurofragmium*sp. And *Trichodermaharzaianum* [24]. Obire and Anyanwu [23] isolated eight mouldsviz; *Aspergillus* sp., *Cephalosporium* sp., *Cladosporium* sp., *Fusarium* sp., *Geotrichum* sp., *Mucor* sp., *Penicillium* sp., and *Trichoderma* sp., and two yeast *Candida* sp. *Rhodotorula* sp. as hydrocarbon utilizers, in their study of impact of various concentrations of crude oil on fungal populations of soil. Fungi belonging to some of these genera were isolated in this study.

Table 4. Cultural Characteristics of Hydrocarbon Utilizing Fungi on SDA

Isolate No	Culture Characteristics	Microscopic Characteristics	Probable genera
HUF1	Powdery green colonies that is yellow at the reverse side	Septate and branched hyphae with conidia in chains	*Aspergillus* sp.
HUF2	White smooth colonies that are yeast like in appearance.	Elongate budding yeast like cells, branched pseudohyphae.	*Candida* sp.
HUF3	Dense white cottony colonies that is white at the reverse	Non-septate hyphae sporagphores, rhizoids, ovoid brown sporangioshores, sporangium containing collumella	*Rhizopus* sp.
HUF4	Whittish cottony colonies	Multi-segmented canoe shaped spore and branched conidiospores	*Fusarium* sp.
HUF5	Creamy glaborous colonies that are yeast like in appearance	Spherical budding yeast like cells.	*Candida* sp.
HUF6	Dark grey cottony colonies that is white at the reverse.	Non-septate hyphae with sporangium that contains black sporangisphores and a subtending columella without rhizoids.	*Mucor* sp.
HUF7	Powdery yellowish green dense mycelia and light yellow at the reverse side.	Long conidiosphore consisting of brown like conidia in chains	*Penicillium* sp.
HUF8	Smooth pink colonies that are yeast like in appearance.	Spherical budding yeast like cells.	*Rhodotorula* sp.
HUF9	Powdery dark brown colonies that is flat on the surface of the medium and brownish at the reverse.	Septate and branched hyphae and conidia in chains.	*Aspergillus* sp.

The bacterial counts for the samples were higher than fungal counts for similar treatments (Figure 3 & Figure 4). The higher counts of bacteria compared to fungi may be as a result of the nutrient status of the soil [14] and the

presence of some toxic components which do not favour fungal growth [9].

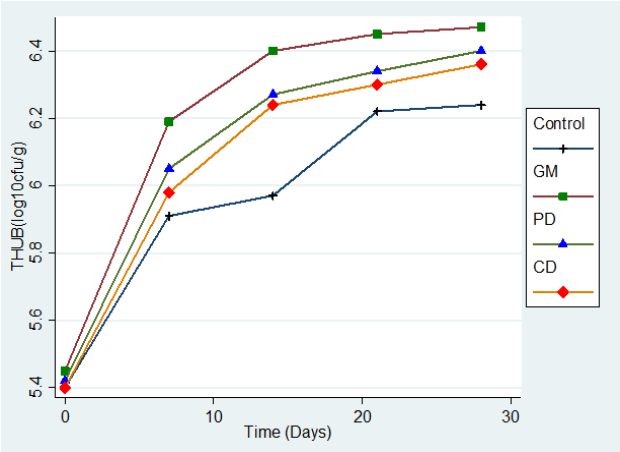

Figure 3. Changes in total hydrocarbon bacterial count of crude oil polluted soil

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

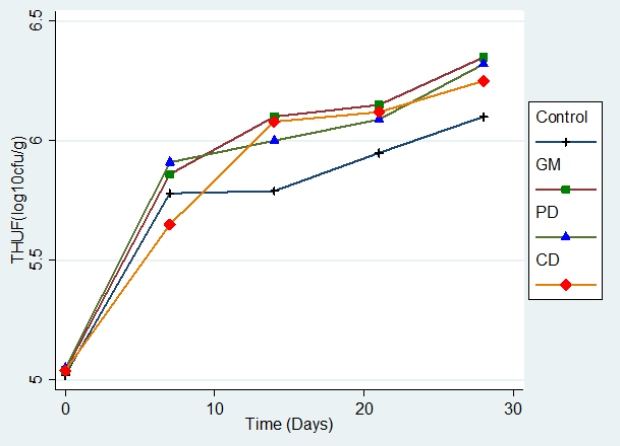

Figure 4. Changes in total hydrocarbon utilizing fungal count of crude oil polluted soil

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

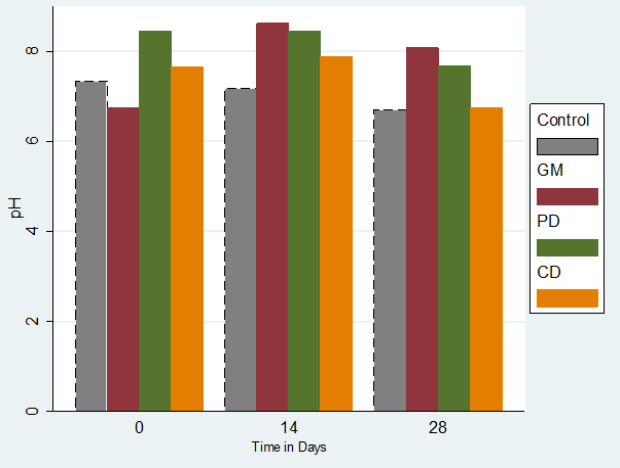

Figure 5. Changes in pH values of soil samples during remediation

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

The pH values for soil with varying treatment ranged from 6-8.5 as shown in Figure 5. Analysis of variance showed that the differences in pH values were insignificant. From the study, there was a fluctuation in value of pH with time. The fluctuation in value of pH may be due to the metabolites produced by the microorganisms during the remediation period. The physicochemical environmental factors (pH, nitrate, phosphate) recorded during the study period were among those reported to affect bacterial growth [1,11].

The changes in nitrate and phosphate were represented in Figure 6 and Figure 7 respectively. The effects of treatment was statistically significant as well as the effect of time at $p < 0.05$ level for both nitrate and phosphate. There was a decrease in the nitrate and phosphate level in all treatment options and control, but the amended options showed appreciably decrease in nitrate and phosphate concentration than the control. This is an indication that the phosphate and nitrate were used by microorganism during the bioremediation.

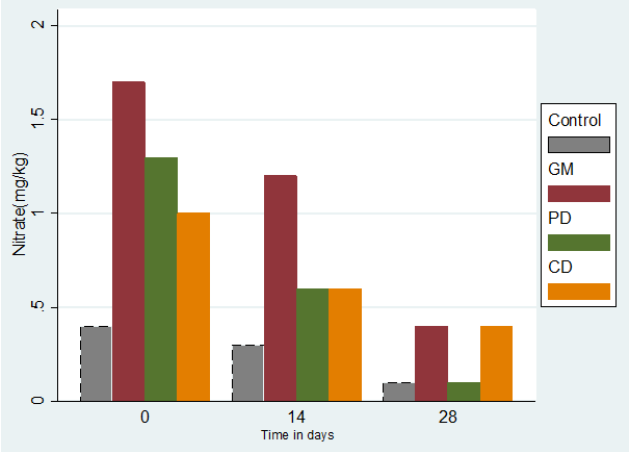

Figure 6. Changes in Nitrate Concentration of soil samples during remediation study

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

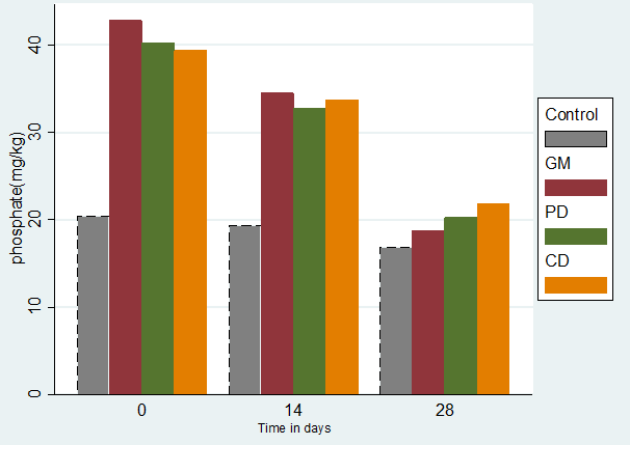

Figure 7. Changes in Phosphate concentration of soil samples during remediation

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

The changes in total organic carbon (TOC) in the soil samples are represented in Figure 8. The effect of time

was statistically significant at p< 0.05, but there was no significant difference at p<0.05 for all the four conditions.

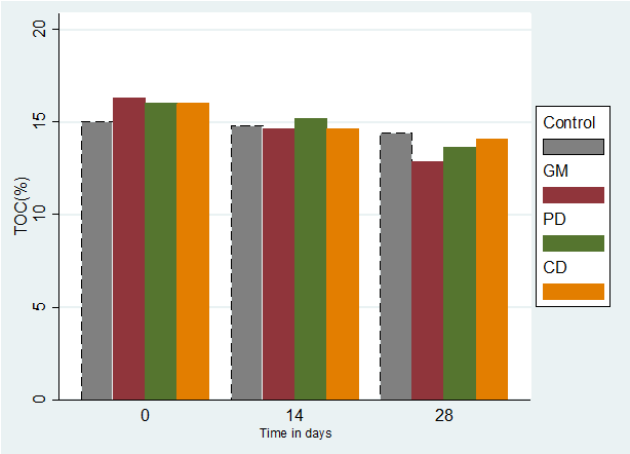

Figure 8. Changes in total organic carbon in soil samples during remediation study

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung

Total petroleum hydrocarbon (TPH) levels reduced with time during the study period (Figure 9). The effect of time was highly significant at p<0.05 level, on the other hand, there was no significant difference at p<0.05 for all the four conditions.

The TPH decreased from 53,966.60mg/kg to 36,667.22mg/kg on the 28th day in the unamended sample. The depletion of hydrocarbon indicated that indigenous bacterial communities in the hydrocarbon impacted soil have the natural capacity to degrade petroleum hydrocarbon, since they could use crude oil as a source of carbon and energy. Rosenberg & Ron [32] in some of the case studies of bioremediation project that took place shortly after Exxon Valdez colossal oil spill, in one of such , the researchers used Inipol EAP22 oleophillic fertilizer to treat the oil impacted shorelines. It was found that C_{18} phytane ratio in the treated plots reduced during the summer of 1989 when the study was done. However the control plots also showed a similar decrease in the ratio of hydrocarbon used as biodegradation index.

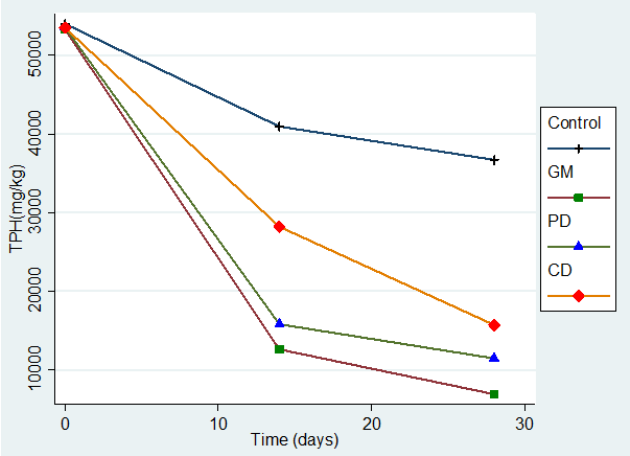

Figure 9. Changes in Total Petroleum Hydrocarbon of soil sample during remediation

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

In this study the amendment of polluted soil with animal waste enhanced the rate of hydrocarbon degradation (Figure 9 & Figure 10). There was a rapid reduction in the total petroleum hydrocarbon within the first 14days of the study in all treatment options and control. At the end of 14days there was 76.4%, 70.5%, 47.3% and 24.0% TPH reduction in soil amended with goat manure, poultry dropping, cow dung and in control respectively (Figure 10). Goat manure treated option showed the highest percentage loss in TPH at 87.1% followed by poultry droppings treated option at 78.6%, then cow dung treatment option at 70.7% and the control at 32.1% TPH loss. On the contrary, Agarry *et.al.* [3] reported 73% and 50% TPH loss for hydrocarbon polluted soil treated with poultry manure and goat manure respectively. The difference in the results might be due to the heterogeneity of soils and crude oil as well as the possible interactions between the soils amendment and the natural soil constituents [16].

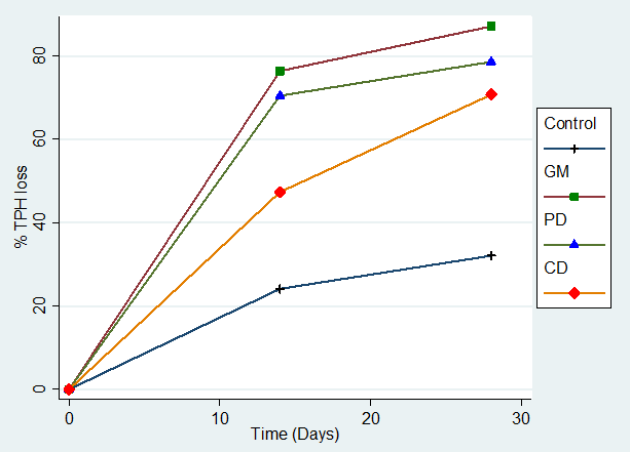

Figure 10. Percentage loss in total petroleum hydrocarbon in soil samples during remediation

GM = Polluted sample amended with goat manure, PD= Polluted sample amended with poultry droppings, CD= Polluted sample amended with cow dung.

4. Conclusion

The results of this study showed that contaminated soil amended with goat manure, poultry droppings, cow dung and the control sample showed 87.1%, 78.6%, 70.7% and 32.1% loss in total petroleum hydrocarbon respectively. Contaminated soil amended with goat manure showed the highest percentage total petroleum hydrocarbon loss. The findings of this study showed that the ratio of biodegradation depends majorly on soil nutrient availability. The low total petroleum depletion in control soil justifies this view. Taking into account that the amount of limiting nutrient such as nitrogen and phosphorous present in the polluted soil is low, the use of organic waste as nitrogen and phosphorous source can enhance bioremediation process, as well as solve the problem of waste management and utilization.

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