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BIOLOGICAL DEGRADATION OF KEROSENE IN SOIL AMENDED WITH POULTRY DROPPINGS

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ABSTRACT
Deliberate or accidental discharges of kerosene and other petroleum products are major contributors to environmental pollution problem globally. The effects of non-sterile and sterile poultry droppings on microbial degradation of kerosene in soil were investigated with an intention to develop low cost strategy for bioremediation of soil ecosystems contaminated with kerosene. Soil deliberately contaminated with kerosene, treated with 200gm non-sterile and sterile poultry droppings were designated TA and TB respectively. Similar contaminated soil treated with 250gm non-sterile and sterile poultry droppings were designated TC and TD respectively, while kerosene contaminated soil without poultry droppings amendment served as control (CON). Samples collected were analysed chemically and microbiologically using standard chemical and microbiological procedures respectively. The mean counts of bacteria and fungi in the polluted soil exhibited an initial decrease between weeks 0 and 2 before assuming a steady increase for the rest of the study period. Hydrocarbon utilizers isolated from poultry droppings were *Pseudomonas aeruginosa, Bacillus* sp, *Alcaligenes faecalis*, *Serratia* sp, *Penicillium chrysogenum*, *Aspergillus niger*, and *Candida* sp. At week 10, the percent degradations of residual kerosene quantified gravimetrically in TA, TB, TC and TD were 96.81, 89.64, 98.59 and 93.04% respectively at which time the corresponding value obtained for control was 39.16%. These results revealed that proper application of poultry droppings especially non-sterile poultry droppings can effectively enhance bioremediation of kerosene polluted soil.

KEYWORDS: Soil pollution, residual kerosene, poultry manure, bacteria, fungi, waste management.

INTRODUCTION

The word kerosene is derived from the Greek word 'kero,' which means wax. It is thin oil distilled from petroleum and chemically composed of 35% paraffins, 60% naphthenes and 15% aromatics by weight. Kerosene serves as illuminant, alcohol denaturant, aircraft gas turbine and jet fuel, spray oil to combat insect on citrus plant as well as source of energy for cooking (Irwin *et al*., 1997; Ikpeme *et al*., 2007; Kalme *et al*., 2008). Despite the several usefulness of kerosene, it also constitutes a major environmental concern globally. Based on the type and concentration of aromatic compound present in kerosene, its acute toxicity to living organisms vary from moderate to high. Kerosene is highly toxic to humans, irritating to skin and mucous membrane. Its spill could cause potential acute toxicity to both aquatic and terrestrial life as well as inhalation hazards (Saratale *et al*., 2007). Chronically, the effects of some of the constituents in kerosene (benzene, toluene, xylene, naphthalene, alkyl benzenes and various alkyl polycyclic aromatic hydrocarbons) include changes in the liver, harmful effects on the kidney, heart, lungs, and nervous system. Also of concern are the increased rates of cancer, immunological, reproductive, fetotoxic, genotoxic effects attributed to some of the compounds found in kerosene and other petroleum products (Irwin *et al*., 1997). In addition to economic damage and aesthetic problem caused by kerosene spills, plants, animals and microorganisms in both land and water are negatively affected (Guzman *et al.,* 2004; Ikpeme *et al*., 2007). Industrialization, accidental and deliberate spill of petroleum products have increased the pollution of hydrocarbon compounds in the soil and water. Basically, kerosene enters the environment through different sources such as accidental spills, pipe leakage, pipe vandalization, deliberate disposal of oily wastes, corrosion of pipes, kerosene seeps and other operational deficiencies (Ikpeme *et al*., 2007; Kalme *et al*., 2008). However, the unavoidable spills of kerosene arising from tank overflow, bunkering and poor vending facilities could be attributed to the importance of kerosene as a major source of energy for cooking and lighting in all sectors of the society in Nigeria (Ikpeme *et al*., 2007).

Application of methods based mainly on metabolic activity of microorganisms have been observed as the most rational way of detoxifying the environment loaded with petroleum derivatives (Leahy and Colwell, 1990). Bioremediation processes utilize naturally occurring microorganisms to treat specific environment polluted with chemicals (Wackett and Hershberger, 2001; Pelczar *et al.* 2002). Stimulation of the natural potentials of microorganisms to degrade and detoxify hazardous pollutants is a welcomed development as it brings about the biotransformation which reduces the complex mixture of noxious materials to simple nutrients in soil and aquatic environments (Ikpeme *et al*., 2007).

Poultry droppings are organic fertilizers. They contain substances of various origin used for soil fertilization as well as source of nutrients and energy for soil microorganism (Hoffmann *et al*., 2007). In addition to the large amount of nitrogen and considerable quantity of

phosphorus found in poultry droppings, they also contain useful hydrocarbon utilizers such as *Pseudomonas* sp, *Micrococcus* sp and *Acinetobacter* sp. which further improved natural biogeochemical cycling (Hoffmann *et al*., 2007; Akinde and Obire, 2008). Bioremediation protocols involving application of nutrients to oil polluted site to stimulate the growth of naturally occurring oil degrading microorganisms can improve the rate of recovery of environments contaminated with petroleum or its products. The presence of essential nutrients for microbial growth and appreciable population of hydrocarbon utilizers in poultry droppings probably confirmed poultry droppings as good biostimulants as well as good source of exogenous hydrocarbon utilizers. Thus the use of poultry droppings for bioremediations is beneficial not only for oil clean-up but also for waste management as the removal and management of poultry droppings is becoming a major problem due to the increasing concentration of fowls on poultry farms (Hoffmann *et al*., 2007). Keeping these points in mind, these study assessed the effects of poultry droppings on microbial degradation of kerosene in soil.

MATERIALS AND METHODS

Source of material

The soil samples used in this study were randomly collected from the upper 25 cm of a plot of agricultural land (measuring 30 x 25 m) beside Bells University of Technology, Ota, Nigeria, using sterile hand auger. The plot of the land from which the soils were obtained has no known history of contamination with crude oil or petroleum related products. The soils collected from different spots on the plot were put together, mixed thoroughly and sieved before use. The kerosene used was bought from a petrol filling station in Ota, while the poultry droppings used were randomly collected from Obasanjo farm in Ota. The poultry droppings collected from different points were combined and crushed thoroughly using sterile mortar and pestle to homogenize it before use.

Experimental design

The experiment was set up in the laboratory using a completely randomized design. The soil samples collected were mixed thoroughly and sieved before use. The poultry droppings collected from different points were combined and mixed thoroughly, half of the quantity collected was sterilised by tyndallisation while the other half was left unsterile. Two kilogram each of the soil contained in five open pans, measured 29.0cm×18.0cm×10.0cm (internal dimension) were separately contaminated with 200ml of sterile kerosene, to give approximately 10% (v/w) pollution. Four of the setups designated Treatments (TA, TB, TC and TD) were treated with poultry droppings, while the fifth setup without poultry droppings treatment was designated Control. While TA and TB were supplemented with 200 gm of non-sterile and sterile poultry droppings respectively, TC and TD were supplemented with 250 gm of non-sterile and sterile poultry droppings respectively. Setups TA and TB were designed to determine the effects of non-sterile and sterile poultry droppings in bioremediation of kerosene contaminated soil, while setups TC and TD were designed to determine the effects of poultry droppings concentrations in bioremediation of kerosene contaminated soil when compared with the setups TA and TB respectively. However, the control was designed to determine the contribution made by microorganisms indigenous to the soil. The four treatments and the control designs were setup in three replicates and kept in the laboratory at room temperature (28±2°C) throughout the investigation periods (10 weeks). They were watered weekly with 200 ml sterile distilled water. Samples were taken at 2-week interval for analysis.

Microbiological and physicochemical analyses

Using the standard plate count techniques, total viable counts of bacteria were performed on nutrient agar plates while that of fungi were done on potato dextrose agar (PDA) plates fortified with streptomycin (0.125g/l) after appropriate dilutions of the samples. Incubation was carried out at 30^0C for 1–3 days (Adebusoye *et al*., 2010). The population densities of hydrocarbon- utilizing organisms were determined by plating on minimal salt agar (MSA) previously described by Nwachukwu (2001). For hydrocarbon-utilizing bacteria, the medium was adjusted to pH 7.2, while it was adjusted to pH5.6 for fungi and further fortified with streptomycin to inhibit bacterial growth. In both cases, sterile kerosene served as the sole carbon and energy source and was made available through vapor phase transfer previously described by Raymond *et al*. (1976). Microbial colonies were counted, screened, and pure cultures obtained by replica plating. Presumptive identification of isolates was done with reference to Bergey's Manual of Determinative Bacteriology (Holt *et al*. 1994), Barnett and Pankhurst (1974) and O'Donnell (1979). The available nitrate, phosphate and sulphate were determined using standard analytical protocols described by APHA (1998). Moisture content was determined using moisture analyzer and the pH by a pH meter (Jenway) according to Nwachukwu (2000).The residual kerosene was extracted twice from the contaminated soil sample (10 gm) using n-hexane: dichloromethane solvent system (1:1) and quantified gravimetrically as described by Nwachukwu (2001). To do this, 10gm of soil sample was randomly taken from each replicate at surface, middle and bottom, and mixed thoroughly before analysis. The kerosene was extracted by mixing the soil with 40ml volume of the solvent system, stirred for 5min and filtered through whatman No 1 filter paper. The procedure was repeated twice and extracts pooled and dried in an oven at 80^0C. The residual kerosene was then obtained by mass difference.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) while significant means were separated with the Duncan's multiple range tests using the SPSS 17.0 statistics.

RESULTS

The physicochemical properties and microbial load of the unpolluted soil and the poultry droppings used as organic manure in the bioremediation of kerosene polluted soil are as shown in Table 1.

TABLE 1: Physicochemical Properties and Microbial load of Soil and Poultry Droppings.

PARAMETER	SOIL	POULTRY DROPPING
Moisture (%)	2.20±0.02	9.75±0.01
pH	5.35	7.31
Temperature (^{0}C)	31	29
Nitrate (ppm)	5.96 ±0.02	4.69±0.01
Phosphate (ppm)	1.16±0.01	11.29±0.02
Sulphate (ppm)	5.83±0.03	4.92±0.01
THM (cfu/gm)	$1.15\pm0.01\times10^{10}$	$7.92\pm0.02\times10^{9}$
HCUM (cfu/gm)	$9.70\pm0.01\times10^{4}$	$6.70\pm0.01\times10^{4}$

THM, total heterotrophic microorganisms; HCUM, hydrocarbon utilizing microorganisms; cfu, colony forming unit

TABLE 2: Mean Bacterial Count for Treatments and Control

Sampling Time (Weeks)	Mean Bacterial Count (cfu/gm ± SD) $\times10^{10}$				
	TREATMENTS				CONTROL
	TA	TB	TC	TD	CON
0	4.41 ± 0.02^{v}	2.99 ± 0.01^{o}	4.46 ± 0.01^{w}	2.79 ± 0.01^{n}	2.45 ± 0.01^{j}
2	1.66 ± 0.01^{d}	1.44 ± 0.01^{c}	1.80 ± 0.01^{e}	1.27 ± 0.00^{b}	1.09 ± 0.01^{a}
4	2.44 ± 0.01^{j}	2.24 ± 0.01^{h}	2.54 ± 0.01^{k}	2.13 ± 0.01^{g}	2.00 ± 0.01^{f}
6	3.61 ± 0.00^{r}	2.56 ± 0.01^{l}	3.74 ± 0.01^{s}	2.74 ± 0.01^{m}	2.41 ± 0.00^{i}
8	4.22 ± 0.02^{u}	3.24 ± 0.01^{p}	4.42 ± 0.01^{v}	3.25 ± 0.01^{p}	2.78 ± 0.01^{n}
10	5.16 ± 0.01^{x}	3.77 ± 0.01^{t}	5.78 ± 0.02^{y}	3.59 ± 0.01^{q}	3.00 ± 0.01^{o}

SD, standard deviation; cfu, colony forming unit; CON, control.
Rows and Columns with the same superscript are not significantly different ($p\leq 0.05$).

The poultry droppings contain considerable amounts of nitrate, phosphate and sulphate essential for microbial growth as well as high population of hydrocarbon utilizing microorganisms($6.70\pm0.01\times10^{4}$cfu/gm).These hydrocarbon utilizers were presumptively identified to be *Pseudomonas aeruginosa, Bacillus* sp, *Alcaligenes faecalis*, *Serratia* sp, *Penicillium chrysogenum*, *Aspergillus niger*, and *Candida* sp. Biodegradation of kerosene in the polluted soil ecosystems were monitored by periodic evaluation of changes in mean microbial population densities and reduction in kerosene concentration analysed gravimetrically. Table 2 presents the mean population densities of bacteria counted in treatments (TA, TB, TC and TD) and control.

TABLE 3: Mean Fungal Count for Treatments and Control

Sampling Time (Weeks)	Mean Fungal Count (cfu/gm ± SD) $\times10^{7}$				
	TREATMENTS				CONTROL
	TA	TB	TC	TD	CON
0	2.80 ± 0.01^{w}	2.31 ± 0.01^{r}	2.89 ± 0.02^{x}	2.30 ± 0.02^{q}	2.31 ± 0.01^{r}
2	1.75 ± 0.01^{i}	1.319 ± 0.02^{b}	1.80 ± 0.01^{j}	1.31 ± 0.02^{c}	1.30 ± 0.01^{a}
4	1.95 ± 0.01^{l}	1.50 ± 0.01^{e}	2.14 ± 0.01^{n}	1.62 ± 0.02^{g}	1.46 ± 0.02^{d}
6	2.24 ± 0.01^{p}	2.01 ± 0.02^{m}	2.47 ± 0.01^{s}	2.16 ± 0.01^{o}	1.60 ± 0.02^{f}
8	2.57 ± 0.01^{t}	2.71 ± 0.02^{u}	$3.29\pm0.01^{b'}$	2.79 ± 0.01^{v}	1.70 ± 0.02^{h}
10	$3.19\pm0.01^{a'}$	2.95 ± 0.02^{y}	$3.85\pm0.02^{c'}$	3.05 ± 0.02^{z}	1.86 ± 0.02^{k}

SD, standard deviation; cfu, colony forming unit; CON, control.
Rows and Columns with the same superscript are not significantly different except those with superscript bearing prime sign ($p\leq 0.05$).

There was an initial decrease in total aerobic bacterial count from $4.41\pm0.02 \times 10^{10}$ to $1.66\pm0.01 \times 10^{10}$, $2.99\pm0.01 \times 10^{10}$ to $1.44\pm0.01 \times 10^{10}$, $4.46\pm0.01 \times 10^{10}$ to $1.80\pm0.01 \times 10^{10}$, $2.79\pm0.01 \times 10^{10}$ to $1.27\pm0.00 \times 10^{10}$ and $2.45\pm0.01 \times 10^{10}$ to $1.09\pm0.01 \times 10^{10}$ respectively for TA, TB, TC, TD and control between weeks 0 and 2, thus, showing the toxic effect of kerosene on the indigenous microorganisms.

However, there was a subsequent increase in bacterial population which was highly significant in treatments (TA, TB, TC and TD) supplemented with poultry droppings compared with the control not supplemented with poultry droppings. Table 3 depicts the mean counts of fungi enumerated in treatments (TA, TB, TC and TD) and control. There was also an initial decrease in mean fungal count between weeks 0 and 2 apparently due to toxic

effect of kerosene. However, the fungal population density later assumed an increasing trend which was more remarkable in treatments (TA, TB, TC and TD) amended with poultry droppings. The mean counts of hydrocarbon-utilizers found to be higher in treatments (TA, TB, TC and TD), especially those fortified with non-sterile poultry droppings (TA and TC) were presented in Table 4.

Table 4: Mean Hydrocarbon Utilizer Count for Treatments and Control

Sampling Time (Weeks)	Mean Hydrocarbon utilizers Count (cfu/gm ± SD) $\times 10^6$				
	TREATMENTS				CONTROL
	TA	TB	TC	TD	CON
0	2.57 ± 0.01^{c}	1.80 ± 0.02^{a}	3.13 ± 0.01^{g}	1.80 ± 0.01^{a}	1.81 ± 0.01^{a}
2	3.71 ± 0.01^{i}	2.94 ± 0.01^{f}	4.54 ± 0.02^{m}	2.91 ± 0.01^{e}	2.25 ± 0.02^{b}
4	4.90 ± 0.02^{o}	3.88 ± 0.01^{j}	6.19 ± 0.01^{v}	3.98 ± 0.01^{l}	2.67 ± 0.01^{d}
6	6.02 ± 0.02^{t}	4.84 ± 0.01^{n}	6.83 ± 0.01^{x}	4.94 ± 0.01^{p}	2.90 ± 0.01^{e}
8	6.43 ± 0.02^{w}	5.45 ± 0.01^{q}	7.51 ± 0.01^{y}	5.78 ± 0.01^{r}	3.59 ± 0.02^{h}
10	8.09 ± 0.01^{z}	5.91 ± 0.01^{s}	$9.02\pm0.01^{a'}$	6.11 ± 0.01^{u}	3.94 ± 0.01^{k}

SD, standard deviation; cfu, colony forming unit; CON, control.
Rows and Columns with the same superscript are not significantly different except those with superscript bearing prime sign ($p \leq 0.05$).

At the end of the investigation period, the mean population densities of hydrocarbon-utilizers counted in TA, TB, TC and TD were $8.09\pm0.01 \times 10^6$, $5.91\pm0.01 \times 10^6$, $9.02\pm0.01 \times 10^6$ and $6.11\pm0.01 \times 10^6$ respectively, while the corresponding mean count of hydrocarbon-utilizers enumerated in control not fortified with poultry droppings was $3.94\pm0.01 \times 10^6$, thus the higher kerosene reduction observed in the treatments. The mean residual kerosene recovered in treatments (TA and TB) and control is presented in Fig. 1, while Fig. 2 revealed the mean residual kerosene recovered in treatments (TC and TD) and control. In both cases, the disappearance of residual kerosene was much more rapid in treatments (TA, TB, TC and TD), especially those supplemented with non-sterile poultry droppings (TA and TC) and differed significantly ($p \leq 0.05$) when compared with the control not amended with poultry droppings. In deed the non-sterile poultry droppings has added additional hydrocarbon-utilizers, thus the much more rapid kerosene disappearance noticed.

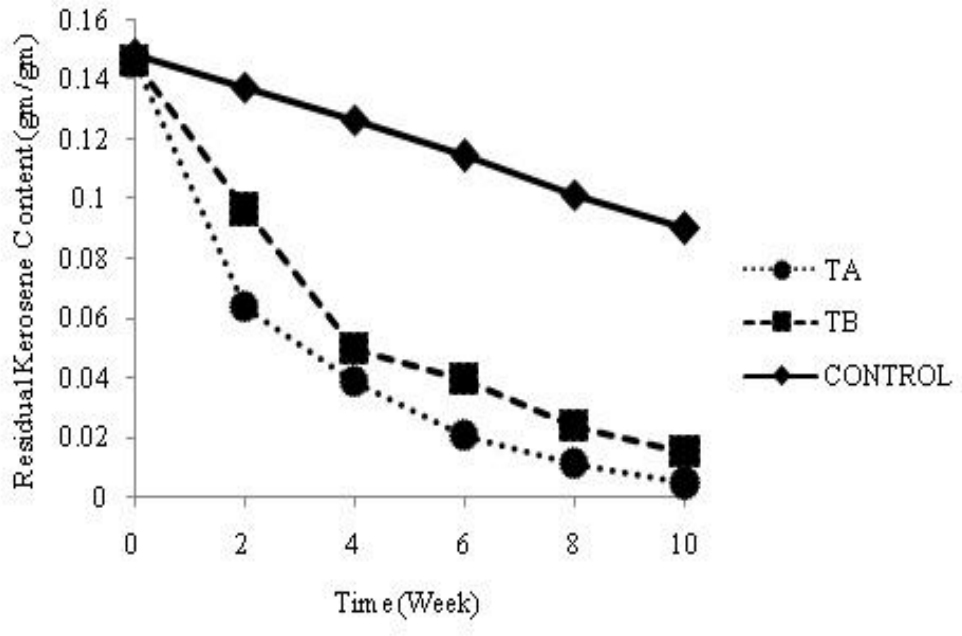

FIGURE 1: Residual kerosene recovered from treatments (TA and TB) and control at two weeks interval for a period of ten weeks.

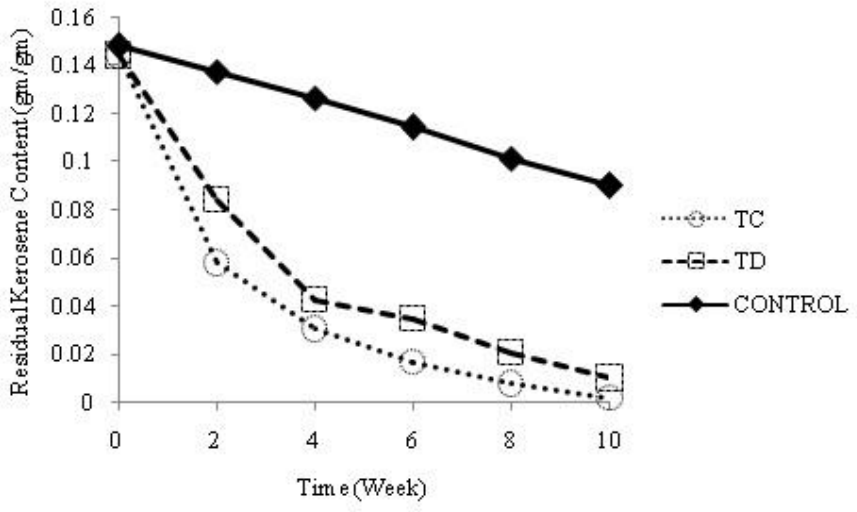

FIGURE 2: Residual kerosene recovered from treatments (TC and TD) and control at two weeks interval for a period of ten weeks.

At week 10, the percent degradations of residual kerosene in TA, TB, TC and TD were 96.81, 89.64, 98.59 and 93.04% respectively at which time the corresponding value obtained for control was 39.16%. We also observed that there were more degradations of kerosene in treatments TC and TD amended with higher quantity of poultry droppings compared with treatments TA and TB. In general, the microorganisms isolated as hydrocarbon utilizers and identified presumptively in this study were species of *Pseudomonas,Bacillus*, *Alcaligenes, Serratia, Micrococcus*, *Acinetobacter, Penicillium*, *Aspergillus*, *Candida*, *Rhodotorula* and *Trichoderma*.

DISCUSSION

Soil and water contaminations by hydrocarbon compounds have increased due to industrialization, accidental and deliberate spill of petroleum products. A major contributor to global environmental pollution problem is the entering of kerosene into our environment through accidental spills, pipe leakage and vandalization, deliberate disposal of oily wastes, corrosion of pipes, kerosene seeps and other operational deficiencies (Ikpeme *et al*., 2007; Kalme *et al*., 2008). Additionally, kerosene spills arising from tank overflow, bunkering and poor vending facilities is almost becoming inevitable in Nigeria as kerosene serves as a major source of energy for cooking and lighting in all sectors of the society (Ikpeme *et al*., 2007).

The low number of indigenous hydrocarbon utilizes in soil and the toxicity of oil pollutant on natural flora could be to an extent the cause of persistence of hydrocarbon pollutants in the environment (Nwachukwu, 2000; Atlas, 1991). Among all strategies to speed up the biological breakdown of hydrocarbons in soil, biostimulation of the intrinsic microorganisms by addition of nutrients is the most frequently used bioremediation technique as the contaminant introduces enormous amount of carbon source which tends to result in rapid depletion of the available nitrogen and phosphorus which are essential for microbial growth (Margesin and Schinner, 2001). In view of this, we investigated the effects of poultry droppings on microbial degradation of kerosene in soil in this study. The physico-chemical properties and microbial loads of soil and poultry droppings as revealed in Table 1 showed that there are essential nutrients in poultry droppings especially nitrate and phosphate necessary for microbial growth. This is in agreement with the work of Adeleye (1991) who documented the presence of nitrate and phosphate in poultry droppings. Additionally, microorganisms such as *Pseudomonas aeruginosa, Bacillus* sp, *Alcaligenes faecalis*, *Serratia* sp, *Penicillium chrysogenum*, *Aspergillus niger*, and *Candida* sp. capable of hydrocarbon utilization were also present in poultry droppings. This is in line with the previous reports by Akinde and Obire (2008) and Obire *et al*. (2008).The initial decrease in both bacterial and fungal population densities (Tables 2 and 3 respectively) observed between weeks zero and two in this study confirmed the toxic impacts of kerosene on indigenous microbial flora which is in accordance with the reports by Nwoko *et al*. (2007), Akoachere *et al*.(2008) and Atlas (1981). Table 4 revealed a steady increase in the population density of hydrocarbon utilizing microorganisms especially in the treatments, thus the higher reduction in residual kerosene observed in the treatments especially those amended with non-sterile poultry droppings as compared to the control (Fig. 1 and 2) and differed significantly ($p \leq 0.05$).The same trend was observed by Calomiris *et al*. (1976) and Nwachukwu (2000), who reported that there is always an increase in the population density of hydrocarbon-utilizers in the ecosystems exposed to crude petroleum and petroleum products. Also, the population densities of hydrocarbon utilizers present in treatments fortified with non-sterile poultry droppings (TA and TC) were higher and significantly different when compared with the treatments amended with sterile poultry droppings (TB and TD) and the control not supplemented with poultry droppings ($p \leq 0.05$). This finding is in accordance with the reports by Obire *et al.* (2008) and Umanu and Nwachukwu (2010). Within the range of quantities of poultry droppings used in this study, our results revealed an increase in kerosene

degradation as the concentrations of poultry droppings applied increases. In conclusion, proper application of poultry droppings especially non-sterile poultry droppings can effectively enhance bioremediation of kerosene polluted soil as it contains essential nutrients such as nitrate and phosphate needed for microbial growth and metabolism as well as significant population of hydrocarbon utilizing microorganisms. Additionally, the use of poultry droppings in bioremediation is also beneficial as it removes and manages poultry wastes thereby reducing environmental pollution.

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