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Biodegradation of Crude Oil Polluted Soil by Co-Composting with Agricultural Wastes and Inorganic Fertilizer

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Abstract.
Pollution of the environment by petroleum products is inevitable due to oil production, transportation and distribution activities. The present study is aimed at examining the extent of bioremediation that can be achieved in crude oil polluted soil after supplementing with organic manure (poultry droppings and goat dung); inorganic fertilizer (NPK 15:15:15) and saw dust respectively.
A bioremediation study was carried out on soil experimentally polluted with Bonny Light crude oil by supplementation with organic and inorganic nutrients (poultry manure, goat dung, saw dust and NPK fertilizer). The efficacy of the treatments was monitored for 112days by the measurement of total hydrocarbon utilizing bacteria load and some physico-chemical parameters. The polluted soil (Control) sample had mean bacterial counts of $8.8x10^4$, $9.0x10^4$, $9.2x10^4$, $9.9x10^4$ and $7.6x10^4$ cfu/g respectively. Sample treated with NPK fertilizer had mean bacterial counts of $4.4x10^4$, $4.6x10^4$, $4.7x10^4$, $4.9x10^4$ and $5.2x10^4$ cfu/g on days 0,28,56,84 and 112 respectively; sample treated with poultry manure had mean bacterial counts of $1.6x10^4$, $1.8x10^4$, $2.0x10^4$, $2.4x10^4$ and $2.7x10^4$ cfu/g on days 0, 28,56,84 and 112 respectively; the saw dust treated sample had mean bacterial counts of $2.0x10^4$, $2.3x10^4$, $2.7x10^4$, $2.9x10^4$ and $3.0x10^4$ cfu/g on days 0,28,56,84 and 112 respectively while that treated with goat dung had mean bacterial counts of $9.3x10^4$, $9.5x10^4$, $9.6x10^4$, $9.8x10^4$and $9.9x10^4$ cfu/g on days 0,28,56,84 and 112 respectively. There were differences in the physico-chemical analyses from the diverse samples. After statistical analysis ($P \leq 0.05$) there was a significant difference between the different treated samples from the control. The results suggest that nutrient supplementation would be effective in the remediation of crude oil polluted soils.
The potentials of various treatment options for the bioremediation of crude oil polluted soils seems to hold the most immediate solution especially for use in areas that would be adversely affected by physical or other removal methods. In this study, the reduction of oil in the treated samples is evident, polluted samples supplemented with fertilizer and poultry manure respectively proved to be the best options during the 112 days study period. This study shows that those organic supplements containing nitrogen and phosphorus have great potentials for the remediation of soils contaminated with petroleum hydrocarbon within a reasonable time.
Keywords: Bioremediation, Crude Oil Polluted Soil, Organic Manure (poultry droppings and goat dung); Inorganic fertilizer (NPK 15:15:15), Saw dust.

1. Introduction

Petroleum products remain the principle source of energy; however, despite its importance and large amounts of usage on land, petroleum products have posed global environmental pollution (Amund, 2000; Plohl et al., 2002; Chikere & Chijioke-Osuji, 2006). In the Niger Delta region of Nigeria, terrestrial and aquatic systems are the main recipients of crude oil spillage, sometimes resulting in large-scale contamination of these environments. Crude oil contamination in this area is gaining more prominence as a result of increased upstream and downstream activities of the petroleum industry hence increased deleterious effect on the ecology of this area (UN Report, 2001.)
The problem of environmental pollution has assumed an unprecedented proportion in many parts of the world (Bank et al., 2003). Soil contamination by petroleum hydrocarbons is one of the world's most common environmental problems (US EPA, 2000). Petroleum products are considered to be recalcitrant to biodegradation and persist in ecosystems because of their hydrophobic nature and low volatility as such, they create a major threat to the environment (Karthikeyah and Bhandari, 2001, Abed et al., 2002, Parrish et al, 2005, Lueprom-Chai et al., 2007;). Contaminants present in soils can enter the food chain and seriously affect animal and human health (Khan, 2005).Total petroleum hydrocarbons (TPHs) are one of the most common groups of persistent organic contaminants (Huang et al, 2005). Therefore, suitable solutions for removal or control of these soil contaminants must be found.
In the past three decades, various physical, chemical and biological methods are suitable for decontaminating relatively small areas while they are expensive to use over large areas such as the ones contaminated by industrial substances, oil products and mining sites (Chekol et al., 2004; Escalante-Espinosa et al., 2005). For example, soil washing, vapor extraction, encapsulation, and solidification/ stabilization have been successful.

These methods are expensive, and may only be partly effective and public pressures may restrict the field utilization of such intensive techniques. In the last two decades, oil spillage have given rise to increased scientific knowledge of behaviour of hydrocarbons and have led to the development of new intervention methods (Chikere and Chijioke-Osuji, 2006) such as bioremediation which have emerged as an effective technology for treatment of hydrocarbon contaminants in soil. A diverse consortium of micro-organisms is capable of degrading a wide range of hydrocarbon molecules. Recent studies on Phytoremediation process is also being used to extract, sequester and detoxify pollutants from the environment. This remediation method is environmentally friendly and visually attractive also the structure of the soil is highly maintained (U.S. EPA, 2000). Also, the use of mycelia of fungi in bioremediation termed Mycoremediation is highly effective (Singh, 2006). It explains why they have been investigated extensively since the mid-1980s for their bioremediation capabilities.

Similarly, Biodegradation is the transformation or breakdown of substances into simpler components through the biochemical reactions of microorganisms such as bacteria, yeasts and fungi although it is often limited by extremes in pH, inadequate concentration of oxygen, nutrients, and high levels of contaminants. Microorganisms involved in the degradation of petroleum hydrocarbons in the environment have been found to be economic, efficient, versatile, as well as environmentally friendly (Margesin & Schinner 1999, Yakubu (2007). Most fungi are generally more tolerant to high concentrations of pollutants. However, complete biodegradation of hydrocarbon mixtures requires a bacterial consortium (Van Hamme et al, 2003). This is because each microorganism has its own specific metabolic capabilities and consequently deficiencies, when presented with a range of structurally unique substrates. The application of bacteria in bioremediation is termed Bioaugmentation; bacterial assemblies may provide a range of metabolic capabilities that cover the full spectrum of reactions required to completely degrade hydrocarbon mixtures and then utilize all of the breakdown products. Therefore, the bacteria benefit from living in association due to synergistic and commensalistic relationships thus faster and more complete biodegradation is possible than by individual species alone.

Of the many remediation methods currently in use, biostimulation is viewed as one of the most promising technologies. Biostimulation involves the use of biological process to return a polluted environment to its original state by increasing the activity of micro-organisms that can degrade the contaminants through the addition of nutrients, oxygen or other electron donors and acceptors (Obire and Anyanwu, 2009; Blaise-Chikere, C. 2012). The actual mechanism involved is biodegradation and it is mediated by about 200 microbial species representing approximately 30 genera of bacteria, yeast and even algae (Yuan et al, 2003; Jop*, et al,* 2008).

The present study is carried out to examine the effect of application of organic manure (poultry dung, goat dung); inorganic fertilizer (NPK 15:15:15) and sawdust on crude oil contaminated soil in order to enhance its bioremediation.

2. Materials & Methods.

2.1 Experimental Layout.

Soil was divided into five treatment cells as presented in Figure 1. Each cell was 2m x 2m.

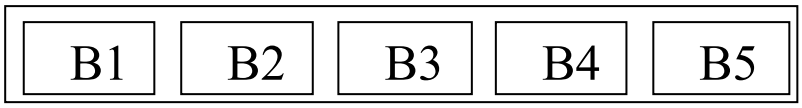

Figure 1: Experimental layout.

Cell B1: Crude oil polluted soil + Dried poultry manure
Cell B2: Crude oil polluted soil + Dried Goat dung
Cell B3: Crude oil polluted soil + Fine saw dust
Cell B4: Crude oil polluted soil + NPK 15:15:15
Cell B5: Crude oil polluted soil. (Control).

2.2 Source of Nutrient Supplements.

The organic nutrient supplement (dried poultry manure) was obtained from the National Root Crops Research Institute, Umudike, Abia State, Nigeria. The dried poultry manure contained 33mg nitrogen and 1.6mg phosphorus per gram. The goat dung was obtained from a commercial livestock farm located in Umuode Nsulu in Isiala Ngwa North L.G.A., Abia State, Nigeria. The sawdust (fine) was obtained from the timber market, in Umuahia, Abia State, Nigeria while the Inorganic fertilizer (NPK 15: 15:15) containing 0.15g N, 0.065g P and 0.125g K was produced by NAFCON (National Fertilizer Company of Nigeria) and obtained from the Ministry of Agriculture and Natural Resources, Umuahia, Abia State, Nigeria.

2.3 Crude Oil Source.

The crude oil used for the study is Bonny light crude obtained from the core analysis laboratory of the Nigeria National Petroleum Corporation (NNPC), Moscow Rd., Port Harcourt, Nigeria.

2.4 Method of Soil Contamination & Treatment.

Twenty litres of Bonny light crude was poured uniformly on each treatment plot including the control to simulate

conditions of a major spill. The plots were left undisturbed for three days, after which the top soil about 3cm depth containing some oil was removed manually to simulate emergency clean up conditions before the treatment applications.

The non in-situ remediation method was adopted as described by Shabir,*et al* 2008 with slight modification. Five wooden porous- bottom boxes (B1, B2, B3, B4 & B5) measuring 30cm by 8cm were constructed and lined with perforated polythene bags. Four of the boxes contained 3kg composite oil contaminated soil sample mixed with 12g of dried poultry manure, dried goat dung, Saw dust and NPK 15:15:15 respectively while the fifth served as control (polluted soil only).Each treatment sample was tilled to mix the nutrient properly with the soil using a long stainless spoon respectively for each sample and after the zero week, samples were taken for analyses. The set-ups were mixed at weekly intervals while exposed to ambient environment conditions. Soil samples were taken every four weeks for analyses, which were carried out in triplicates.

2.5 Microbiological Analyses.

2.5.1 Isolation and identification of bacteria

Determination of heterotrophic bacterial load was carried using ten-fold serial dilution method and plating on oxoid nutrient agar (NA) using spread plate technique and incubated at room temp 28^{o}C for 24hrs. The total heterotrophic bacterial counts for the various samples treatments were taken from Nutrient agar plates. The hydrocarbon utilizing bacterial counts of the diverse samples were taken using mineral salt medium (MSM) and vapour phase transfer method of Thijsse and VanderLinder, (1961). The bacterial colonies were isolated and identified to their species level using conventional microbiological and biochemical tests as described by Cheesebrough (1998) , Taiwo and Oso (2004).

2.6 Physico-Chemical Analyses.

The physico-chemical parameters measured was carried out after all soil samples were air-dried, ground to fine dust and sieved to pass through a 2mm-mesh sieve. Particle size distribution was determined by the hydrometer method (Okalebo et al., 2002), total nitrogen was determined by the micro Kjeldahl method (Bremner & Mulvaney, 1982). Oil and Grease content was determined as described Okolo et al 2005.The color was described using color chart for dry and moist soils; the odor was by perception. The texture was detected using hand feel between the fingers and the structure was determined by soil ability to form clumps while water permeability was examined as described by Victorova, (1986). Total organic carbon was determined by the Walkley and Black, (1934) wet oxidation method as modified by Nelson and Sommers, (1982). Total hydrocarbon content was determined using the method of Chaineau, *et al* (1995). Ten grams of the sieved samples were suspended in 10 ml of water-acetone (50/50-v/v) pH 7.0 solution. Soil suspension was held at room temperature for 2 hours, and then 50 ml of acetone was added. Soil suspension was shaken and centrifuged at a speed of 5000 rpm for 10 minutes. The supernatant was poured into clean, oil free flask. Hexane (50ml) was added to the solid residue and separated after shaking. Water-acetone and hexane layers were mixed together in a separator funnel and extraction carried out with hexane. The extract was collected in a pre-weighed beaker and left to evaporate under the fume-hood overnight. The residue was weighed together with the solvent blank beaker. The difference was calculated as total hydrocarbon content (APHA, 1985).

3. Results & Discussion.

3.1 Bacterial and Fungal Analyses.

The total heterotrophic bacterial count in the control was observed to be low on the first day which is the period of adaptation (Fig. 1). There was a rapid multiplication in bacteria population, reaching a peak at day 84 with a decline at day 112.

The increase in the hydrocarbon-utilizing bacterial counts was as a result of utilization of the hydrocarbon in the crude oil as the source of carbon and energy by these bacteria. The results from the treatment plots when compared with that from the control showed that the increase in hydrocarbon utilizing bacterial counts was due to the various treatments applied as there was no significant difference in the results from the control with time when tested with ANOVA at $P \leq 0.05$.The result of the changes in hydrocarbon utilizing bacterial counts is presented in Figure 2. The count increased consistently in all the treatment options over the study period but there was no increase in the control. Previous reports has it that high numbers of certain oil degrading microorganisms from oil-polluted environment is evidence that these microorganisms are vigorous degraders of the pollutants in the environment (Okerentugba and Ezeronye, 2003). Other reports associated the increase in proliferation of not only hydrocarbon degraders but additional populations that utilize the resultant products from hydrocarbon breakdown (Atlas and Bartha, 1992; Okpokwasili and Nnorom, 1990).

The characterization and identification of fungal isolates are as shown in Table 1. The fungal isolates obtained from this study agree with those obtained earlier from an oil-polluted sample collected around Uzere Flow Station in Warri (Chikere and Chijioke–Osuji, 2006). The fungal genera: *Trichoderma*, *Penicillium* and *Aspergillus* are major hydrocarbon utilizers (Jobson *et al.,* 1972; Nwankwo, 1984; Odu, 1981; Chikere and Chijioke–Osuji, 2006), these were similar to those obtained in the present study.

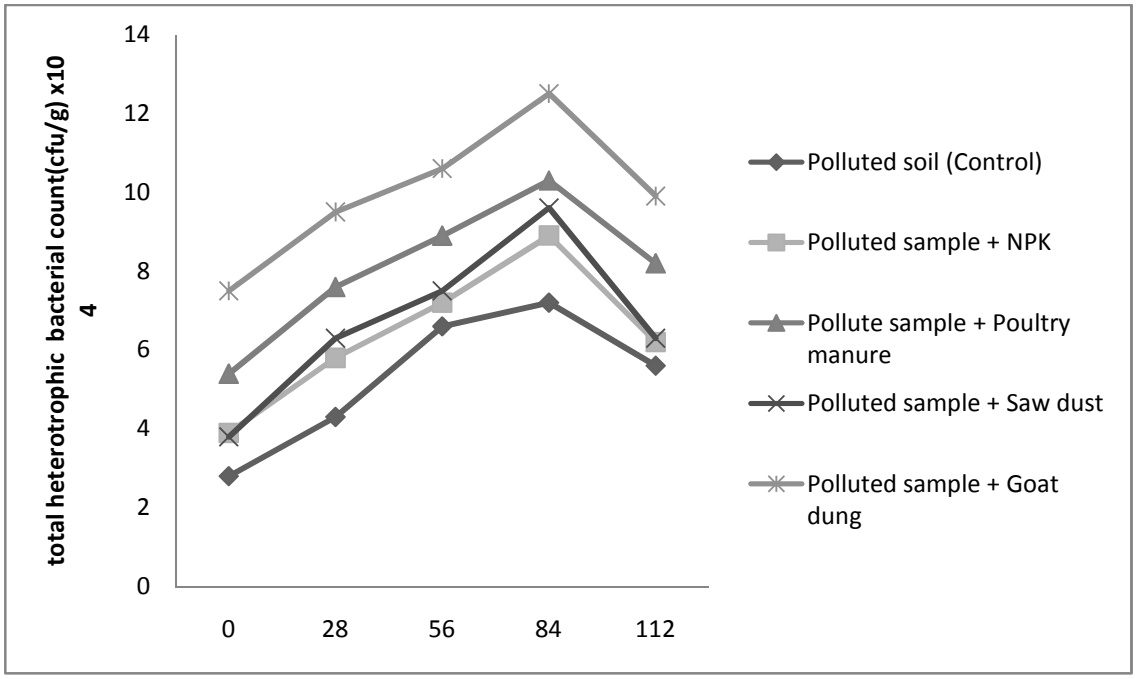

Fig 2. Total Heterotrophic bacterial count in Untreated and treated soil.

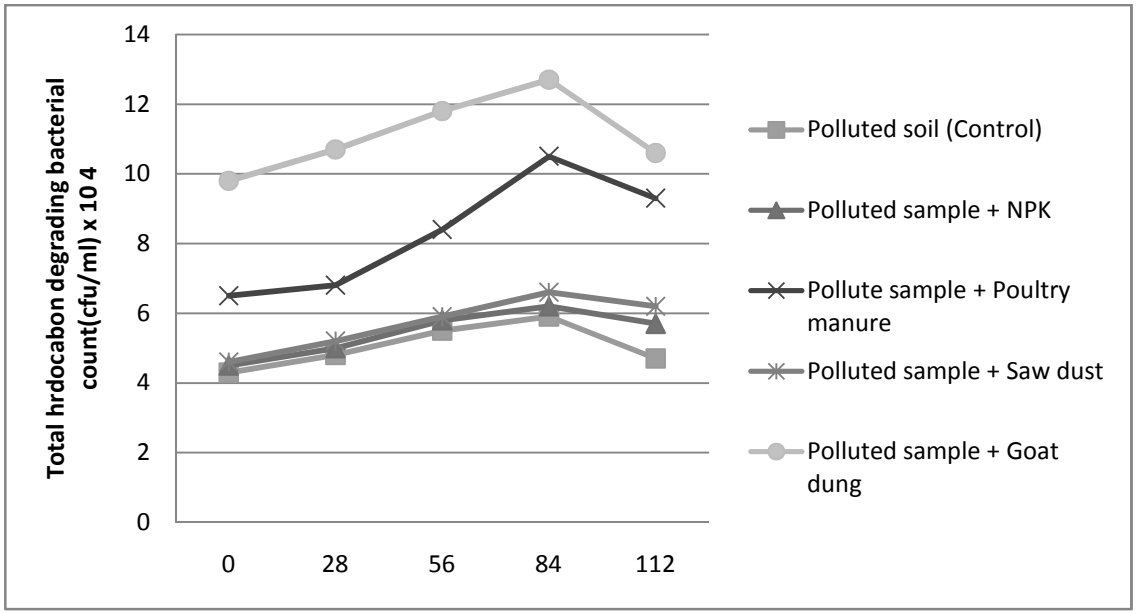

Fig 3. Total Hydrocarbon degrading bacterial count in both Untreated and treated Crude oil polluted soil.

Table 1: Characterization and Identification of Fungal Isolates from Polluted Soil and treated soil

Sample source	Cultural Characteristics	Microscopy	Tentative identification
Polluted soil	White-velvety to powdery with central tuft 3cm in 7days darken after about 2wks	Singly on aerial hyphae or terminally in groups of 2-3 short conidiophores cylindrically often with a slightly swollen base	*Candida* sp
Polluted soil	White & fluffy spreading with irregular spores	Posses sporulating filaments, spores are produced from tips of phialides	*Trichoderma sp*
Polluted soil	Irregular white & fluffy colonies	dark nucleus and spores	*Cladosporium sp*
Polluted soil	white fluffy colonies , diameter 6 cm within 7 days with conidiophore	Densed brushlike, Ellipsoidal – cylindrical conidia dark green in mass. Phialids were often solitay	*Penicillium* sp
Polluted soil	At 25^{o} C ± 2 colonies on PDA showed diameter of 3cm, within 7days consisting a dense felt brownish conidiophores. brown conidial heads split into several loose colums,	Phialides were borne directly on the vesicles. Conidia were often globose –subglobose.	*Aspergillus flavus*
Saw dust + soil	Irregular white & fluffy colonies	dark nucleus and spores	*Cladosporium sp*
NPK + soil,	white fluffy colonies ,diameter 6 cm within 7 days with conidiophore .	Densed brushlike, Ellipsoidal – cylindrical conidia dark green in mass. Phialids were often solitay	*Penicillium* sp
NPK + soil,	White & fluffy with greenish nucleus	Irregular spreading with spores, filaments bound with rope like structures	*Acremonium* sp.
NPK + soil,	White , circular & pasty with raised elevation and a nucleus	Only one-celled conidia	*Geomyces* sp
Poultry dropping	Colonies showed diameter of 3cm within 12-14days,colour was grey-greenish with aromatic odour.	Conidia showed ellipsoid-cylindrical metulae bearing 3-6 slender phialides with dense brush-like spores.	*Penicillium* sp
Poultry dropping	White & fluffy with greenish nucleus	Irregular spreading with spores, filaments bound with rope like structures	*Acremonium* sp.

3.2 Physico-Chemical Analyses.

The results of the Total Organic Carbon content from the sites during the study period are presented in Figure 4. It was observed that while there was a general decrease in the Total Organic Carbon content in the treated samples through out the study period (0-112days), there was no appreciable drop in the control. Apart from the oil utilizing bacteria in animal dung, it has been noted that any other organic matter that can improve the soil texture (Yakubu 2007).

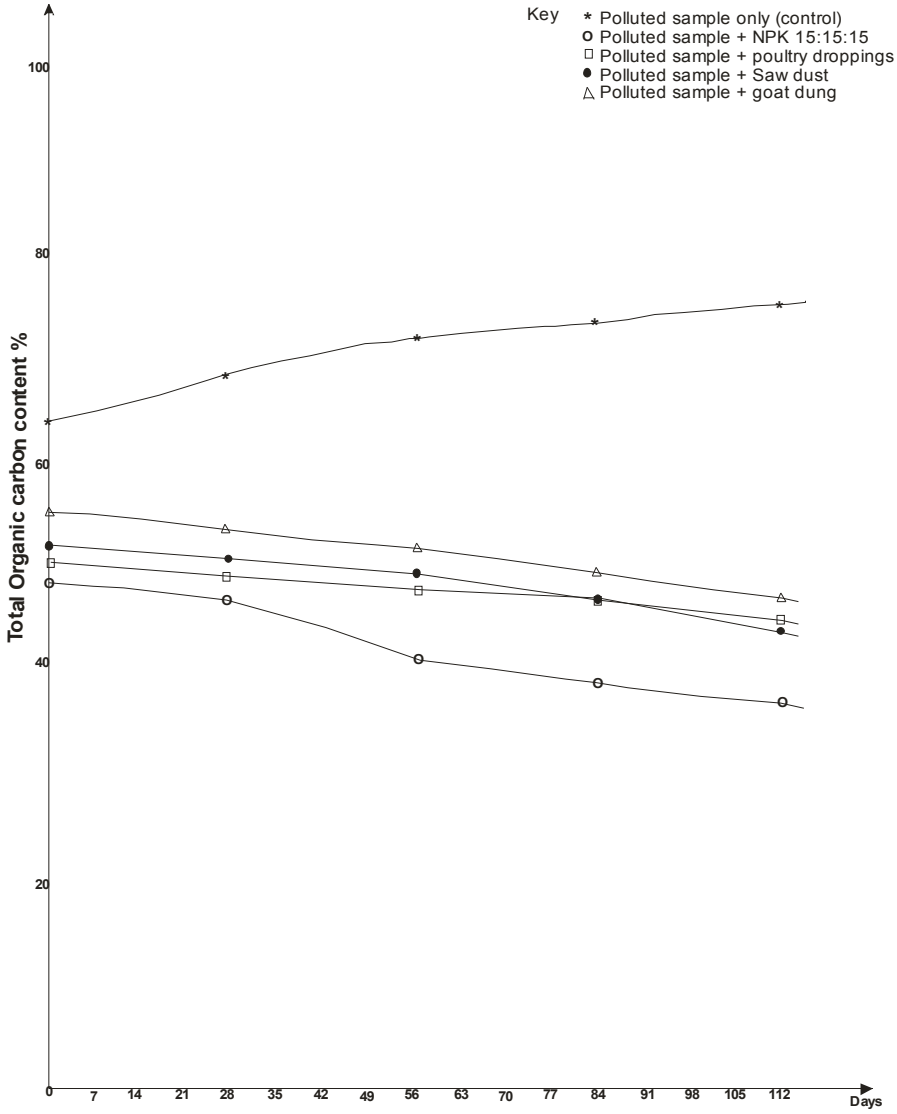

Figure 4: Changes in the total organic carbon content of the various sample over the 112 days study period

Total Nitrogen (N) of various treatment options increased with the period of remediation (Figure 5). However, in the control total Nitrogen value kept reducing. The plots treated with NPK fertilizer had the highest phosphate and nitrogen concentration. Verstracte *et al* (1976) reported that adjustment of C: N: P ratio in an oil-contaminated pod soil was highly beneficial to biodegradation. Poultry manure was observed to have enhanced nitrogen content due to its nutrient composition. It is reported to have a high nitrogen content, which may offset the initial nitrogen deficiency in crude oil-compost mixture (Atagana 2014). Improvement of nitrogen content by composting was least in the sawdust treated crude, this contrasts the findings of Atagana (2014) and may be attributed to the slow rate of breakdown of wood material. Previous studies have again shown that nitrogen is essential for cellular protein and cell wall configuration, while phosphorus is needed for the nucleic acids, cell membrane and ATP formation (Swindell et al., 1988). Therefore, bioremediation of contaminated crude oil sample requires an adequate supply of these elements which in turn are needed by crude degrading microorganisms for their active growth and metabolic performance (Van Hamme et al., 2003). Nitrogen is the most important nutrient element for plants and it correlates with organic carbon indicating that the reserve of this element is mostly from organic carbon (Okusami, 1980), also the total content of phosphorus in soil is important for plant nutrition and is represented by its organic and mineral compounds; the phosphate level in the control is below the mineral level for crop growth especially maize (Agboola and Obegbesan, 1974).

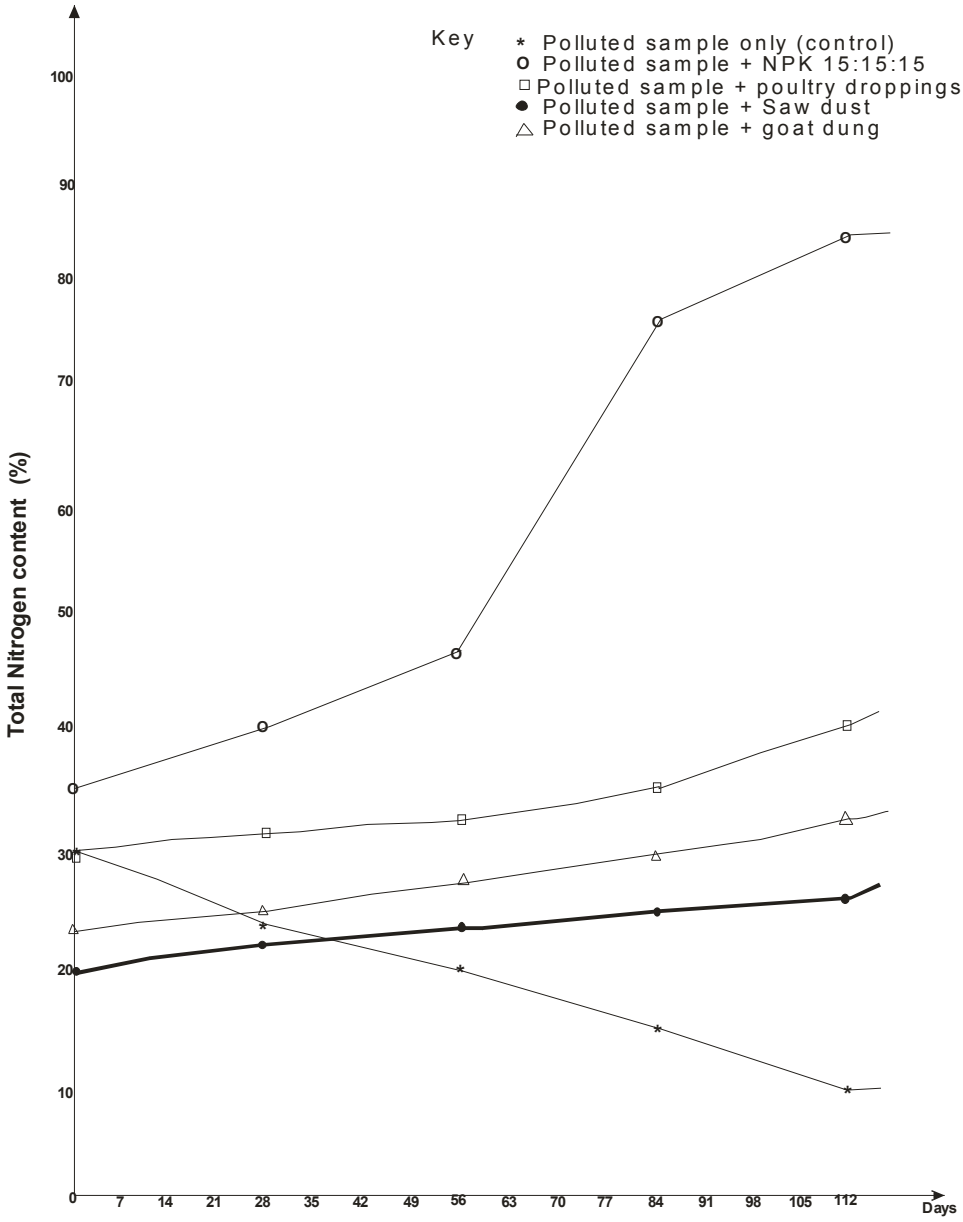

Figure 5: Changes in total Nitrogen content of the various samples over the 112 days study period

There was a marked decrease in total hydrocarbon content in all the treatment options except the control. The rate of hydrocarbon loss is shown in Figure 6. Poultry dropping best supported reduction rate of hydrocarbon (60.7%) with its peak at day 112 than saw dust and goat dung treatments while NPK least supported the hydrocarbon. However, the degradation rate was low in the first week of the experiment with all treatments. The experimental design of this work (simulating soil contamination) rather than use of age long contaminated soil encouraged the inherent usage of hydrocarbon. The plots treated with NPK fertilizer had the highest phosphate and nitrogen concentration. This agrees with findings of Agarry et al .,(2010) who simulated soil contamination with mixed petroleum products and got up to 39% hydrocarbon reduction, similarly other authors (Ijah and Antai, 2003, Okolo *et al* 2005) had reported degradation of crude oil using poultry manure.

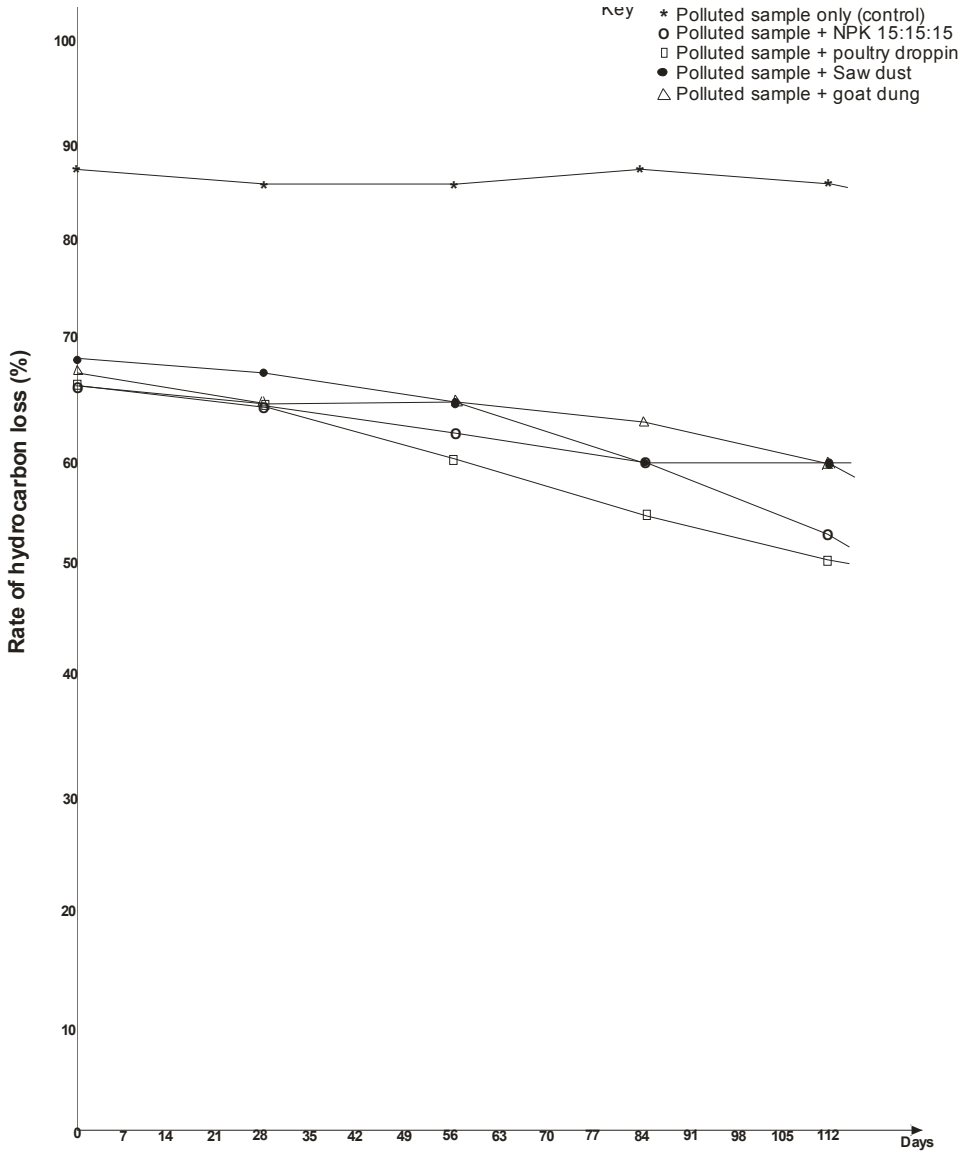

Figure 6: Changes in Rate of hydrocarbon loss from the various samples over the 112 days study peri od.

Figure 7 shows the changes in the oil and grease content of the sites during the study period. It was observed that the oil and grease content decreased from day 0 to 112 due to the various treatments while the control showed no significant variation at $P \leq 0.05$. After the first 28days poultry manure was only able to reduce oil and grease at rate of 27.3% but it later showed the best support for degradation of crude oil contaminated soil with 88% O&G reduction. However the least activity was found in Polluted soil + saw dust treatment. The decrease in oil and grease content could be attributed to utilization of hydrocarbon by increased bacterial counts. The control plot did not show significant reduction of O &G.

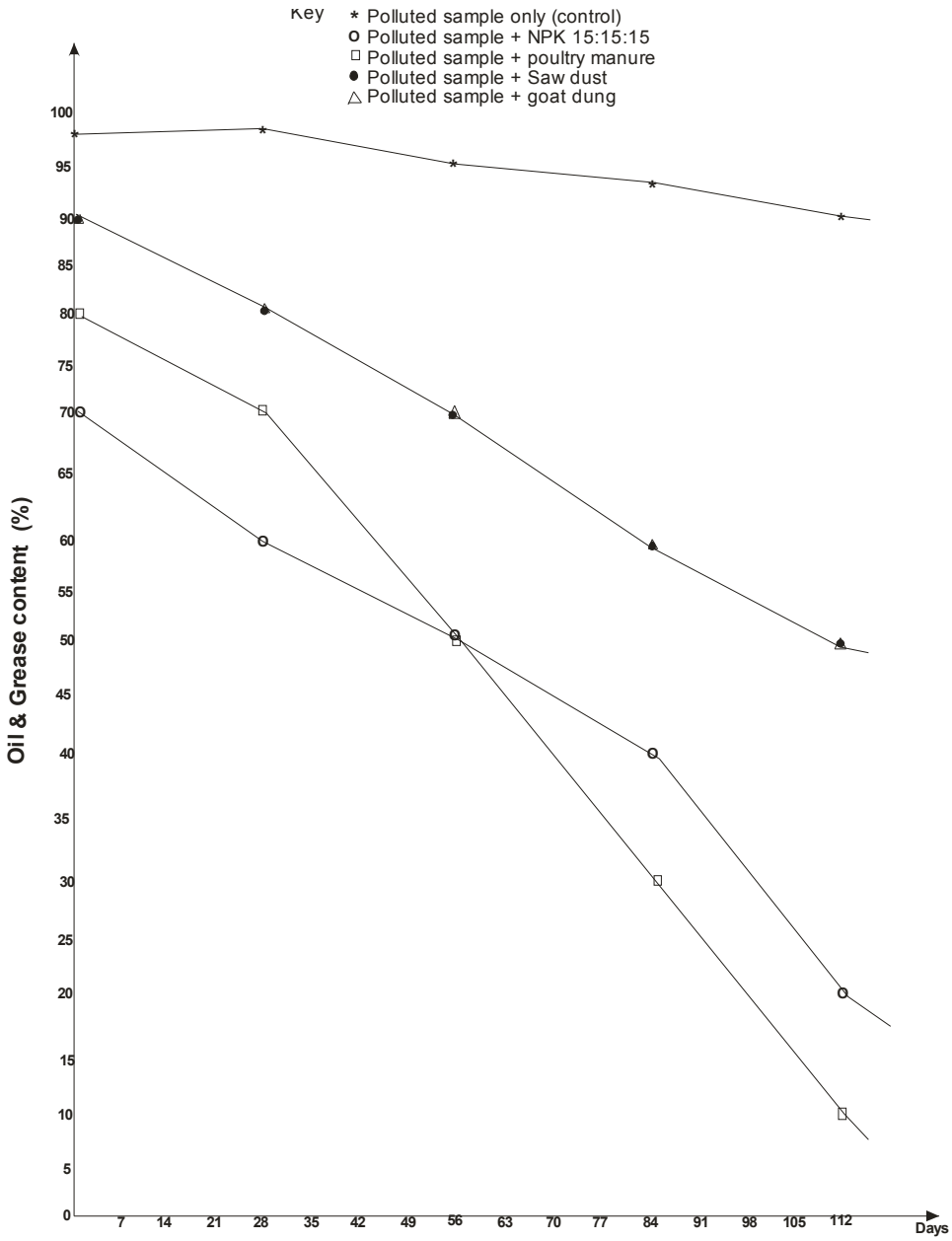

Figure 7: Changes in oil and grease content of the various samples over the 112 days study period.

The potentials of various treatment options for the bioremediation of crude oil polluted soils seems to hold the most immediate solution especially for use in areas that would be adversely affected by physical or other removal methods. In this study, the reduction of oil in the treated samples is evident; polluted samples supplemented with fertilizer and poultry manure proved to be the best options during the 112 days study period. This corroborates with previous works done respectively by Adieze *et al*, (2003); Odokuma and Dickson, (2003); Nweke and Okpokwasili, (2004); Eze, (1998); Atuanya and Ibeh, (2004).

Victorova, (1986) emphasizes that the granular composition of a soil depends on the number and size of its mechanical elements or particles after all the compounds holding them together have been destroyed. The polluted sample has fine grains while the treated samples have bound grains; this is as a result of degradation of the soil in the polluted area. Water permeability being the capacity of a soil to allow water through, is a function of its texture, aggregation and swelling; the soil in polluted area has lost its colloid state and coagulating property thus its lack in permeability. This is expressed in Table 2.

Table 2: Physical characteristics of the control and treated soil samples.

	Water permeability	Odour	Texture	Structure	Particle size(mm)	Colour
Polluted soil (Control)	Negative	Crude oil	Fine/loose	Evident peds Not durable	0.5-1.0	Black/brown
Polluted soil + NPK	Positive	Not distinct	bound	durable	1.2	Undefined
Polluted soil + Poultry manure	Positive	Not distinct	bound	durable	1.2	Black
Polluted soil + saw dust	Positive	Not distinct	bound	durable	1.2	Undefined
Polluted soil + Goat dung	Positive	Not distinct	bound	durable	0.9	undefined

- All results represent average of triplicate experimental samples

4. Conclusion.

This study shows that those organic supplements containing nitrogen and phosphorus have great potentials for the remediation of soils contaminated with petroleum hydrocarbon within a reasonable time. In addition, climatic conditions play an important role in accelerating the rates of biodegradation. Treatment of polluted soil with nutrient supplements would result in bioremediation of such soils over time; that is, the use of the right types and quantities of nutrients and provision of favorable environmental conditions for the growth of the oil-eating microbes.

The use of poultry manure yielded the greatest degree of bioremediation in this study; it is also a cheap method to use.

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