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Bioremediation of Hydrocarbon Contaminated Soil Using Organic Wastes as Amendment

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ABTRACT The soil and aquatic environments in most under developed and developing Countries are usually contaminated by diesel. The negative impact of petroleum products cannot be overemphasized due to its harmful effect on human life as well as the environment. An alternative Green technology is bioremediation, the remediation of such hydrocarbon contaminated soil. It mainly involves biostimulation where organic or inorganic components are introduced to enhance indigenous microbial growth that directly degrades the contaminants. The aim of this paper was to identify potential organic wastes in enhancing the biodegradation of used diesel in contaminated soil. To achieve the objective, cow dung and goat manure were selected as the organic components to be added individually into the 10% (w/w) diesel-contaminated soil. Each set up with 1.5kg of diesel-contaminated soil were added with 10% of the organic matter (cow dung and goat manure) and left for degradation for 28 days in plastic vessels. Periodic sampling of soil from each vessel was carried out at 7 days interval , physicochemical analysis, isolation and enumeration of bacteria were done. Results indicated that after 28 days of exposure to the organic matters, biodegradation of diesel in the soil were much higher than that of the control set-ups. Cow dung amended set-ups showed 26% biodegradation while goat manure amendment gave23%, as compared to the control set-up (16%). This might be as a result of the presence of additional nutrient in the organic matter amended soil which enhanced the capabilities of the indigenous microorganisms degradation. The biodegradation rate of the two organic matters differed due to the differences in the nutrient content, particularly of available nitrogen and phosphorus. In addition, cow dung amended-soil was found to have improved soil physiochemical characteristics that enabled speedy adaptation by the microbes to the contaminated soil. Statistical analysis indicated a significant difference at $P<0.05$ .The total heterotrophic bacterial counts were as follows: cow dung amended soil, $2.25\text{-}6.4\times10^{7}$ cfu/g while goat manure amended soil $2.1\text{-}5.7\times10^{7}$ cfu/g. On the other hand, the control set ups was within this range ($1.1\text{-}2.3\times10^{6}$cfu/g).Cow dung and goat manure can be effective organic amendments for the biodegradation of diesel contaminated soil. Both organic matters proved to enhance the multiplication of indigenous microbes thus enabling rapid biodegradation of the contaminant in the soil.

Keywords: Bioremediation, cow dung, goat manure, diesel

Introduction

Government and Scientists have been showing keen interest in oil pollution in aquatic and terrestrial systems due to discharge of large amounts of oil in the water bodies and soil (Vidal, 2010). The remediation technique might be as simple as introducing certain chemicals into the system, to a highly complex process involving numerous chemical and biological processes. This is typically relevant to areas polluted with pesticides, metal and metalloid contamination, petroleum pollution, etc (Floch *et al*., 2011; Li *et al*., 2013). Soil pollution has been described to be the most threatening of all the life support systems due to the fact that pollutants have the capacity to affect the native organisms that reside in the soil as well as do away with the food chain (Floch *et al*., 2011; Moreno *et al*., 2009).

Petroleum contamination of the environment is mostly caused due to leakage of petroleum hydrocarbon during handling process which has a great impact on the ecosystem (Williams and Odokuma, 2013). Among all, hydrocarbons that exist, diesel has a great potential to resist its degradation because of the nature of its composition. The carbon member of diesel oil hydrocarbon ranges between 11 and 25 and the distillation range is between 180ºc and 380ºc, which chemically constitute of 46% cycloalkanes, 24% n-alkanes, and 30% aromatics (Gavrilescu *et al*., 2010). As the use of petroleum product is increasing, it is a potential threat to the environment. The conventional technique used for remediation includes digging up contaminated soil and removing it to landfill (Agarry, 2010). There are several ways to remove hydrocarbon from the soil and they include: bioaugmentation, biostimulation, mycoremediation, phytoremediation, biosparging, bioventing and composting. For over 30 years, bioremediation has been the most common method used for hydrocarbon removal. Bioremediation is effective, economic and ecofriendly; it leads to the complete mineralization of hydrocarbon. Among others, research has shown that the use of animal manure which includes poultry manure, goat manure, cow manure (dung) has been effective in the cleaning up of soil contaminated by petroleum products (Agarry *et al*., 2010).

Biostimulation is the addition of substrates, vitamins, oxygen and other compounds that stimulate microorganisms' activities so that they can degrade the petroleum hydrocarbons faster. Stimulation of microorganisms by the addition of nutrients brought large quantities of carbon sources which tend to result in a rapid depletion of the available pools of major inorganic nutrients such as Nitrogen and Phosphorus (Sang-Hwan *et al*., 2007). An example of this is the addition of fertilizer to an oily wastewater. This works by supplying nutrients that are limiting the growth of the bacteria for the oil contaminated wastewater such as nitrogen and phosphorous. With this addition, the organisms can rapidly degrade the oil utilizing it as the carbon source and the fertilizer as the nitrogen and phosphorous source .Combinations of inorganic nutrients are often more effective than single nutrients where a low level of macronutrients and a high level of micronutrients were required to stimulate the activities of indigenous microbes (Sutherland *et al*., 2000 ; Liebeg *et al*., 1999).

Organic waste utilization

Addition of nitrogen and phosphorus to an oil polluted soil has been shown to accelerate the biodegradation of the petroleum in soil (Ijah *et al*.,1997). It was reported that 18.7% and 31.2% higher crude oil biodegradation was obtained from soil amended with chicken droppings and fertilizer, respectively, compared to un-amended control soil after 10 weeks. Degradation of crude oil in soil amended with melon shells as source of nutrients was 30% higher than those of un-amended polluted soil after 28 days (Ijah *et al*., 1997; Abioye *et al*., 2009). The rate of pollutant degradation is enhanced by the addition of a carbon source as a nutrient in contaminated soil and this stimulates the growth of microorganisms responsible for biodegradation of the pollutant. Therefore, utilization of organic waste in the bioremediation of soil seems a highly potential area of interest. This will reduce the amount of organic waste sent to landfill, thus reduce the emission of landfill gases and also provide a cheap source of organic additive for the remediation purpose. Nevertheless, extensive research is crucial in order to identify suitable organic wastes to be utilized in the soil bioremediation. Therefore, the aim of this study was to identify potential organic wastes in enhancing the biodegradation of used diesel in contaminated soil.

Materials and Methods

Organic waste collection

Cow dung and goat manure were collected from Dickson and Sons Company Limited farm in Enugu State and used as the organic components added individually into the 10% (w/w) diesel-contaminated soil. Used Diesel was collected from Chinda filling station located close to Rivers State University of Science and Technology Round-about Mile 3 in Port Harcourt, Rivers State.

Microcosm set-up

Top soil (0-15cm) depth was collected from a farm within Rivers State University of Science and Technology farm located in Port Harcourt, using a ditch auger and bulked to form a composite sample. About 1.5kg of soil (sieved with 2mm mesh sieve) was placed in plastic vessels labeled A, B, C and D. The soil was then polluted with 10% (w/w) used diesel (150g) and left undisturbed for two (2) days. Vessel A (an extra control) consisting of autoclaved soil and 0.5% (w/w) sodium azide (NaN_3) was set up to observe the used diesel in the soil as a result of non-biological factors. Vessel B served as control containing only soil and used diesel. After two days, 10% of each organic waste viz Cow dung (CD) and Goat Manure (GM) were individually supplemented into the oil polluted soil labeled C and D respectively and mixed thoroughly. The moisture content was adjusted to 60% by the addition of water, and the content was tilled for aeration three times a week throughout the experimental period. Triplicates of the plastic vessels were incubated at room temperature (30 ± 2°C).

Determination of physicochemical properties of samples

Methods for the determination of physicochemical properties of samples (cow dung, goat manure, diesel polluted and non-polluted soil) were used as outlined by APHA (1985). The pH meter used was pocket-sized HANA pHep + HI 98108 with automatic temperature compensation. Conductivity values were determined using conductivity meter (Jenway 4010 UK.).Total organic carbon (TOC) was determined by dichromate wet oxidation method of Walkley and Black as modified by Dhyan *et al*., (1999). Nitrate content was determined using the macro Kjeldahl digestion method of Brady and Weil (1999) and available phosphorus was determined using the method reported by Olsen and Sommers (1982). Sulphate was determined using the turbidometric method while total hydrocarbon content (THC) was determined by the partition gravimetric method. Potassium was determined using flame photometric method.

Total Petroleum Hydrocarbon (TPH) Determination

Residual hydrocarbon contents of the soil samples were determined by toluene cold extraction method of Adesodun and Mbagwu (2008). 10 g of soil sample was weighed into 50 ml flask and 20 ml of toluene (AnalaR grade) added. After shaking for 30 minutes on an orbital shaker (model N-Biotek-101M), the liquid phase of the extract was measured at 420 nm using DR/4000 Spectrophotometer. The total petroleum hydrocarbon (TPH) in soil was estimated with reference to a standard curve derived from fresh used lubricating oil diluted with toluene. TPH data were fitted to the first-order kinetics model: $C = C\ o\ e - k\ t$, (1) where C is the hydrocarbon content in soil (g kg^{-1}) at time t , $C\ o$ is the initial hydrocarbon content in soil (g kg^{-1}), k is the biodegradation rate constant (d^{-1}), and t is time (d).

Enumeration and Identification of soil bacteria

Periodic sampling from each oil polluted soil was carried out at 7 days intervals for 28days for the enumeration of total Heterotrophic Bacteria (THB). Samples were diluted and an aliquot from each sample was placed on nutrient agar medium (Oxoid) for isolation of THB with the addition of 50µg/ml nystatin to suppress the growth of fungi. Plates were incubated at 30°C for 24 hours before the colonies were counted. Hydrocarbon utilizing bacteria (HUB) in the soil samples were enumerated using mineral salt medium as described by Mills *et al*. (1978) and modified by Okpokwasili and Amanchukwu (1988). The agar plates were incubated at 30°C for 5-7 days before the colonies were counted. The bacterial colonies were randomly picked, and pure culture was obtained by repeated sub-culturing on nutrient agar (Oxoid) and mineral salt agar plates. The bacterial isolates were characterized using microscopic techniques (Gram staining) and biochemical tests.

Statistical Analysis

Results were analyzed using analysis of variance (ANOVA) and means were compared for significance at $p \leq 0.05$ using Duncan's multiple range analysis.

Results

Results of the physicochemical analysis

The physicochemical properties of the soil and organic waste used for bioremediation are shown in Table 1.

Table 1: Physicochemical Properties of Soil and Organic Wastes used prior Bioremediation.

Parameter	**Soil**	**Cow Dung (CD)**	**Goat Manure (GM)**
pH	5.90	7.21	7.10
Nitrogen (%)	0.18	0.56	0.45
Phosphorus (mg/kg)	8.35	635.46	455.20
Potassium (mg/kg)	70.10	78.6	72.90
TOC (%)	4.35	9.85	7.8
Moisture (%)	3.20	16.50	16.50
C: N	30:1	16:1	17:1

Biodegradation of used diesel

The biodegradation of used diesel in soil throughout the period of study (28 days) are shown in Fig. 1. The results showed high biodegradation of used diesel at the end of 28 days with soil amended with organic wastes compared to the control soil treatment.

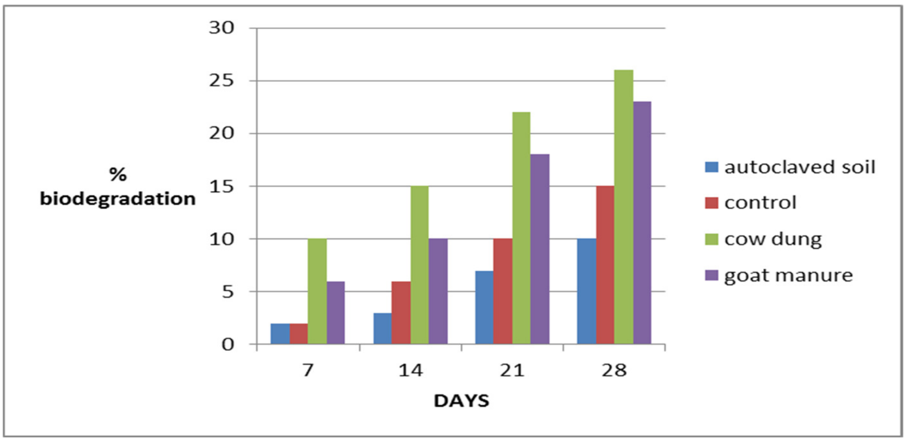

Fig 1: Percentage biodegradation of petroleum hydrocarbon in soil contaminated with used diesel and amended with organic wastes

Microbial count

The counts of total heterotrophic bacteria (THB) in soil contaminated with used diesel oil and amended with CD ranged from 2.25 x 10^7 CFU/g to 6.4 x10^7 CFU/g while that of contaminated soil amended with GM ranged from 2.1 x 10^7 CFU/g to 5.7 x 10^7 CFU/g (Fig. 2). The un-amended control soil had the count of THB ranging between 1.1 x 10^6 CFU/g and 2.3 x 10^6 CFU/g. Higher THB counts in diesel oil contaminated soil amended with CD might be due to the presence and bioavailability of more N and P into the soil that contributed to the stimulation of the microbial flora in the soil. The presence of the macro and microelements in the CD served as a source of nutrient for the increase and maintenance of the microorganisms (Lee *et al*., 2003).

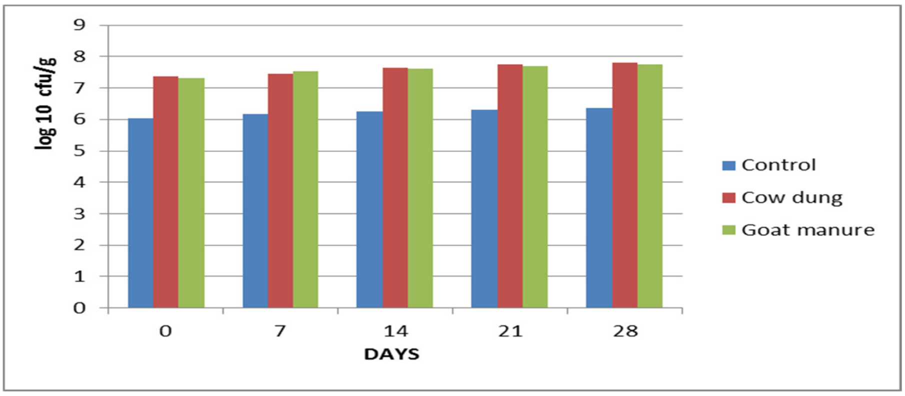

Fig 2: Counts of Total Heterotrophic Bacteria population in soil contaminated with used diesel amended with organic wastes.

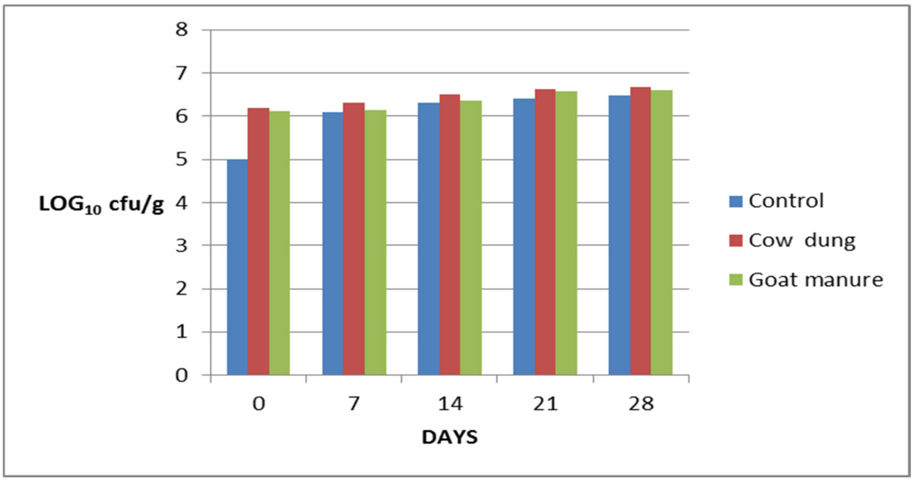

Fig 3: Counts of Hydrocarbon Utilizing Bacteria population in soil contaminated with used diesel amended with organic wastes.

Discussion

The initial soil pH value used for the bioremediation experiment was quite acidic in nature (5.90). Since the survival of most microbial species is dependent on a certain pH range, soil pH is essential. Moreover, availability of nutrients can be affected by soil pH. At the end of 28 days, used diesel contaminated soil amended with CD showed the highest percentage of oil biodegradation with 26% followed by soil amended with GM which is 23% compared to the un-amended control soil that showed 16% of biodegradation of oil at the end of 28 days. Diesel contaminated soil amended with organic wastes have greater oil biodegradability compared to un-amended control soil in this study.

The main difference of oil biodegradation between the soil amended with organic wastes and un-amended soil treatment occurred during the 14-28 days, where biostimulation resulted in significant increase of oil biodegradation. The addition of nutrients stimulates the degradative capabilities of the indigenous microorganisms thus allowing the microorganisms to break down the organic pollutants at a faster rate (Ausma *et al*., 2002).

In this study, the diesel contaminated soil amended with CD recorded the highest oil biodegradation of 26% compared to GM which was 23%. The reason for the results obtained might be due to differences in the nutrient contents, particularly Nitrogen and Phosphorus in the two organic wastes in stimulating the native microbes. Cow Dung (CD) with the highest concentration of Nitrogen and Phosphorus (Table 1) explained its highest enhanced biodegradation of used diesel in the soil compared to GM. Nitrogen and Phosphorus are known as most important nutrients needed by hydrocarbon utilizing bacteria to carry out effective and efficient activities of biodegradation of xenobiotics in the soil environment. Similar results were observed in hydrocarbon contaminated soils amended with poultry and pig manure compost (Adesodun, 2008).

There was 10% oil biodegradation in autoclaved soil. This biodegradation might be due to non-biological factors like evaporation or photo-degradation. The addition of sodium azide to the autoclaved soil completely sterilized and poisoned the microorganisms left in the soil, thus, its biodegradability was low in comparison to all other treatments. From the statistical analysis, there was significant difference at $P<0.05$ between the amended soil and the un-amended polluted soil in the treatments showing the positive contribution of organic wastes to the biodegradation of used diesel oil in the soil.

The presence of significant quantities of nitrogen and phosphorus in the organic wastes especially high nitrogen content required for biodegradability activities of bacteria resulted in higher counts of THB and HUB in the CD and GM amended soil compared to the un-mended polluted soil (Adedosun, 2008) . The higher microbial counts (Fig. 2 and Fig. 3) in used diesel oil contaminated soil amended with CD and GM is accompanied by significant oil biodegradation (Fig. 1) which indicates that the native soil microbes utilized a portion of the carbon supplied by the diesel fuel as a potential nutrient source.

Higher counts in THB and HUB might as well be due to the abilities of the organic wastes to neutralize the toxic effect of the oil on the microbial population by rapidly improving the physicochemical characteristics of the soil (Floch, 2011). The organic wastes might have helped in improving the soil aeration, thus providing adequate oxygen required by the microbial community which as a result favored the growth of indigenous bacteria in the soil.

Conclusion

Bioremediation could be a feasible and efficient response to soil contamination with petroleum hydrocarbons. Biodegradation of used diesel oil was enhanced positively by the amendment of organic wastes (CD and GM). From the study, it was observed that cow dung and goat manure have high nitrogen and phosphorus content which are known as most important nutrients needed by hydrocarbon utilizing bacteria to carry out effective and efficient activities of biodegradation of xenobiotics in the soil environment. Due to the high content of these nutrients, hydrocarbon utilizing bacteria (HUB) counts were 10% higher in all organic wastes amended soil compared to the un-amended control soil throughout the period of study. Hence, Cow dung and Goat Manure can be harnessed and used as bioremediating agents in polluted sites.

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