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Biodegradation of Spent Lubricating Engine Oil in Soil Using Organic and Inorganic Amendments

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Abstract

The potentials of organic (Eggshell and Plantain peel) and inorganic (NPK) amendments as biostimulating agents of autochthonous microflora for hydrocarbon biodegradation used singly and in combination were investigated in microcosms containing soil spiked with spent lubricating oil (3%wv^{-1}) and amended with 12% of Plantain peel (P), Eggshell (E) and NPK (N) singly and in combination. The rates of biodegradation of the spent lubricating oil were studied for a remediation period of 42days under laboratory condition. At the end of the biodegradation, the highest percentage of oil biodegradation (81.7%) was recorded in soil amended with NPK and the least percentage of biodegradation (14.4%) was recorded in autoclaved soil without amendment. Hydrocarbons utilizing bacterial counts were high in the amended soils ranging from 1.04×10^5 to 1.89×10^7cfu/g than those in unamended and autoclaved control soil that ranged between 2.7×10^3 to 1.72×10^6cfu/g. There was a positive correlation between the microbial growth biodegradation rate in the presence of Eggshell, Plantain peel and NPK singly and in combination in each of the microcosms. The biodegradation data fitted well into first order kinetic model used to determine the rate of biodegradation of spent lubricating oil and revealed that NPK has the highest biodegradation constant of 0.040day^{-1} and half-life of 17.3days. The genera of hydrocarbon utilizing bacteria found were *Acinetobacter, Micrococcus, Enterobacter, Staphylococcus, Proteus, Corynebacterium, Nocardia, Bacillus* and *Pseudomonas.* The use of organic amendments is efficient, environmental friendly, cost-effective and can complement the use of more expensive inorganic fertilizer in the remediation of hydrocarbon contaminated soil.

Introduction

Petroleum-based products are a major source of energy for industries and daily life. However, leakages and accidental spills that occur regularly during the exploration, refining, transportation and storage create serious environmental problems. The contamination associated with such leakages and accidental spills has become a global concern with potential consequences on ecosystem and human health (Onwurah *et al*., 2007). Furthermore, the rise in consumption of petroleum-based products such as automotive lubricating oil is fast becoming a worldwide problem, resulting in large volume of used oil and its waste (Koma *et al*., 2003).

Keywords: Biodegradation, spent lubricating oil, biostimulation, amendments

Despite efforts in some countries to recover and recycle these used motor oils, significant amount of lubricants are still deposited into the environment (Battersby, 2000). Contamination of the soil by spent lubricating oil is prevalent in oil producing and industrialized countries of the world. However, the problem is more severe in developing countries where there are no effective regulatory policies on the environment (Onuoha *et al*., 2011). Like any other developing country, Nigeria is faced with similar challenges. The high demand for petroleum and associated products during the last ten decades coupled with ineffective regulatory policies has made environmental pollution inevitable consequence.

While complete eradication of these problems is difficult to achieve, the aim is often to minimize ecological ruin (Nwachukwu, 2003). Many remedial approaches have been explored to clean up polluted soils. Physical, chemical and biological methods are being employed in the remediation of polluted soils (Okoh, 2006; Erdogan and Karaca, 2011). However, the physical and chemical methods are associated with high cost, very laborious and often achieve incomplete removal of the contaminants from the environment (Eckenfelder and Noris, 1993), producing undesirable ecological consequences (Adebusoye *et al*., 2007) therefore, biological method is preferred. The process relies upon microbial enzymatic activities to achieve the cleanup process of the contaminants from the environment (Philip *et al*., 2005). Biodegradation describes the process of using microorganisms to remove hazardous components of waste from the environment (Dua *et al*., 2002). Biodegradation is favoured as a good option for the remediation of polluted sites mainly because it uses inexpensive equipment, environmentally friendly and simple. However, lack of essential nutrients such as nitrogen and phosphorus are a major factor affecting biodegradation of hydrocarbon by microorganisms in soil and water environment. Therefore, addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Okolo *et al*., 2005; Okpokwasili, 1994; Olabisi *et al*., 2009).

Studies have reported various nutrient sources such as inorganic fertilizer, Urea, sawdust, compost manure and biosolids (Cho *et al*., 1997; Namkoong *et al*., 2002). Mushroom compost and spent mushroom compost (SMC) has been applied in treating organo pollutant contaminated sites (Eggen, 1999; Trejo-Hernandez *et al*., 2006; Lau *et al*., 2003). Organic wastes like Banana skin, spent mushroom compost and brewery spent grain were found to enhance the biodegradation of used lubricating oil up to 90% loss of oil within the period of 3 months (Abioye *et al*., 2009b, 2010). According to Dadrasnia and Agamuthu (2013), there was 39%, 42% and 58% total petroleum hydrocarbon (TPH) reduction in soil amended with tea leaf, potato skin and soy cake in soil amended with 10% diesel oil after 56 days. In other studies, bioremediation of hydrocarbon contaminated soil was reported to be enhanced in the presence of poultry manure (Okolo *et al*., 2005), composting process (Boudiella *et al*., 2007), cassava peels and poultry dropping (Chikere *et al*., 2009; Ebere *et al*., 2011). Thus, the study was aimed at examining the efficacy of plantain peel, Eggshell and NPK as biostimulating/amendment agents in remediating spent lubricating oil polluted soil.

Material and Methods

Study Site

Sites of sample collection for this study were randomly selected around University of Ibadan, South Western, Nigeria.

Sample Collection

Soil sample was randomly collected from four points at the Department of Microbiology, University of Ibadan, with hand-dug soil auger at a depth of 0-15 cm. NPK fertilizer (12:12:17) was obtained from the International Institute for Tropical Agriculture (IITA), Ibadan. The spent lubricating oil, eggshell and plantain peels were obtained in University of Ibadan environ and were immediately taken to the laboratory for analysis.

Physicochemical Analysis

The pH, total nitrogen, phosphorus, potassium, organic carbon, moisture, texture (sand, silt, clay) and exchangeable cation (K^+, Ca^{2+}, Mg^{2+}, and Na^+) were determined according to methods from APHA (1998).

Soil Microcosm Study

The soil was spiked with 3% (w/v) spent lubrication oil and thoroughly mixed together to achieve complete artificial contamination. After 3days of post contamination, the amendments were added. The moisture was maintained at 60% water holding capacity by the addition of distilled water. The experiment was set up in triplicate. The experimental set up was kept and monitored for 42 days. The design of the experiment is as described below:

Biodegradation Experimental Design

Treatments	Details of Treatment
A	100g soil +3.0% oil + 12%(w/w) P
B	100g soil +3.0% oil + 12%(w/w) E
C	100g soil +3.0% oil + 12%(w/w) N
D	100g soil +3.0% oil + 6%(w/w) PE
E	100g soil +3.0% oil + 6%(w/w) PN
F	100g soil +3.0% oil + 6%(w/w) EN
G	100g soil +3.0% oil + 4%(w/w) PEN
H	100g soil +3.0% oil (Unamended soil)
I	100g(autoclaved soil) + 3.0% oil

Where E = Eggshell, P = Plantain peel, N = NPK, PE= Plantain peel + Eggshell, PN= Plantain peel + NPK, EN= Eggshell + NPK, PEN= Plantain peel + Eggshell + NPK.

Determination of Bacterial Counts

Heterotrophic bacterial counts were determined by plating serially diluted soil samples on Nutrient agar using the spread plate technique of Odokuma and Ibor (2002). Hydrocarbon utilizing bacteria in the soil sample were enumerated using modified mineral salt medium of Mills *et al*. (1978).

Isolation of oil degrading bacteria

The vapour phases transfer method (Amanchukwu *et al*., 1989) was used for the isolation. Filter paper (Whatman No.1) saturated with sterile spent oil was aseptically placed on mineral salt medium and incubated at (28 ± 2^0C) for 7 days. Colonies of different hydrocarbon utilizing bacteria were randomly picked and sub-cultured severally on the basis of their colonial characteristics to obtain pure culture. The pure cultures were identified using the method in Bergey's Manual of Determinative Bacteriology (Holt *et al*., 1994).

Determination of Total Hydrocarbon Content

The hydrocarbon content in the amended and unamended soil was determined gravimetrically by toluene cold extraction method of Adesodun and Mbagwu (2008). Soil sample (10g) was weighed into 50ml flask and 20ml of toluene was added to extract the hydrocarbon in the soil. After shaking for 30minutes on an orbital shaker, the mixture was allowed to stand for 10minutes and then was filtered through Whattman no.1 filter paper. The liquid phase of the extract was measured at 420nm absorbance using a spectrophotometer. The total hydrocarbon content in the soil was estimated with reference to standard curve that was derived from fresh spent oil diluted with toluene. Percent degradation (D) was calculated using the formula:

$$D = \frac{TPHi - TPHr}{TPHi} \times 100$$

Where, TPH_i and TPH_r are the initial and residual TPH concentrations respectively.

Bioremediation Kinetics and Half-Life

The total hydrocarbon content obtained was fitted into the first order kinetic model of Yeung *et al*. (1997).

$$Y = ae^{-kt}$$

Where, Y = residual hydrocarbon content in soil (mg/kg)
a = initial hydrocarbon content in soil (mg/kg)
k = biodegradation rate constant (day^{-1})
t = time (days)

The model was used to estimate the biodegradation rate and half life of the hydrocarbons in soil relative to treatments applied. Half life was calculated from the model of Yeung *et al*. (1997) as: Half life, $t_{1/2} = \frac{1n2}{k}$

This model is based on the assumption that the degradation rate of hydrocarbon positively correlated with the hydrocarbon pool size in soil (Yeung *et al.*, 1997).

Determination of the Efficiency on Individual Amendments Applied to the Soil

Assessment of the efficiency of individual amendment applied to the spent lubricating oil contaminated soil was evaluated by determining the "net percentage loss" due to the individual amendment.

It was calculated thus:

Net % loss = (% loss in THC of oil polluted soil with amendment) – (% loss in THC of unamended polluted soil).

The effectiveness of each biostimulation agents was therefore tested. Through evaluation of unamended soil microcosm (natural attenuation) and amended soil microcosm (biostimulation), biostimulant efficiency (B.E) was calculated at the end of the day-42 remediation period using the equation according to Zahed *et al.* (2011):

$$\%B.E = \frac{\%TPH(S) - \%TPH(U)}{\%TPH(S)} \times 100$$

Where; %B.E = Biostimulant Efficiency; $\%TPH_{(S)}$ Is the Removal of Used Oil in the Amended Soil, and $\%TPH_{(U),}$ the Removal of Used Oil in the Unamended Soil.

Results

Physicochemical Properties of Soil and Organic Wastes

The physicochemical properties of the soil and organic amendments (Plantain peel and Eggshell) used in this bioremediation study are presented in Table 1 below. The pH of organic amendments and the soil were approximately neutral. The soil had a low concentration of Nitrogen, Phosphorus and Carbon of 0.81%, 0.67% and 1.22% respectively. The Carbon to Nitrogen ratio (C: N) of the soil used was 3:2. The organic amendments were also low in Nitrogen, Potassium and Carbon concentration. However, the Phosphorus content was very high. The inorganic fertilizer used in the bioremediation studies had an NPK value of 12:12:17 and some trace amounts of MgO, S and Ca. The NPK value of the inorganic fertilizer was higher than both the soil and organic amendments.

Table 1: Physicochemical properties of soil and organic amendments

Parameters	Soil	Eggshell	Plantain peels
pH	6.9	7.4	6.9
Nitrogen (%)	0.81	0.62	1.22
Phosphorus (mg/kg)	6.71	836.55	686.4
Organic carbon (%)	1.22	0.95	11.63
Calcium (%)	0.25	44.8	1.03
Potassium (cmol/kg)	0.57	0.13	0.91
Moisture (%)	11.1	1.0	4.7
Magnesium (cmol/kg)	1.29		
Sand (%)	87.8		
Silt (%)	3.4		
Clay (%)	8.8		

Bacterial Counts

The total heterotrophic bacteria counts are presented in figure 1 below. All the additives increased the microbial counts during the period of the study as compared to that of the unamended control except for the autoclaved soil that had a lower microbial population compared to that of the unamended control. The hydrocarbon utilizing bacteria counts had a similar trend to that of the heterotrophic bacteria count (Figure 2).

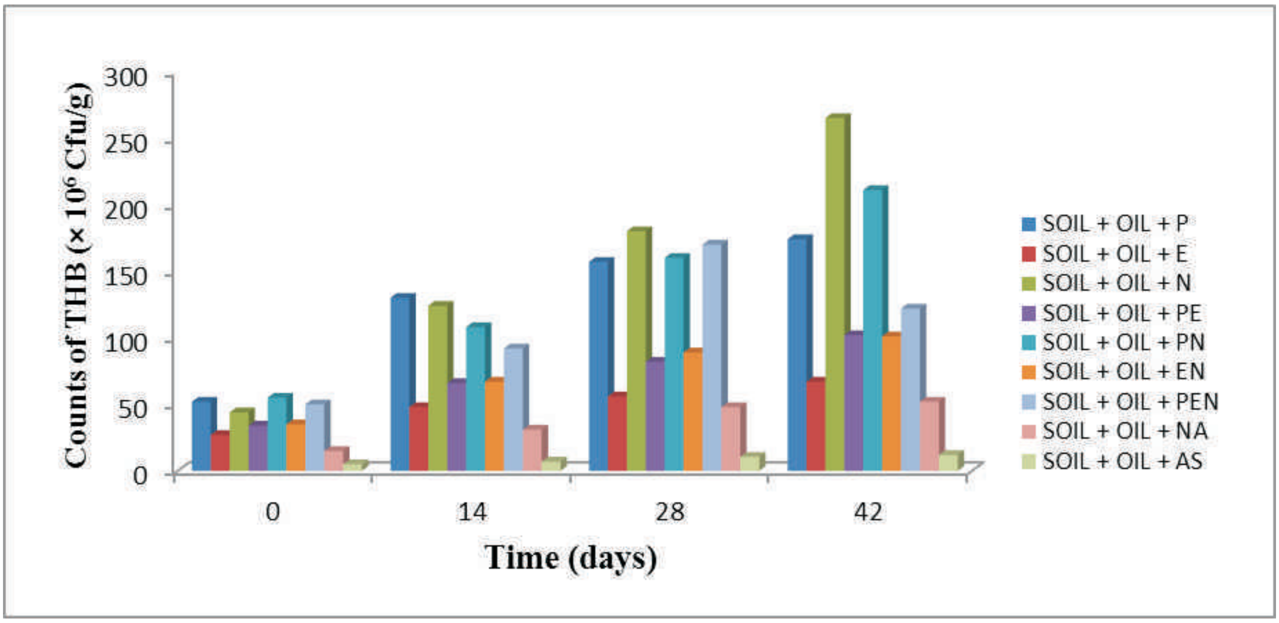

Figure 1: Total Heterotrophic Bacteria in soil spiked with 3% spent lubricating oil and amended with Plantain peel, Eggshell and NPK

Key:Plantain peel and Eggshell (PE); Plantain peel and NPK (PN); Eggshell and NPK (EN); Plantain peel, Eggshell and NPK (PEN); Un-amended control soil (NA); Autoclaved soil (AS)

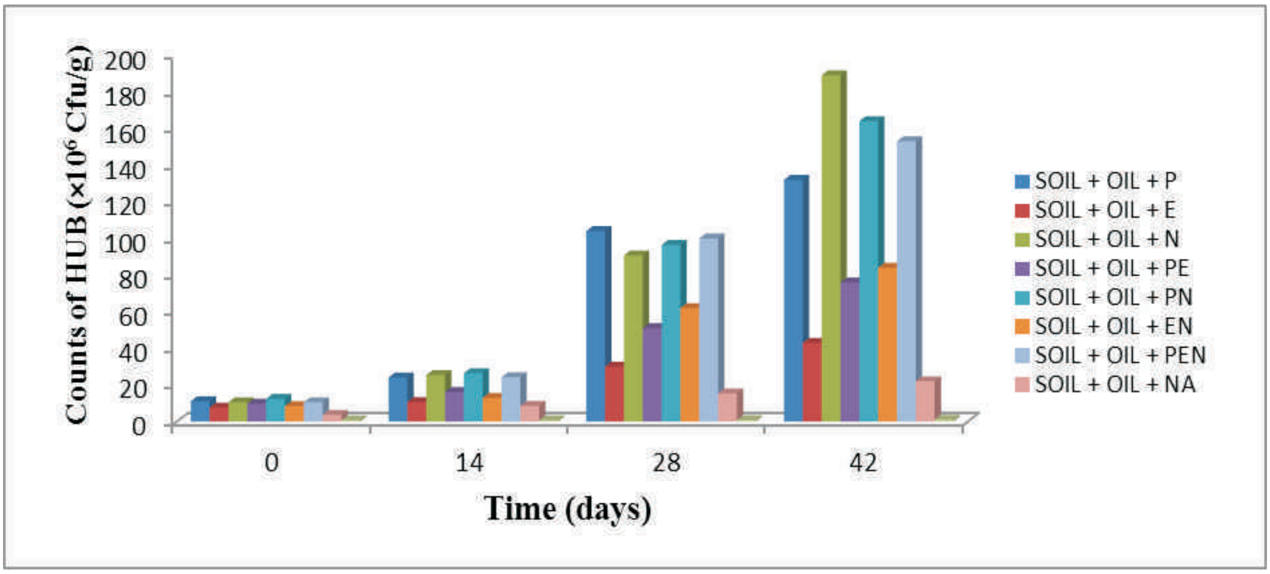

Figure 2: Hydrocarbon-utilizing bacteria (HUB) in soil contaminated with 3% spent lubricating oil and amended with Plantain peel, Eggshell and NPK.

Key:Plantain peel and Eggshell (PE); Plantain peel and NPK (PN); Eggshell and NPK (EN); Plantain peel, Eggshell and NPK (PEN); Un-amended control soil (NA); Autoclaved soil (AS)

Biodegradation Pattern of Spent Lubricating Oil in Soil with Amendments

The level of degradation of spent lubrication oil with and without amendments throughout the study period is presented in figure 3. There was a marked reduction in the total hydrocarbon content within the period of the study with the addition of the organic amendments and inorganic amendment both singly and in combination. From observation, the rate of degradation in both amended and

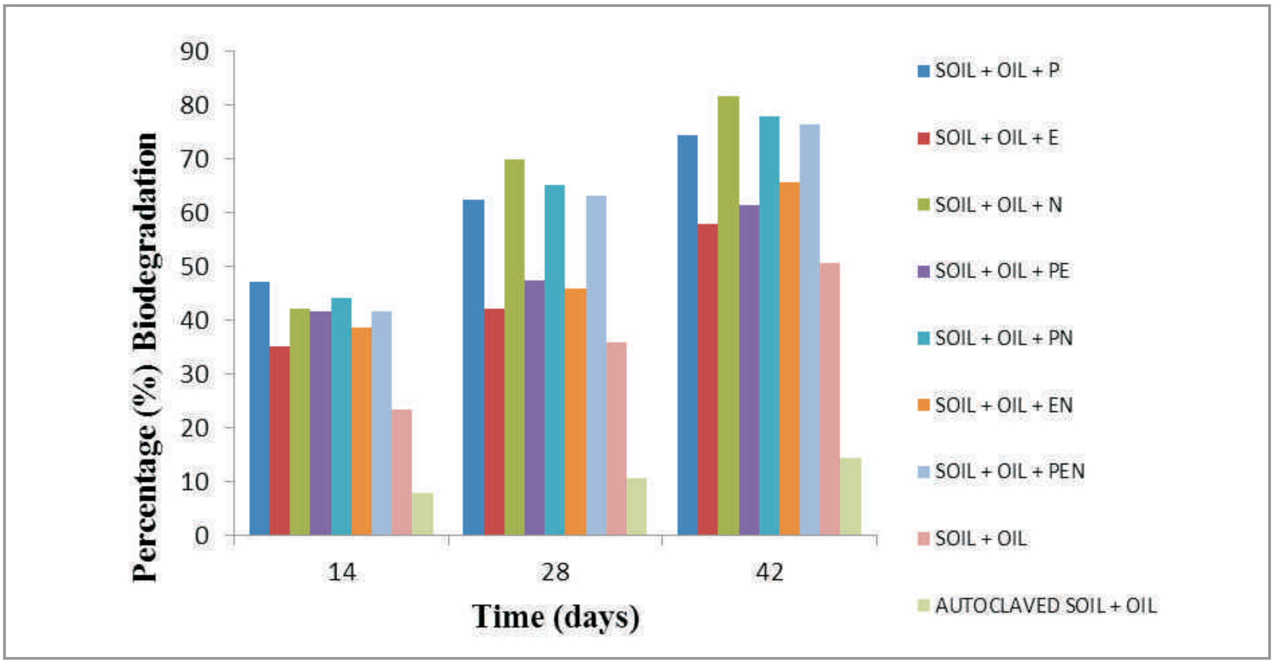

Figure 3: Biodegradation of petroleum hydrocarbon in soil contaminated with 3% spent lubricating oil amended with Plantain peel, Eggshell and NPK singly and in combination.

Key:Plantain peel and Eggshell (PE); Plantain peel and NPK (PN); Eggshell and NPK (EN); Plantain peel, Eggshell and NPK (PEN); Un-amended control soil (NA)

unamended soils at the end of the study was N>PN>PEN>P>EN>PE>E>NA>AS. The net percentage loss of spent lubricating oil in the contaminated soil indicated the effectiveness of each treatment (table 2).

Table 2: Net Percentage Loss of Total Petroleum Hydrocarbon on Soils during Bioremediation

Treatments	14th day	28th day	42nd day
P + 3% SPO	23.7	26.5	23.6
E + 3% SPO	11.7	6.20	7.30
N + 3% SPO	18.7	34.0	31.0
PE + 3% SPO	18.2	11.6	10.7
PN + 3% SPO	20.8	29.2	27.3
EN + 3% SPO	15.3	9.90	15.0
PEN + 3% SPO	18.3	27.3	25.8

Key: P = Plantain peel, E = Eggshell, N = NPK, PE = Plantain peel + Eggshell, PN = Plantain peel + NPK, EN = Eggshell + NPK, PEN = Plantain peel + Eggshell + NPK and 3% SPO = 3% of Spent Lubricating Oil.

Biodegradation Rate Constant and Half-Life of Hydrocarbon in Soil Spiked with Oil

The biodegradation rate constant (k) and half-life ($t_{1/2}$) (Table 3) for the different treatment soil amended with NPK (N) has the highest biodegradation rate constant of 0.04 day^{-1} and lowest half-life of 17.3 days at 3% concentration of spent lubricating oil as data for the sampling periods were combined before this model could be used. Autoclaved soil (AS) had the lowest biodegradation rate constant of 0.0037 day^{-1} and highest half-life of 187.2 days.

Efficiency of the Amendments Applied to the Soil

The efficiency of the amendments in degrading the hydrocarbon at the end of 42 days is shown in table 4. The bio-stimulant efficiency (% BE) lies between 12.6% and 37.9%. There were relative variations in the bio-stimulation efficiency of the different amendments with NPK fertilizer, N having highest B.E (37.9%) followed by PN (35.0%), PEN (33.7%), P (31.8%), EN (22.8%), PE (17.4%) and E (12.6%) respectively.

Table 3: Biodegradation Rate Constants (k) and Half-life ($t_{1/2}$) for Spent Lubricating Oil Contaminated Soil

Microcosm Code	Biostimulation Treatment	k (day^{-1})	$t_{1/2}$	Coefficients of determination (R^2)
P	Plantain peel	0.032	21.7	0.994
E	Eggshell	0.021	33.0	0.952
N	NPK fertilizer	0.040	17.3	0.948
PE	Plantain peel, Eggshell	0.023	30.1	0.948
PN	Plantain peel, NPK fertilizer	0.036	19.3	0.981
EN	Eggshell, NPK fertilizer	0.025	27.7	0.930
PEN	Plantain peel, Eggshell, NPK fertilizer	0.034	20.4	0.981
NA	Unamended soil	0.017	40.8	0.997
AS	Unamended autoclaved soil	0.037	187.2	0.991

Table 4: Percent Degradation of Spent Lubricating Oil and Biostimulants Efficiency after 42 days

Microcosm Code	Biostimulation treatment	% degradation	BE (%)
NA	Unamended soil	50.7	-
P	Plantain peel	74.3	31.8
E	Eggshell	58.0	12.6
N	NPK fertilizer	81.7	37.9
PE	Plantain peel, Eggshell	61.4	17.4
PN	Plantain peel, NPK fertilizer	78.0	35.0
EN	Eggshell, NPK fertilizer	65.7	22.8
PEN	Plantain peel, Eggshell, NPK fertilizer	76.5	33.7

Discussion

The soil used for the bio-stimulation had C/N ratio of 1.5:1 which is a low C/N ratio for effective biodegradation of oil in the soil, hence the need for addition of organic and inorganic amendments as a source of nutrient. Addition of nutrients is a standard practice for increasing hydrocarbon degradation (Atlas and Bartha, 1998). By adding nutrients, the C/N and C/P ratios of the soil are brought closer to the bacterial C/N and C/P requirements. Rolling *et al*. (2002) reported that stimulated biodegradation of hydrocarbon in soil amended with 2.5g of Nitrogen, N per kilogram gives C/N ratio greater than 300. According to Okoh (2006) and Kim *et al*. (2005), Nitrogen, (N) is an important limiting agent for effective bioremediation to take place. Thus, knowledge of bioavailability of nutrients is necessary in the planning of an efficient bioremediation strategy. The addition of bulking agents to soil has been reported to increase oxygen diffusion and mineral nutrient availability as well as carbon source quality and mechanical support surface for bacterial adsorption, and improve soil physicochemical characteristics so as to speed up microbial adaptation and selection (Jorgensen *et al.,* 2000, Molina-Barahona *et al*., 2004).

Okpokwasili (1994) observed that the use of NPK fertilizer and poultry droppings effectively stimulated bacterial growth into utilization of crude oil. Agarry and Jimoda (2013) reported that the use of plant residues and animal dung used alone or in combination improved the rate of petroleum hydrocarbon degradation in contaminated soils. Similar observations have been reported in the use of starch, glucose, plants and animal organic wastes in the biodegradation of petroleum hydrocarbons in soil (Teng *et al*., 2010; Ibiene *et al*., 2011; Agbor *et al*., 2012; Nduka *et al*., 2012; Abioye *et al*., 2012). In this study, NPK fertilizer has relatively higher biostimulation efficiency as compared to the organic amendments. In contrast, Agarry and Jimoda, (2013) reported that a combination of organic amendments (animal dung wastes and plant residues) had a higher biostimulation efficiency than the inorganic (NPK fertilizer). However, this was subject to the amount of the organic and inorganic nutrients that was used in the remediation process.

The hydrocarbon-utilizing bacteria genera identified in this study include *Bacillus, Pseudomonas, Micrococcus, Proteus, Nocardia, Corynebacterium,* *Acinetobacter, Enterobacter* and *Staphylococcus.* These bacteria species has been implicated in hydrocarbon degradation by several researchers (Abioye *et al*., 2009a; Onuoha *et al*., 2011; Agbor *et al*., 2012; and Onuoha, 2013).

This present study revealed that the rates of oil degradation in the soil increased with time. The amended hydrocarbons in the polluted soils degradation rate as compared to the unamended control and autoclaved soil. This could be attributed to the nutrients present in the organic and inorganic amendments which might have been released easily into the soil by the oil degraders. It is possible that hydrocarbon utilizers inherent in the amendments have also played a role in the biodegradation process (Williams *et al*., 1999; Onuoha, 2013). It is worthy to note that the observed reduction in spent lubricating oil may not only be attributed to the biodegradation process induced by nutrient additions and hydrocarbon utilizers but other processes such as volatilization, adsorption to organic compounds and other abiotic factors. This is seen in the case of the unamended controlled soil and the autoclaved soil where 50.7% and 14.4% reduction respectively were observed. The coefficient of determination reveals that the first order kinetic model used in this study fits with high correlation coefficient that lies between 0.948 and 0.997. The first order degradation kinetics has been reported in earlier studies based on petroleum hydrocarbon degradations (Namkong *et al*., 2002; Abioye *et al*., 2012; Dadrasnia and Agamuthu, 2013). According to Agarry and Jimoda (2013) the higher the biodegradation rate constant, the higher the rate of biodegradation and the lower the half-life of degradation as observed in this study.

Conclusion and Recommendation

This study showed the potentials of locally available organic wastes as nutrient supplements for remediation of soil contaminated with spent lubricating oil. The decrease in total hydrocarbon content after degradation is an index that the application of organic wastes was effective in laboratory scale of hydrocarbon contaminated soil. Although application of NPK fertilizer was better, notwithstanding,l other amendment regimes both singly and in combination showed potential in remediation of spent lubricating oil contaminated soil. The application of biostimulation strategy can reduce the duration of remediation and cost. However, the success and efficiency of such biostimulation technique vary considerably and vary from one site to another since there is no universal soil treatment regimen for the bioremediation of all petroleum hydrocarbon-contaminated soils. The effectiveness of any soil treatment applied for such purpose has to be evaluated on a case-specific basis.

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