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Full Length Research Article

STIMULATED BIOREMEDIATION OF SOIL CONTAMINATED WITH SPENT ENGINE OIL USING ORGANIC WASTES

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ABSTRACT
In this study, the role of some organic wastes in stimulating the removal of petroleum hydrocarbon from contaminated soil was assessed. The concentration of hydrocarbon in soil before, during and after bioremediation and volume of carbon dioxide released from soil were investigated. The contaminated soil was amended with chicken droppings (CD), cow dung (CDU), and rice husk (RH) for sixty (60) days under laboratory conditions. The soil was mixed thoroughly every ten (10) days to enhance aeration, and 60% water holding capacity (WHC) of the soil was maintained to supply bacteria with adequate moisture. The initial pH value of soil was 4.7, while CD, CDU and RH had values of 6.9, 7.1 and 7.6 respectively. Water holding capacity (WHC) of contaminated soil was 15%, CD showed 31%, CDU (43%) while RH had 40.7%.The percentage of nitrogen in soil was very low (0.92%) compared to Organic carbon (10.5%), and Phosphorous (19.2mg/kg).CD showed the highest value of nitrogen (2.4%) and phosphorous (17.6mg/kg) followed by CDU with 1.7%;17.1mg/kg and 0.89%;15.21mg/kg in RH. Highest concentration of organic carbon (11.4%) was recorded in RH; CDU had 10.9% while CD was with 10.1%. Concentration of spent engine oil in soil before bioremediation was 7.525mg/g. The initial count of Hydrocarbon degrading Bacteria (HDB) in contaminated soil was 5.7×10^5CFU/g, and species of HDB isolated from soil were *Acinetobacter, Bacillus, Micrococcus, Flavobacterium, and Pseudomonas.* Total petroleum hydrocarbon in soil amended with CD was reduced by 86.7%, CDU and RH treated soils showed 80.05% and 66.75% reduction respectively, while untreated soil recorded lowest reduction (46.8%) at the end of 60days period. Largest volume of carbon dioxide was evolved from soil amended with CD (15.77cm^{-3}).Soil treated with CDU produced 14.02cm^{-3}, and RH soil had 11.72cm^{-3}. Lowest volume (3.78cm^{-3}) of carbon dioxide was produced from untreated soil. This result demonstrated that these organic wastes could stimulate biodegradation of spent engine oil in the order CD>CDU>RH.

Keywords: bioremediation, soil, engine oil, organic waste.

INTRODUCTION
Increase in the use of automobile engines and machines has resulted into rise in use of lubricating (engine) oil hence increase in rates of environmental contamination by used engine oil (Abioye *et al*., 2012).Used engine oil contains harmful substances such as heavy metals, aliphatic and aromatic hydrocarbons, Benzenes as well as sulphur and nitrogen (Mohammed *at el*., 2011). These substances cause negative effects to the soil and soil micro flora such as abridged growth rate and reproduction, destitute health and mutagenesis, thus alter population dynamic, disrupt tropical relations and edifice of natural groups within ecosystems (Samuel *et al*., 2015).

The spent oil is generated when the engine oil is exposed to high temperature and mechanical stress as the engines perform their function (Agarry *et al.,* 2010). Spent oil is disposed into the environment in high amount during manual oil changing operation either intentionally as in the case of automobile and generator mechanics or accidentally through spill. The oil is dumped into water bodies, farmlands and open vacant plots use as mechanic workshops hence polluting both soil and water (Ikhajiagbe and Anoliefo, 2011). Osubor and Anoliefo (2003) conveyed that approximately 20 million gallons of spent oil are accumulated every year from mechanic workshops across Nigeria and disposed carelessly into the environment. They further reported that oils are release into the milieu from the exhaust systems during engine operations as well as engine leaks.

The chemical impurities in spent engine oil are toxic and can cause cancer and mutation in living cells (Udeani*et al.,* 2009; Ajao *et al.,* 2011).These chemicals seep into the water table, contaminate the ground water and subsequently get into the human body through the food web from plants (Adams *et al.,* 2014) . The spent engine oil floats as a scum on the surface hence prevent sunlight and oxygen from penetrating the water thereby affecting water animals like fishes, frogs, crabs and water plants (Agarry and Oladipupo, 2012; Adams *et al.,* 2014). Pollution of soil by spent oil results in immobilizations of soil nutrient, loss of water-retaining capacity, low pH, reduced soil catalase enzyme action, as well as inhibition of nitrate reductase activity of plants; thus, soil dependent activities such as agriculture are affected (Imam *et al.,* 2011). Contaminations of exposed vacant spaces and farmland with petroleum product and grease is nowadays widespread than crude oil spill especially in the municipal areas (Osaigbovo*et al*, 2013). The present study aims to use some organic wastes to stimulate bioremediation of soil contaminated with spent engine oil.

MATERIALS AND METHODS
Study Area
This research was carried out in Lapai town, Niger State, Nigeria. Lapai town is the capital of Lapai Local Government Area. It is positioned on Latitude 9°03'00" N and longitude 6°34'00" E. It has a land mass of 3051km^2 and a population of 110127 at the 2006 census (Map data, 2020).The National Population Commission reported that Lapai town had a human population of over 184,000 as at the year 2006 (NPC,2006).

The influence of Ibrahim Badamasi Babangida University, Lapai have resulted into increased human population thence increase in the use of more machinery such as cars, motorcycles and generators. This however resulted into pollution of land and water by petroleum products from the mechanic workshops.

Collection of soil sample

Soil specimens were collected from ten different location from automobile workshops near the central motor park Lapai. The soil samples were placed in polythene bags and conveyed to the microbiology research laboratory of IBB University, Lapai for analysis. The soil samples were bulked together to form a composite, mixed thoroughly, crushed and passed through a 2mm sieve. One-hundred-gram (100g) sub sample of the sieved soil was measured and placed in transparent polythene bags for the physico-chemical analysis.

Collection of organic stimulants

The organic materials used as stimulants were Rice husk, cow dung and chicken droppings. Cow dung and chicken droppings were sourced from IBB University, Lapai school farm, while the rice husk was obtained from a rice milling engine located near Baddegi market Lapai. The agricultural wastes (rice husk) were collected using rack and shovel, air dried, grinded and kept in clean polythene bags, while cow dung and chicken droppings were collected into a pan using hand (gloved), dried under the sun for three (3) days, grinded and store in a plastic container. All processed samples were transported to the laboratory for further analysis (Abioye *et al.,* 2010).

Collection of spent engine oil

Freshly drained used engine oil was obtained during motor service from the central mechanic workshop located beside the central motor park opposite United Bank for Africa (UBA), Lapai. The oil was collected into a clean five-liter gallon and transported to Microbiology research laboratory, IBB University, Lapai.

Determination of physico-chemical properties of the experimental soil.

The parameters analyzed include pH, soil texture, water holding capacity, total organic carbon, nitrogen and phosphorus composition of the soil. These parameters were assessed before and after contaminating the soil with spent engine oil so as to record changes that might occur as a result of contamination.

Soil pH was determined using pH meter in accordance with the protocols of Ijah et al (2003). The physical properties of soil such as clay, silt and sand was determined using hydrometer method (Vidali, 2001).The soil type was determined by comparing the values of clay, silt, and sand with the soil triangular chart (Vidali,2001).Total nitrogen, available phosphorous, organic carbon and exchangeable bases were estimated following Kjeldahl digestion procedure as described by Macgill (2002) while the water holding capacity of the soil was in accordance with the techniques of Vidali (2001)(Agarry and Oladipupu,2012).

Sterilization of organic stimulants

One hundred gram of each organic waste was mixed with water to about half its water holding capacity and sterilized in the autoclave at 121°C for 15 minutes. Each organic waste was mixed carefully with distilled water inside glass Petri dish and covered with a lid, then rapped with foil paper before placing in the autoclave (Ijah et al 2003).

Enumeration of hydrocarbon degrading bacteria in spent engine oil

Total counts of Hydrocarbon degrading bacteria (HDB) in the contaminated soil were carried out in accordance with the procedures described by (Mandri and Lin, 2007). Serial dilution of the soil ranging from 10^{-1} – 10^{-10} was prepared by dissolving one gram of soil sample in 9 mL sterile water and sequently transferring 1 mL to 9 mL distilled water. The procedure was repeated until 10^{-10} dilution was achieved. One (1) mL of each dilution was inoculated into triplicate plates of freshly prepared mineral salt medium (composition in gram/liter; 1.8g K_2HP0_4, 4.0g NH_4Cl, 0.2g $MgS0_4$. $7H_20$, 1.2g KH_2Po4, 0.01g $FeS0_4.7H_20$, 0.1g NaCl, 20g agar) supplemented with1% spent engine oil and incubated at 30˚C for 5 days. Bacteria colonies that emerged on the agar surface were counted and the total count expressed as colony forming unit/gram (CFU/g) of the soil (Abioye *et al*., 2012).

Isolation of HDB present in the spent engine oil contaminated Soil

The colonies that emerged on the MSM plates after incubation were distinguished into types based on their cultural properties such as color, size and shapes, consistency and texture. The distinguishable colonies were picked and streaked on nutrient agar plates to obtain pure isolates as demonstrated by Abioye et al., 2010.

Identification of hydrocarbon degrading bacteria

The HDB isolated were characterized microscopically through Gram staining to establish their cellular morphology. Their biochemical characteristics were determined as described by Holt *et al.* (1999). The isolated organisms were identified by comparing their features with those of known taxa described in Bergey's manual of determinative bacteriology and confirmed with microgen confirmatory kit (Agarry *et al.,* 2010). The HDB Isolated from soil was subjected to Gram's staining procedure. While Catalase, Oxidase, Coagulase, Motility, Urease, Methyl, Citrate utilization, Voges-proskauer, Hydrogen sulphide, Starch hydrolysis and sugar fermentation tests were done to confirm their identity (Abioye *et al*., 2012).

Bioremediation Study

This phase of the study was devoted to assessing the effects of three organic wastes on the biodegradation of petroleum hydrocarbon in the spent engine oil. The soil sample was first contaminated with 10% weight per weight spent engine oil, mixed rigorously and allowed to stay for seven days so that the soil microorganisms can acclimatize to the new condition (Das and Muhkheriji 2007).

One kilo gram (kg) of the contaminated soil placed into four (4) different two (2) liter capacity plastic containers and labeled A-D. One hundred (100) gram of each organic waste was introduced into containers A, B and C separately and mixed thoroughly. Each treatment was replicated 3 times. The vessels labeled D containing only contaminated soil (no organic waste) served as control. The content of each vessel was mixed manually twice per week to enhance aeration and kept moist by adding sterile distilled water at half the WHC of the soil. Ten (10) gram subsamples of were taken from each container after one (1) week of incubation and subsequently at 10 days intervals for the determination of residual hydrocarbon content.

Table 1: Experimental design for the study

Treatment	Contents
1	1000g soil + 10% CD + 60% dH_2O
2	1000g soil + 10% CDU + 60% dH_2O
3	1000g soil+ 10% RH + 60% dH_2O
4	1000g soil + 60% dH_2O

CD=Chicken droppings, CDU=Cow dung, RH=Rice husk, dH_2O = distilled water

Determination of Residual spent Engine oil in Contamination soil

Remaining hydrocarbon composition of the soil samples were measured through toluene cold extraction method described by Adesodun and Mbagwu (2008). Ten (10) gram of microcosm soil was dispensed into a 50mL capacity flask containing 20mL toluene. The flask was shaken on an orbital shaker for 30 minutes and allow to stay for 10 minutes. The supernatant was filtered through Whatman filter paper No.1 into another beaker. The extract was place in an Airone –RS fume cupboard for 10 minutes to remove Ethane by evaporation. Absorbance was then taken at 420nm using Agilent technologies carry series UV, vis spectrophotometer. The total petroleum hydrocarbon (TPH) in the soil was estimated with reference to a standard curve derived earlier from fresh spent engine oil diluted with toluene. The total amount of oil removed after study period, was estimated by subtracting the residual value from the initial concentration, thence express in percentage.

Determination of biodegradation rate constant and half-life of spent engine oil

TPH data obtained as the concentration of residual oil was fitted to first-order kinetics model of Yeung *et al.* (1997) to determine biodegradation rate constant and half-life of the contaminant using the formula below:

$$R = Ie^{-kt}$$

Where R = residual hydrocarbon content in soil (g/kg^{-1})
I = initial hydrocarbon content in soil (g/kg^{-1})
K = Biodegradation rate constant (day-1)
t= time (day) (Abioye *et al.,* 2009)

Measurement of carbon dioxide evolved from contaminated soil during biodegradation of hydrocarbon.

The rate of degradation of hydrocarbon in spent engine oil was assessed by measuring the volume of carbon dioxide released during bioremediation using method of cornfield, 1961(Abioye, *et al.*,2012). One hundred (100) gram of contaminated soil was measured in to 5 (500milliliter capacity) bottles and labeled A, B, C, D and E. Ten (10) grams of chicken droppings, cow dung, and rice husk were added to bottles A, B, and C respectively. Bottle D containing only contaminated soil (no amendment). This served as negative control, while bottle E contain uncontaminated soil to serve as positive control. Twenty (20) mL of sterile distilled water was added to the soil in each bottle and mixed thoroughly. Twelve (12) glass vials containing 0.5g of barium peroxide and 4.5 mL of sterile distilled water was placed in each bottle and covered very well with lid. The bottles were incubated at room temperature for 28 days. At 10 days interval, a set of three (3) vials were withdrawn from each bottle and the content titrated using 1M hydrochloric acid and the titrant values were recorded. The volume of CO_2 evolved during biodegradation was calculated using cornfield method of determining mineralization of carbon.

RESULTS

Soil physicochemical properties

The physical and chemical property of soil and organic wastes used for bioremediation study is shown in Table 2.The hydrogen ions (pH) of the soil sample falls below neutral level (4.7). pH of chicken droppings, cow dung and rice husk fall within the neutral level of 6.9, 7.1 and 7.6 respectively. The values of nitrogen, phosphorous and organic carbon in contaminated soil prior to bioremediation were 0.92%, 19.2 mg/kg and 10.5%mg/kg respectively. Water holding capacity of the soil sample (15%) was lower than that of CD, CDU and RH with 31.0%, 43.0% and 40.7% respectively. Particle size composition of soil (soil texture) was found to contain sand (72.2%), silt (15.9 %) and clay (11.9%), hence confirmed to be sandy loam. The organic wastes contain higher quantities of carbon, nitrogen and phosphorous when compare with soil sample, except CD that had lower organic carbon (10.1%) compared to 10.5% in soil. Phosphorous was higher in Chicken droppings (17.6mg/kg), while Cow dung and Rice husk had 17.1mg/kg and 15.21mg/kg respectively. CD, CDU and RH recorded nitrogen content of2.4%, 1.7% and 0.82% respectively.

Table 2: Physico chemical and bacteriological properties of experimental soil and organic wastes

Parameter	Contaminated Soil	CD	Organic waste CDU	RH
pH	4.7	6.9	7.1	7.6
Nitrogen (%)	0.92	2.4	1.7	0.82
Phosphorous (mg/kg)	19.2	17.6	17.1	15.21
Organic carbon (%)	10.5	10.1	10.9	11.4
WHC (%)	15	31.0	43.0	40.7
Sand (%)	72.2			
Silt (%)	15.9			
Clay (%)	11.9			
Soil texture	Sandy loam			
HDB Count(CFU/g)	$5.7x10^5$			

Key:
WHC=water holding capacity, CD=Chicken droppings, CDU=Cow dung, HDB=hydrocarbon degrading bacteria

In this study, the hydrocarbon degrading bacteria identified in contaminated soil were *Bacillus* sp*, Pseudomonas* sp*, Micrococcus* sp*, Acinetobacter* sp *and Flavobacterium* sp. The initial count of HUB in the soil was $5.7x10^5$ CFU/g.

Total Petroleum Hydrocarbon in soil assessed for 60 days is shown in figure 1. The concentration of TPH present in soil at the beginning of this study was 7525mg/kg. The result revealed that soil treated with CD had the least residual oil (1.0mg/kg) after 60 days. 1.5mg/kg in CDU, while the soil treated with RH had 2.5mg/kg. The highest concentration (4.0 mg/kg) of TPH in soil after 60days was recorded in control soil. The result showed a statistically significant difference (p=0.02) at $p0<0.05$ between amended soils and control.

Percentage loss of total petroleum hydrocarbon in soil during bioremediation is shown in figure 2. There was a rapid reduction in the amount of TPH within the first 10days of bioremediation in the amended soils. At the end of bioremediation period (60days), soil amended with CD showed the highest mean reduction in concentration of spent engine oil (86.06%). Soils amended with CDU and RH showed a percentage reduction (80.05% and 66.75%) respectively, while the unamend soil showed 46.8 % mean reduction of TPH. The result showed a statistically significant difference at P<0.05 among different treatments and control. The difference in total loss of oil among treatments is more pronounce between the control and amended category. However, there is no difference in the mean loss between CD and CDU.

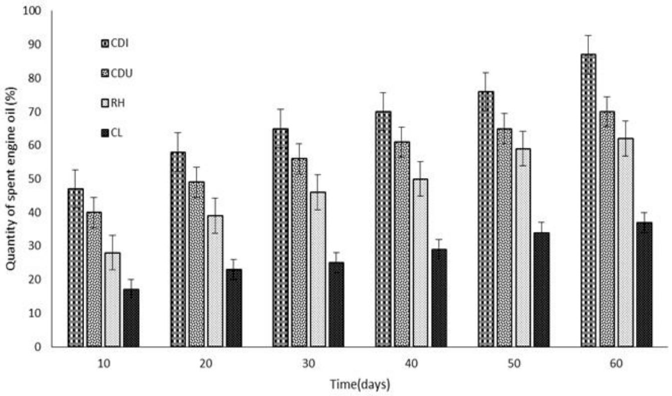

Figure 1: Effect of bio stimulants on residual TPH in soil during bioremediation (g/kg)

Key: CD = Chicken Droppings CDU = Cowpea Haulm RH = Groundnut Haulm CL = Control

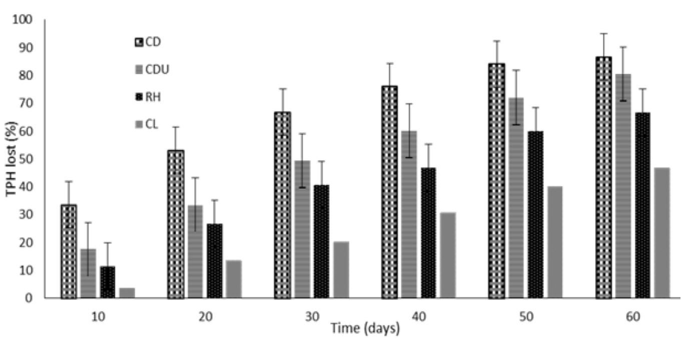

Figure 2: Reduction of total petroleum hydrocarbon (TPH) in soil (%)

Key: CD = Chicken Droppings CDU = Cowpea Haulm RH = Groundnut Haulm CL = Control

Biodegradation rate constant (K day $^{-1}$) and half-life ($t_{1/2}$)

Table 3 shows the biodegradation rate constant and half-life of petroleum hydrocarbon in soil contaminated with spent engine oil. The result revealed that soil amended with CD had the highest biodegradation rate constant of 0.125kg/day. CDU and RH had 0.123kg/day and0.119kg/day respectively, while the lowest biodegradation rate constant (0.010kg/day) was recorded in unamend soil (control).Half-life is the time taken to degrade half of total concentration of chemical substances by bacteria. In this study, it took only 5.55 days for spent engine oil in soil treated with CD to reduce to half its concentration. The half-life of spent engine oil in soil treated with CDU and RN were 5.63days and 5.82 days respectively. While in the control soil where the bioremediation happened naturally, a calculated half-life of 69.31 days was recorded.

Table 3: Biodegradation rate and half-life of TPH in soil amended with organic wastes

Treatment	Biodegradation Rate Constant (Kday^{-1})	Half-life (t ½/day)
CD	0.125	5.55
CDU	0.123	5.63
RH	0.119	5.82
CL	0.010	69.31

Carbon dioxide evolution during bioremediation

The volume of CO_2 liberated from experimental soils in this study is presented in figure3. Soil amended with chicken droppings liberated the largest mean volume of CO_2 (15.77cm^3). CDU treated soil showed 14.0cm^3 and RH liberated11.72cm^3 volume of carbon dioxide. Lowest volume of CO_2 was recorded in control soil (3.78cm^3). Statistical analysis revealed that there is significant difference (p=0.048) at P<0.05 between treatments and control. However, there is no significant difference in the amount of CO_2 evolved among treatments at P>0.05.

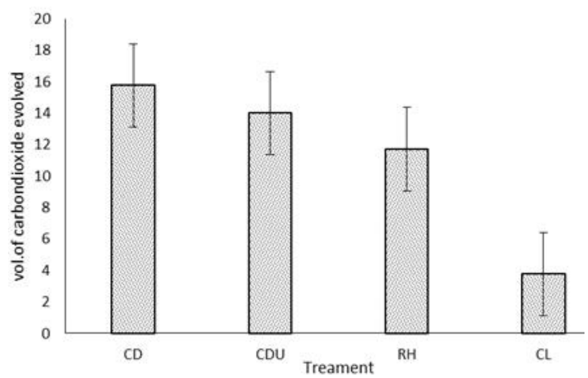

Figure 3: Mean carbon dioxide evolved from soil during bioremediation

DISCUSSION

Spent engine oil contains high concentration of petroleum hydrocarbon which slows biodegradation rate and high rate of bio accumulation 9Yeung et al., 1997). Presence of lubricating oil in soil can affect its physico-chemical properties as well as the population of natural flora. This conforms with the study of Abioye *et al* 2012 who recorded drop in pH on addition of spent engine oil to soil. Lower moisture content of the polluted soil (15%) could be attributed to some soil pores where water will occupy were blocked with spent oil (Atlas, 1981).

To avoid the problem of slow rate of biodegradation and persistence of spent engine oil in soil, Water holding capacity of contaminated soil (15%) in this study is within the optimum range for the growth of HUB and effective degradation of oil (Vidali, 2001).Abioye (2009) also reported that soil moisture for effective degradation of TPH is within the range of 15%-60%. Omotayo *et al*

(2012) is of opinion that soil with adequate moisture content support better microbial growth.

The findings of this study showed that contaminated soil is low in essential nutrients like carbon, nitrogen and phosphorous, hence do not fulfill the requirement for efficient degradation of spent engine oil. The soil had 0.92% nitrogen, 10.10.5% total organic carbon (TOC) and 19.8 phosphorous which were expected to be more for effective bioremediation; hence the need for addition of supplements. Studies by Ijah and Antai, 2003; Anjana and Meenal 2009; Abioye *et al.,* 2012 used organic wastes like wood chips and sewage sludge, Banana skin, spent mushroom compost, brewery spent grain, chicken droppings and food wastes compost as supplements to arouse the degradation of spent engine oil in soil. Orji *et al* (2012) earlier used cow dung while Dads and Muhkheriji (2007) used tea leaf, soy cake and potato skin to enrich soil so as to fasten the rate of biodegradation. Following same technique, this study used chicken droppings, cow dung and rice husk were used as organic supplements to improve the soil microbial population so as to accelerate the rate of biodegradation of TPH in contaminated soil.

Species of hydrocarbon utilizing bacteria isolated from contaminated soil include *Acinetobacter, Bacillus, Flavobacterium, Micrococcus* and *pseudomonas.* The isolation of these species is an indication of their tolerant to the hydrocarbon contents of the soil and thus can be supported with organic wastes to stimulate their bioremediation potential. Unlike the present study, Adam *et al* (2014) isolated *Pseudomonas*, *E.coli, Arthrobacter* and *Corynebacteria* from oil contaminated soil. In the study of Ajao *et al* (2011), bacteria species of *Flavobacterium, Bacillus, Pseudomonas* and *Serratia* were isolated from used motor oil.
The count of hydrocarbon utilizing bacteria in contaminated soil before bioremediation commenced was $5.7x10^5$CFU/g, this shows that HUB can strive even in extreme conditions and high concentration of TPH (Rahman *et al.,* 2002.Onifadeet *al*., 2007; Omotayo *et al*., 2012; Adams *et al*., 2014. High count of HUB in soil indicates that the soil in mechanic workshop is exposed to petroleum hydrocarbon.

The quantity of spent engine oil lost during bioremediation process in this study varies with treatment. Higher loss was recorded in soil treated with CD followed by CDU and RH as indicated in Figure 3.The quantity lost in control soil was least when compared to the amended soil. Higher lost in CD can be attributed to higher population of HUB in CD. This could be due higher percentage of nitrogen present and bioavailability of the nutrients in CD to bacteria species (Abioye *et al*., 2009).Result of this study revealed higher degradation of spent engine oil from soil stimulated with organic wastes compare to concentration degraded in control. However, highest percentage degradation was recorded in soil amended with chicken droppings (86.7%) while 80.05% and 66.75% were recorded in cow dung and rice husk respectively. This is clear evidence that CD is a better stimulant of bacterial growth owing to it high nitrogen composition as indicated in table 2 which is essential for the degradation of engine oil by the bacteria. The differences in the concentration of oil loss within the same period of bioremediation might be due to differences in the composition of nutrients in organic wastes and number of HUB counts (Abioye *et al*., 2012).

The slow rate of degradation of TPH in control soil is in conformity with the report of Brandit *et al* (2008) Abioye *et al* (2010) who revealed that rate of bioremediation is slow under natural conditions. Godslead *et al* (2015) suggested that addition of nutrient sources markedly improve moisture content of soil hence improves rate of degradation. This work also agrees with the work of Abioye *et al* (2012) who revealed that soil amend with brewery spent grain had 92% degradation, while soil treated with banana skin and spent mushroom compost had 84% and 79% rate of degradation within 12 weeks of bioremediation. The result of this work is also similar to the result of Adams *et al* (2013) who reported that the rate of degradation of spent oil in soil amended with poultry manure, cow dung and goat dung increases with time. Even though the percentage biodegradation of hydrocarbon in treated soil is higher in this study, amount of oil reduced in the control (46.8%) is reasonable. This could be due to the fact that biodegradation process is not only induced by nutrient addition, but other abiotic processes also such as volatilization and absorption can cause degradation of oil (Adams *et al*., 2013).

Data obtained as the residual spent engine oil in soil within the period of study was combined to determine biodegradation rate constant and half-life for each treatment. The data was subjected to first order kinetics model of Yeung *et al* (1997) and Abioye *et al* (2009). The highest biodegradation rate (0.125g/day) and shortest half-life (5.55days) recorded in soil amended with CD can be related to higher quantity of nitrogen and phosphorous. Soil amended with CDU and RH had 0.123 and 0.119 biodegradation rate constant. The smallest biodegradation constant and the longest half-life (69.31days) observed in control soil shows that even after 60days of study period, petroleum hydrocarbon in control soil was still not reduce to half its initial concentration. This is an indication that biodegradation of hydrocarbon under natural condition is very slow hence need for the improvement of soil condition to hasten the rate of degradation.
There is a linear correlation between percentage degradation and volume of CO_2 evolved during bioremediation of soil contaminated with spent engine oil in this study. This result supports the work the work of Ijah and Antai (2003), who affirmed that higher volume of CO_2 was measured in soil treated with chicken droppings compared to the unamend soil. It also agrees with the result of Abioye *et al* (2009) where higher volume of CO_2 was evolved from treated soil compared to control.

Conclusion
Spent engine oil polluted environment like the mechanic workshop can be cleaned up effectively and efficiently using indigenous hydrocarbon degrading bacteria if the environmental conditions such as pH, water holding capacity, soil texture, temperature are favorable. However, the process of bioremediation can be enhanced by addition of organic wastes because they contain nutrients such as nitrogen, and phosphorous which stimulate bacterial activities. However, organic wastes can be utilized effectively to remediate soil contaminated with spent engine oil. The release of Carbon dioxide from the soil during bioremediation process indicates the presence of hydrocarbon in Spent Engine Oil is degraded into harmless by products like carbon dioxide and water. Statistical analysis of residual oil and percentage reduction data showed that there is significant difference between the initial concentration and the residual concentration ($p=0.02$ at $p0<0.05$) between amended soils and control at the end of research period.

A significant difference at P<0.05 was observed in percentage loss however, there was no significant difference between CD and CDU. The findings of this study also revealed a significant difference (p=0.048 at P<0.05) in the amount of carbon dioxide released during biodegradation of oil between treatments and control.

Recommendation

1. Mechanic workshops should be located far away from residential areas to avoid coming into contact with the harmful substances in spent engine oil.
2. Ministry of environment should create public awareness programs to enlighten people on the dangers of the component of spent engine oil
3. Organic wastes that are usually thrown away, should be utilize in stimulating bioremediation processes and sorption of heavy metals.

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