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A Comparism of the Effect of Some Organic Wastes on the Rate of Bioremediation of Soil Contaminated with Spent Engine Oil Using Gas Chromatography/Mass Spectrophotometer

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Keyword: *Biodegradation, Bioremediation, Indigenous Hydrocarbon Utilizing Bacteria, kinetics_Model, petroleum hydrocarbon, Biostimulation-Efficiency*

Abstract

Potential of some organic wastes (chicken droppings, cowpea haulms and groundnut haulms) to stimulate the biodegradation of petroleum hydrocarbons in spent engine oil by the indigenous bacteria was investigated. The rate of biodegradation was studied for twelve weeks. Percentage Nitrogen and Phosphorous content in the contaminated soil do not fulfil the requirement for efficient degradation of spent engine oil because they are low in contaminated soil, hence the need for addition of supplements. The Hydrocarbon Utilizing Bacteria found in the soil belongs to the genera Bacillus, Micrococcus, Staphylococcus and Flavobacteria. The gravimetric analysis of TPH in spent engine oil showed 84.42%, 71.81%, 70.85% degradation in CD, CPH and GNH amended soils respectively, while 47.36% was recorded in control. Biostimulation efficiency (B.E %) of the organic wastes was determined and result revealed that the organic wastes can stimulate biodegradation of TPH in the following order CD (43.89)>CPH34.04)>GNH (33.24). Dynamics of soil bioremediation was also determined and high values of Correlation determination (R^2): (CD=0.9687, *CPH=0.9579, GNH=0.937; CL=0.9883) obtained showed that biodegradation data fitted well into the first-order kinetic model. The chromatograms of oil extracted from soil before and after bioremediation using Gas Chromatography Mass Spectrophotometry (GCMS) analysis of extracted oil, showed varied peaks representing different hydrocarbon compounds as well as alcohols and fatty acids. At the end of this study, highest percentage degradation of hydrocarbon was recorded in soil treated with chicken droppings (73.9 %), 63.18% and 53.8% reduction was observed in CPH and GNH treated soils respectively, while control soil had the lowest (33.28%) percentage hydrocarbon loss after bioremediation. The organic wastes used in this study, can complement the use of more expensive and unfriendly physical and chemical methods of remediating contaminated soil.*

Introduction

Industrialization and urbanization which cause rise in consumption of petroleum- based products such as lubricating oil (engine oil) have resulted into pollution of the environment by large volume of used oil. Contamination of soil by spent engine oil through leakages and accidental spill has become a serious concern globally because of its consequences on the ecosystem and human health (Ogunjobi and Ekanem, 2016).Onuoha *et al*. (2011) stated that contamination of soil by spent engine oil is prevalent in oil producing and industrialized countries, but the problem is more severe in developing countries like Nigeria where there are no effective regulatory policies on the environment. Spent engine oil is a mixture of polycyclic Aromatic Hydrocarbons (PAHs), performance enhance additives, decomposition products and heavy metals such as lead, vanadium, Nickel, Arsenic mercury and many more that come from engine parts as they wear down (Agarry and Oladipupo, 2012). These chemical toxins in spent engine oil can cause various forms of health problems like kidney damage, various kinds of cancer, brain damage, reduced growth and development((Agarry and Oladipupo, 2012).

The physical and chemical technologies like thermal treatment, dig and dump and chemical methods were used earlier for the treatment of soil contaminated with petroleum products are expensive and not environmentally friendly, involve complex technologies which could destroy soil texture (Ogunbayo *et al.,* 2012). However, some researchers have reported that some microorganisms have the potential to cleanup soil polluted with organic pollutants such as hydrocarbons through bioremediation. These microorganisms are very unique and can naturally degrade large hydrocarbons and utilize them as food source (Mandri, and Lin, 2007; Ajao *et al.,* 2011). The bioremediation process is very slow because of hydrophobicity and low solubility of contaminants in aqueous media, depletion of essential nutrients such as nitrogen in the soil, reduced soil oxygen and moisture as well as decrease in bioavailability of pollutants to microbes (Fatuyi *et al.,* 2012).Hazardous hydrocarbon compounds can be degraded into nontoxic substances by the hydrocarbon degraders. The contaminant is first oxidize into fatty alcohol, aldehyde, and carboxylic acid, which are metabolized further by beta-oxidation into water, carbon dioxide and energy (Anthony, 2006).

Lack of nutrients such as nitrogen and phosphorous is a major factor affecting biodegradation of petroleum hydrocarbon by the microorganisms. Therefore, addition of organic and inorganic wastes is an effective way to stimulate the bioremediation process (Ogunjobi and Ekanem, 2016).

Materials and Methods

Study area

The study area for this research was the metropolitan Minna, the capital of Niger State. Niger State is located in the North Central Zone of Nigeria, while Minna town is located in the Eastern Senatorial district of the state at latitude N 09^0 36’ 05” and longitude E 06^0 32’ 24.8” with a landmass of $1664km^2$.According to 2006 census, Minna town had a human population of 3488180 persons (National Population Commission, 2006).

Soil sample collection

Soil samples with a long history of petroleum contamination were collected from a mechanic workshop with a coordinate of 9^o 36’19.97N 6^o 32’14.49 E, in “mechanic village” located at Ketaren Gwari, Minna, Niger State, Nigeria. The contaminated soil samples were collected from ten (10) spots hundred feet away from each other and pooled together to get a composite sample.

Collection of organic wastes

The organic materials used as stimulants were groundnut haulms and cowpea haulms as well as chicken droppings. These were collected from the school farm and poultry in College of Education Minna. The agricultural wastes were collected using rake and cutlass, air dried, milled and kept in clean polythene bags, while the chicken droppings was collected into a pan using hand (gloved), dried in the sun for three (3) days, milled and stored in a plastic container. All processed samples were transported to the laboratory for further analysis (Abioye *et al.,* 2010).

Physicochemical analysis

The pH, total nitrogen, available phosphorous, texture, water holding capacity and exchangeable cations (K, Ca, Mg and Na) were determined using the methods of Vidali, 2001. Concentration of Total hydrocarbon was determined gravimetrically by toluene cold extraction method of Abioye *et al*., 2012.

Microcosm study

One hundred gram (100g) of each organic waste was added to one thousand gram (1kg) of contaminated soil inside a two liter capacity plastic container and 60% pre-determined water holding capacity (WHC) of the soil was added. Each treatment was prepared in triplicate and soil was obtained from the treatments on a two weekly basis to determine the HUB count and the amount of petroleum hydrocarbon removed. The microcosm was studied for twelve (12) weeks.

Bioremediation Experimental Design

TREATMENT	CONTENT
CD	1kgsoil+100g CD+ distilled water
CPH	1kgsoil+100g CPH+ distilled water
GNH	1kgsoil+100g GNH+ distilled water
CL	1kgsoil+ distilled water

CD=Chicken droppings, CPH=Cowpea Haulms, GNH=Groundnut Haulms, CL=Control

Enumeration of Hydrocarbon Utilizing Bacteria

Total counts of Hydrocarbon utilizing bacteria (HUB) in the contaminated soil was determined by plating one mL of serially diluted soil sample on freshly prepared mineral salt medium (composition in gram/liter; 1.8g K_2HPO_4, 4.0g NH_4Cl, 0.2g $MgSO_4$. $7H_2O$, 1.2g KH_2PO_4, 0.01g $FeSO_4.7H_2O$, 0.1g NaCl, 20g agar) supplemented with1% spent engine oil (Mandri and Lin, 2007).

Isolation of Hydrocarbon degrading Bacteria from the soil sample

Discrete colonies that appeared on the surface of solid mineral salt medium were picked and streaked on nutrient agar plates to obtain pure isolates. The pure isolates were sub cultured onto nutrient agar slants. Subsequently, 24 hour culture isolates were gram stained and their biochemical characteristics were determined by carrying out the appropriate tests as described by Holt *et al.*, (1999).

Determination of petroleum hydrocarbon content

The hydrocarbon content of soli was determined by toluene cold extraction method of Adesodun and Mbagwu (2008). Ten (10) gram of microcosm soil was weighed into a 50mL capacity conical flask and 20mL toluene was added. The mixture was shaken on an orbital shaker for 30 minutes and allowed to stand for 10 minutes. The supernatant was filtered through Whatman filter paper No.1 into another beaker. The absorbance was then determined at 420nm using a spectrophotometer (Agilent, USA). The total petroleum hydrocarbon (TPH) content in the soil was estimated with reference to a standard curve derived from fresh spent engine oil diluted with toluene. The total hydrocarbon content was fit into the first order kinetic model of Yeung *et al*., 1997:

$$C_i = C_o e^{-kt} \; (K = C_i / C_o \times t)$$

Where C_i = residual hydrocarbon content in soil (mg/kg)

C_o = initial hydrocarbon content in soil (mg/kg)

K = Biodegradation rate constant (day-1)

t= time (day) (Abioye *et al.,* 2009).

Half-life is the time taken to reduce the initial concentration of spent engine oil to half. The formula below was used to determine the half-life:

$$t_{1/2}=\ln 2/k.$$

Percentage degradation of total petroleum hydrocarbon in soil

The Percentage degradation (D) was calculated using the formula below:

$D=TPH_i - TPH_r / TPH_i \times 100$

Where D=Biodegradation (%)

TPH_i=initial concentration (mg/kg)

TPH_r =residual concentration (mg/kg)

Biostimulation Efficiency (B.E %)

The effectiveness of each organic waste used in stimulating biodegradation of petroleum hydrocarbon was determined using the formula:

$$B.E = \%TPH_t - \%TPH_u / \%TPH_t \times 100$$

Where B.E=Biostimulation Efficiency

$\%TPH_t$=amount lost in treated soil (%)

$\%TPH_u$=amount lost in untreated soil (%)

Determination of kinetics of soil bioremediation using correlation of determination (R^2).

Goodness of fit of first-order kinetics model of Yeung *et al.* (1997) was used to determine the biodegradation rate during bioremediation was tested by calculating correlation of determination (R^2). The acceptable value of R^2 lies between 1-0.95 This was achieved by plotting logarithm of residual concentration against time, which indicates the variability of data that cannot be explained due to error and also show if the kinetic model used is reliable and can be used to extrapolate concentration of spent engine oil before and after the bioremediation period using the equation: y=mx+ c where y=slope, m=concentration, x=time, c=constant

Total Petroleum Hydrocarbon in soil before and after bioremediation using Gas Chromatography-Mass Spectrophotometer (GC-MS).

A standard profile was first determined by injecting one (1) millilitre of hydrocarbon standard in to the Gas Chromatography Mass Spectrophotometer. The chromatogram generated served as a calibration window with which the test samples were compared. Spent engine oil in experimental soils was extracted by adding 100mL of n-hexane to 10gram of soil sample. The oil extracted from soil was concentrated to 1mL by evaporating the solvent. One (1) microlitre of the concentrated oil extract was injected in to the injection port of the gas chromatography and volatilized into gas form. The gases then pass through a column which is coated with material to attract the various components of the oil at varying degrees. The level of attraction at this stage causes components to separate and elute at different times which is shown as peaks on the resulting chromatogram. The chromatograms generated for all the samples were analyzed by chromeleon 7.0 program and a library (NIST, 2007) search was performed for identification of chromatogram peaks. Hydrocarbon compounds in each sample was determine by comparing peaks of its chromatogram with the library generated from the standard.

Results

Physicochemical properties of contaminated soil and organic wastes

PARAMETER	SOIL	CD	CPH	GNH
PH	6.6	6.46	7.04	7.20
NITROGEN	0.5	11.16	10.10	8.89
PHOSPHORUS	11.8	17.44	11.20	11.92
ORGANIC CARBON	10.23	12.34	10.21	10.41
WATER HOLDING CAPACITY	30	17	22	20
SAND	80			
SILT	10			
CLAY	10			
TEXTURE	Sandy loam			

Hydrocarbon utilizing bacterial counts of soil during biostimulation with organic amendments

The initial counts of HUB in contaminated soil was $4.1x10^6$ cfu/g. Subsequently, the count was observed every 2 weeks from week two (2) for the period of 12 weeks and counts in soil amended with Chicken droppings ranged between 1.28×10^6 and $2.35x10^7$ cfu/g, while that of soil amended with CPH and GNH ranged between $1.01x10^6$ to $1.52x10^7$ cfu/g and $9.1x10^6$ to $1.43x10^7$ cfu/g respectively. The unamended soil (control) had a range of

$4.7x10^6$ to $5.8x10^6$ cfu/g. Statistical analysis of mean count at $p<0.05$ revealed that there was significant difference (P=0.00) in the count of HUB among soil treated with organic wastes compared to the control

Table 2: Range of hydrocarbon utilizing bacteria in soil (cfu/g)

TREATMENT	RANGE	MEAN
CD	1.28×10^7_2.35×10^7	1.97×10^7
CPH	1.01×10^6_1.52×10^7	1.35×10^6
GNH	9.1×10^6_1.43×10^7	1.30×10^6
CL	4.1×10^6_5.8×10^6	4.48×10^6

Hydrocarbon Utilizing Bacteria (HUB) isolated from soil contaminated with spent engine oil.

In this study, the hydrocarbon utilizing bacteria isolated soil from contaminated with spent engine oil belonged to the genera *Bacillus, Staphylococcus, Micrococcus and Flavobacteria* (Table2).The highest Percentage frequency of occurrence in soil was recorded in *Bacillus* (41.46%), *Staphylococcus* and *Micrococcus* had 19.51% and 29.29% respectively, while Flavobacteria was the least occurring bacteria (9.70%).

Table 3: Distribution of Hydrocarbon degrading bacteria in soil contaminated with spent engine oil

GENUS	NUMBER (%)
BACILLUS	17(41.46)
MICROCOCCUS	12(29.27)
STAPHYLOCOCCUS	8(19.51)
FLAVOBACTERIUM	4(9.76)
TOTAL	41(100)

Biodegradation of petroleum hydrocarbon in soil during bioremediation(%)

The initial concentration of petroleum hydrocarbon in contaminated soil was 7007mg/kg. Within the first 14days of bioremediation, a rapid reduction in the concentration of TPH was observed in the treatments, however reduction in

TPH progressed gradually in all the treatments following decreasing in the order: CD>GNH>CPH>CL from week 2 to week 6. From week 8, the rate of reduction became faster in Cowpea haulms till the end of bioremediation period, and the highest cumulative percentage reduction of 84.42% was recorded in the soil treated with chicken droppings. A significant difference in reduction of hydrocarbon among treatments compared to the control was observed (p0<0.05).

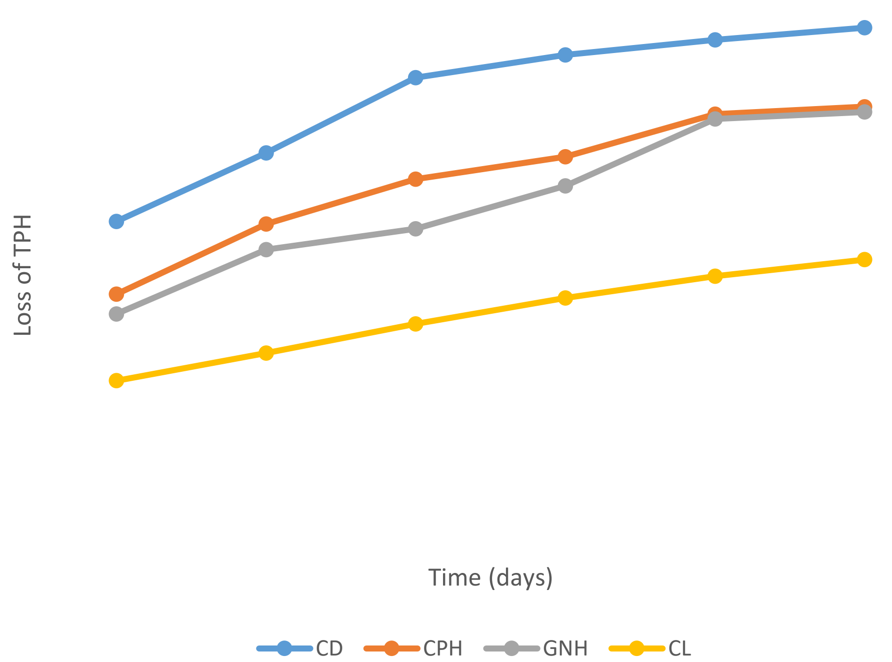

Figure1: Reduction (%) of Total petroleum hydrocarbons in soil during bioremediation

Key: CD=chicken droppings, CPH=cowpea haulms, GNH=groundnut haulms, CL=control

Bio stimulation efficiency of organic wastes after the bioremediation study (B.E %)

The bio stimulation efficiency (B,E) ranged from 33.24%_ 43.89% (Table 4) Chicken droppings showed the highest B.E (43.89%), followed by Cowpea haulm (34.04%) and least value was recorded in groundnut haulm (33.24%).

Table.4: Bio stimulation efficiency of each organic waste after eighty four (84) days

TREATMENT	TPH REDUCED (%)	B.E (%)
CD	84.42	43.89
CPH	71.81	34.05
GNH	70.95	33.24
CL	47.36	

Key: CD=chicken droppings, CPH=cowpea haulms, GNH=groundnut haulms, CL=control

B.E=Bio stimulation Efficiency

Biodegradation rate constant and half-life

Table 3 shows the biodegradation rate constant and half-life for the hydrocarbon in soil. The result revealed that soil amended with chicken droppings had the highest biodegradation rate constant of 0.0704kg/day and the lowest half-life (9.845 days). The unamend soil showed lowest biodegradation rate constant (0.0395 kg/day) and the highest half-life (17.54 days).

Correlation determination (R^2) under the influence of different organic wastes is also presented in table 5. Soil treated with Chicken droppings, and Cowpea haulm had 0.9687 and 0.9579 respectively, while under the natural bioremediation process (control), the value of R^2 was 0.9883. Even though the value of R^2 in Groundnut haulm treated soils (0.937) was also high, it falls below the acceptable limit of 1-0.95.

Table.5: Biodegradation Rate Constant and Half-life of TPH as well as Correlation determination of bioremediation under the influence of natural condition and organic wastes (R^2)

TREATMENT	BIODEGRADATIONRATE CONSTANT(KDAY-1)	HALF-LIFE(T½/DAY)
CD	0.0704	9.85
CPH	0.0599	11.56
GNH	0.0592	11.71
CL	0.0395	17.54

Key: CD=chicken droppings, CPH=cowpea haulms, GNH=groundnut haulms, CL=control

Before bioremediation, GCMS analysis revealed that, spent engine oil in the soil sample contained (21) hydrocarbon compounds with carbon range between C_8-C_{40} representing both straight chains alkanes, unsaturated alkenes, polycyclic aromatic hydrocarbons, hexadecanoic acid, oleic acid and 2-propylheptanol. After 84 days of bioremediation, soil treated with chicken droppings had twenty three (23) peaks of hydrocarbon compounds (C_9-C_{20}).Six (26.1%) out of the 23 compounds belong to the hydrocarbon group of C_8-C_{10}, 4(17.4%) are various types of alcohol, and 11(47.85%) compounds are fatty acids. The chromatograms of TPH from soil treated with cowpea and groundnut haulms showed 19 and 26 peaks with carbon ranges of C_9-C_{18} and C_8-C_{18} respectively. The cowpea treated soil contain 7(36.82%) hydrocarbons, 5(26.3%) alcohols and 6(36.82%) fatty acids, while groundnut haulms soil had 12(46.2%) hydrocarbons, 6(23.1%) and 8(30.8%) fatty acids. The oil extracted from untreated soil showed hydrocarbons compounds with carbon range of C_8-C_{22} [12(66.72%)], 2(11.12%) alcohol and 4(22.24%) fatty acids (Table 6)
Figure 2 shows the total loss of TPH in soil using GCMS. Soil treated with chicken droppings had 73.9 % loss, while 63.18% and 53.8% reduction was observed in CPH and GNH treated soils respectively. The control soil had the lowest (33.28%) percent hydrocarbon degradation after bioremediation.

Table 10: Total Petroleum Hydrocarbon in soil before and after bioremediation using GC-MS

TREATMENT	NO OF PEAKS	CARBON RANGE	HYDROCARBON COMPOUND N(%)	ALCOHOL N(%)	FATTY ACID N(%)
IS	21	C_8-C_{40}	18(85.68)	1(4.76)	2(9.52)
CD	23	C_9—C_{20}	6(26.1)	5(21.75)	12(47.85)
CPH	19	C_9-C_{18}	7(36.82)	5(26.3)	7(36.82)
GNH	26	C_8-C_{18}	12(46.2)	6(23.1)	8(30.8)
CL	18	C_8-C_{22}	12(66.72)	2(11.2)	4(22.24)

N=number of compounds

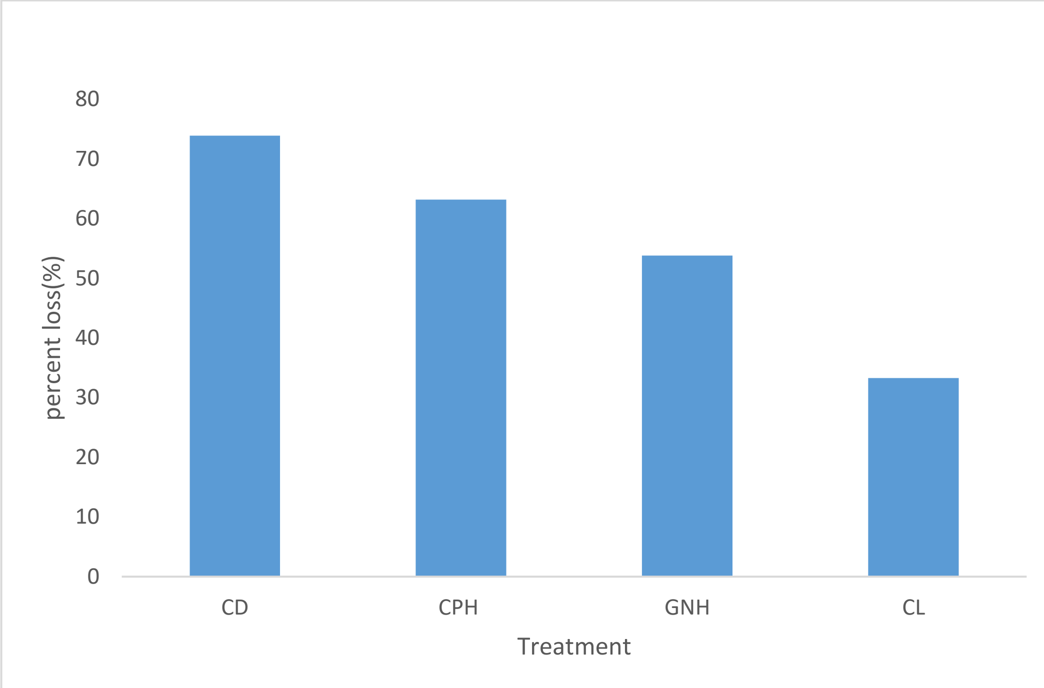

Figure 2: Total hydrocarbon loss in soil using GC-MS

Discussion

The pH of contaminated soil (6.46) though falls within neutral level was lower (6.6) than that of the uncontaminated soil (7.4). This may be as a result of the presence of TPH which have the ability to release hydrogen ions. This is in conformity with the study of Abioye *et al* 2012 who reported drop in pH after adding spent engine oil to soil. Uchendu and Ogwo, 2014 are of the opinion that contamination of soil with spent engine oil can alter the physical and chemical properties of like pH. The neutral pH level of the contaminated soil might be the reason why HUB count was high in contaminated soil since HUB proliferate better in alkaline soil (Abioye *et al*., 2009). This result agrees with the result of study by Abioye *et al.* (2012) where soil amended with banana skin with pH 8.27 had highest count compare to control with PH 6.87.

Low water holding capacity (WHC=30%) of contaminated soil could be due to the fact that some soil pores where water will occupy were blocked with spent oil (Atlas, 1995). However, improvement in the WHC of contaminated soil after bioremediation (38%) can be attributed to the degradation of petroleum hydrocarbon during bioremediation, thence provided more space for water. Abioye *et al.* (2009) reported that soil moisture for effective degradation of TPH is within the range of 15%-60%. Analysis of soil in this study showed that contaminated soil is low in nutrients like, nitrogen and phosphorous hence do not fulfil the requirement for efficient degradation of spent engine oil, hence the need for addition of supplements. Atiku *et al*. (2018) used cow dung and poultry litter while Dadrasnia and Agamuthu, (2013) used tea leaf, soy cake and potato skin to enrich soil so as to fasten the rate of biodegradation.

Higher percentage loss of TPH in soil can be attributed to higher count of HUB, due higher percentage of nitrogen and phosphorous in soil and bioavailability of the nutrients to the hydrocarbon degrading bacteria (Abioye *et al*., 2009). The highest value of Biostimulation Efficiency (43.36%), biodegradation rate constant as well as the lowest half-life recorded in the soil amended with chicken droppings could be as a result of the high concentration of nitrogen and phosphorus in this organic wastes and better bioavailability of the nutrients to bacteria species in soil. There was also a similar trend observed with the findings of Onuaha, 2013 where soil treated with poultry manure had a higher biodegradation rate compare to those with cow dung and goat dung. Ogunjobi and Ekanem, (2017) discovered that NPK Fertilizer is a better bio stimulant when compared to plantain peel and eggshell. The highest R^2 (0.9883) value recorded in soil under natural process of bioremediation (control) may be due to noninterference of the process by the organic wastes in the treated category.

Presence of various hydrocarbon compounds as well as alcohols and fatty acids such as hexadecanoic acid and 2-propylheptanol is a clear evidence that the petroleum hydrocarbons in the soil were in various stages of biodegradation even after the study period. Microorganisms breakdown the contaminant through the oxidation of alkanes into alcohol and fatty acids before it is metabolized further by beta oxidation into harmless substances such as carbon dioxide and water (Munna and Dipa, 2014;Lateef,2016).These compounds have also been detected by several researchers (Dadrasnia and Agamuthu, 2013; Lantiwo and Agarry,2015; Ogunjobi and Ekanem, 2017). Hussein *et al. (*2012) reported that separation and identification of organic compounds in lubricating oil using GC/MS revealed that the oil contain various compounds of hydrocarbon belonging to alkanes, alkenes, alcohols and carboxylic acids. The highest percentage reduction of hydrocarbon compounds (73.9%) and high percentage fatty acids (47.85%) in chicken droppings treated soil after 84days of bioremediation can be attributed to the faster rate of biodegradation of petroleum hydrocarbon in this soil.

Conclusion

Spent engine oil polluted environment like the mechanic workshop can be cleaned up effectively and efficiently using indigenous hydrocarbon utilizing bacteria if the environmental conditions such as pH, WHC, soil texture, temperature are favorable. However, the process of bioremediation can be enhanced by addition of nutrients such as nitrogen, and phosphorous. The findings of this research work have proven that chicken droppings, groundnut and cowpea haulms can stimulate the rate at which the indigenous hydrocarbon utilizing bacteria will degrade petroleum hydrocarbon in spent engine oil. In this study, the HUB isolated include the genus of *Bacillus*, *Micrococcus*, *Flavobacteria*, and *Staphylococcus*.

Soil from Keteran Gwari mechanic workshop was found to be contaminated with high concentration (7007mg/kg) of spent engine oil containing some toxic petroleum hydrocarbons compounds. Chicken droppings amended soil showed the highest reduction (84.42%) of petroleum hydrocarbon compared to cowpea haulm (71.81%) and groundnut haulm (71.95%) treated soils while lowest value was recorded in control soil (47.36%). Chicken droppings is a better stimulant of petroleum hydrocarbon degradation in this study (43.36%), the less effective stimulant among the organic wastes was GNH (33.24%).The chicken droppings treated soil also showed the highest biodegradation rate constant (0.0704day-1) and the shortest half-life (9.845days) of petroleum hydrocarbon in soil when compare with CPH (0.0599 and 11.56days) and GNH (0.0592 day-1 and 11.92 days) treated soils. Chicken droppings showed a R^2 of 0.9687, cowpea haulm had (0.9579), and control (0.9883).The value obtained in soil amended with groundnut haulm was also high (0.9325) but it is below the acceptable limit of 0.95-1.The GC MS result obtained showed that the spent engine oil contain various hydrocarbons compounds at different stages of degradation. The higher number of hydrocarbons peaks and lower number of fatty acids and alcohol in the control category is an indication that biodegradation is slower in control soil.

Recommendation

After observing the results of the objectives of this research, and realizing that keteran Gwari mechanic workshop contain high concentration of spent engine oil and hazardous chemicals, i therefore suggest that there should be periodic monitoring of the mechanic workshop to ascertain that concentration of spent engine oil and heavy metals do not exceed permissible limits. Organic wastes that are usually thrown away should be use as a very cheap source of nitrogen and phosphorous for hydrocarbon utilizing bacteria to facilitate biodegradation of petroleum hydrocarbon in contaminated soil. The ability of bacteria species isolated from the contaminated soil to degrade petroleum hydrocarbon should be tested to determine the best.

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