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Biodegradation of Spent Automobile Engine Oil in Soil Microcosms Amended with Cow Dung

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ABSTRACT: The discharge of spent engine oil in terrestrial and aquatic environments constitutes public health and socio-economic hazards. In this study, the potentials of organic waste (cow dung) amendments as biostimulating agents of the indigenous microflora for hydrocarbon biodegradation in soil microcosms deliberately contaminated with spent engine oil (5%v/w) was investigated for a period of 6 weeks. Physico-chemical and microbiological analysis of soil samples was determined using standard methods. A microcosm constructed consists of 8 trays containing 1kg of soil, artificially contaminated with 50ml of spent engine oil and treated with 50g, 100g and 150g of cow dung. Spent engine oil degradation was assessed gravimetrically at weekly interval and chromatographically after 6 weeks of biodegradation treatment. Results of the physico-chemical analysis showed that the pH of soil was 6.56 while nitrate, moisture content, phosphate and total organic content were 0.82mg/kg, 9.28%, 0.73mg/kg and 3.60mg/kg respectively. Microbiological analysis of the soil sample showed that the total heterotrophic bacteria were $3.6x10^6$cfu/g, while total heterotrophic fungal and hydrocarbon utilizing bacteria (HUB) were $2.2x10^4$cfu/g and 7.9 $x10^4$cfu/g respectively. The mean value of the total viable counts (TVC) population of hydrocarbon-utilizers was higher in biostimulated soil which ranged from ($2.10x10^5$-$5.30x10^9$cfu/g) compared with that of control ($1.20x10^5$-$3.10x10^8$cfu/g). Residual oil concentration showed a more remarkable decrease throughout the incubation period (0.400-0.259mg/g, 0.420-0.218mg/g and 0.410-0.220mg/g for treatments 1, 2 and 3 respectively) when compared to that of control which ranged from 0.400-0.304mg/g. At the end of 6 weeks of microcosms biodegradation studies, percentage degradations of the spent engine oil were 23.81%, 35.29%, 45.45% and 44.94% for CON, T1, T2 and T3 respectively. The result obtained from this study showed that cow dung can be effectively used as a biostimulant during bioremediation of spent engine oil polluted site to enhance biodegradation ability of the indigenous microbial population.

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Today, all forms of Petroleum-based products are the major source of energy for industry and daily life (Buraimoh *et al.*, 2017). However, indiscriminate discharge of spent engine oil in the environment by automobile mechanic workshops has contributed immensely to pollution of terrestrial and aquatic environment which pose a serious threat to health of plants, animals and microbial community (Haytham *et al*., 2016; Ajao *et al.*, 2011; Yakubu, 2007). Spent engine oil has been recognized as one of the most hazardous wastes that are discharged into the environment without being treated to remove its toxicity especially in developing countries such as Nigeria (Udeani *et al.*, 2009; Onuoha *et al*., 2011, Ogunjobi and Ekanem, 2017). It contains some toxic metals and polyaromatic hydrocarbons (PAHs) that could contribute to chronic hazards including mutagenicity and carcinogenicity (Haytham, 2016; Ajao *et al.*, 2011). The greatest cause of spent engine-oil pollution in water bodies and terrestrial environment comes from anthropogenic sources such as drains and urban run-off caused by improper disposal of spent engine oil. Spent motor-oil discharged on land reduces soil productivity. Improperly disposed spent oil ends up in landfills, sewers, backyards, or storm drains where soil, groundwater and drinking water may become contaminated. Engine oil is the oil used for lubrication of various internal combustion engines which include motor or other road vehicles such as cars and motorcycles, heavier vehicles, etc. They are derived from petroleum-based and non-petroleum synthesized

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chemical compounds (additives) (Koma *et al.,* 2003). Apart from the main function to lubricate moving parts, motor oil also cleans, inhibits corrosion, improves sealing and cools the engine by removing heat away from moving parts of the engine (Salam, 2016). Furthermore, it is composed of a mixture of base lubricant oil and additives, and the base oil contains long-chain (C16–C36) saturated hydrocarbons and more than 75% cyclic alkanes including some aromatic hydrocarbons (Haytham, 2016; Koma *et al.,* 2003). Most of the lower hydrocarbon chain constituents can be removed by some physical method such as photo-degradation. However, the major method of removal of these hydrocarbons from a polluted environment is through microbial degradation. Cow dung is undigested residues of consumed food material being excreted by herbivorous bovine animal species (Gupta *et al.,* 2016). It has been reported to contain bacterial species such as *Pseudomanas, Acinetonacter, Stenotrophomonas, Rhodobacter* e.t.c. that are known degraders of petroleum hydrocarbons (Girija *et al.,* 2013). Secondly, cow dung is rich in nitrate and phosphate which makes it serve as a good biostimulant when applied during bioremediation of hydrocarbon-contaminated sites. It is noteworthy that limiting nutrients is one of the major factors that affect the rate of petroleum hydrocarbon removal from contaminated environment. Therefore, the addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Abioye *et al.,* 2012; Walworth *et al.,* 2007). Biodegradation is the process of using microorganisms to remove hazardous components of waste from the environment (Ogunjobi and Ekanem, 2017; Dua *et al*., 2002). Biodegradation has been described as the best technique for the remediation of polluted sites because it's environmentally friendly and effective. However, one of the major factors that affect the rate of biodegradation in the removal of pollutants from the environment is limiting amount of essential nutrients that support microbial growth such as nitrogen and phosphorus (Rodrigues *et al.,* 2020; Ogunjobi and Ekanem, 2017, Buraimoh *et al.,* 2017). Therefore, addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process (Okolo *et al*., 2005; Olabisi *et al*., 2009). The objective of this study was to determine the potential of cow dung as a biostimulant for enhanced biodegradation of spent engine oil in soil microcosms.

MATERIALS AND METHODS

Collection of soil samples and cow dung: Soil Samples were collected at the depth of 0 – 3 cm from Obasanjo farm Ota, Ogun State in polythene bags and kept in the refrigerator at 4 ^{o}C in the Biological Sciences Laboratory prior to microbiological and chemical analysis. The organic stimulant cow dung was collected from cattle abattoir along Idioroko road Ota, Ogun State.

Source of spent engine oil: Spent engine oil was collected from mechanic workshop at Bells University of Technology, Ota junction using a sterile plastic bottle and stored at room temperature (28±2 ^{o}C) prior to microcosm set up.

Physicochemical and microbiological analysis of soil samples: Physicochemical and microbiological analyses of soil sample were determined using standard methods. Moisture content was determined according to the method described by Obayori *et al.* (2008), pH was determined using pH meter (Jenway 3051) in 1:10 of the soil sample in a distilled water, organic carbon was determined according to Schumacher (2002) while total heterotrophic bacteria, hydrocarbon utilizing bacteria (HUB) and total fungi population counts were determined according to the method described previously by Amund and Nwokoye (1993). All analyses were carried out in duplicate.

Preparation of soil microcosms: Soil microcosm used in this study was prepared according to the method described by (Vidali, 2001). Briefly, soil samples (1kg) was weighed and placed in 8 aluminum trays (10cm diameter and height). Each weighed soil sample (1kg) was contaminated with 50ml of spent engine oil to give 5%v/w spent engine oil contamination. The first 6 trays were treated with 50g, 100g and 150g of cow dung in duplicates and were designated T1, T2 and T3 respectively. The last 2 trays were not treated with cow dung to serve as control. To each of the treatment and control, 60ml of sterile distilled water was added to moisten the soils. The microcosms step-up were kept on the laboratory bench to minimize loss of moisture via evaporation for 6 weeks.

Treatment 1 (T1) = 1kg soil + 50ml spent engine oil + 50g cow dung.
Treatment 2 (T2) = 1kg soil + 50ml spent engine oil + 100g cow dung.
Treatment 3 (T3) = 1kg soil + 50ml spent engine oil + 150g cow dung.
Control = 1kg soil + 50ml spent engine oil.

Determination of total hydrocarbon utilizing bacteria: The population of total hydrocarbon utilizing bacteria was enumerated on minimal salt media (MSM) formulated as described by Kastner *et al.* (1994). Briefly, 0.1mL of serially diluted soil samples were plated on oil agar prepared from mineral salt medium

containing Na_2HPO_4, 2.13g; KH_2PO_4, 1.30g; NH_4Cl, 0.50g; $MgSO_4.7H_2O$, 0.2g; Agar, 15g; Nystatin, 50µg/ml; pH, 7- 7.3 in 1 litre of distilled water, to which 1% spent engine oil was added (Abioye *et al.,* 2012). Inoculated plates were incubated at 35 ºC for 3-5 days. Colonies were counted after the incubation and results were recorded.

Determination of residual oil concentration (ROC): Residual oil in soil microcosms was determined gravimetrically according to the method described by Obayori *et al.* (2008). Briefly, residual oil was extracted from 5 g of soil sample collected weekly for analysis from each treatment and control using n-Haxane (30ml twice) (Sigma Aldreich). Soil sample and n-haxane was shaken vigorously in a conical flask for about 5 min to ensure that all the residual engine oil was extracted. The mixture was separated by decanting the solvent through filter paper into a new conical flask (100ml). The procedure was repeated and the solvent was evaporated from the extract by heating in an oven set at 50ºC. Residual oil was determined by mass difference using a sensitive weighing balance.

Hydrocarbon analysis using chromatography (GC-MS): The spent engine oil extracted before the bioremediation treatments and the residual spent engine oil extracted after 6 weeks of bioremediation treatments were subsequently subjected to Gas chromatography-Mass Spectrometry (GC-MS) analysis to identify the hydrocarbons and determine their abundance or intensities in the spent engine oil before and after 6 weeks of bioremediation treatments with cow dung. A Hewlett-Packard 6890 Gas Chromatograph (GC) equipped with 5973 Mass Spectrometer (MS) with HP 5MS (30 m × 0.25 mm I.D × 0.25 µm) fuse-silica capillary column was used for analysis.

The column temperature program was set at 100 ºC hold for 1 min, 15 ºC/min to 160 ºC and 5 ºC/min to 300 ºC and hold for 7 min. The GC injector was held isothermally at 280 ºC with a splitless period of 3 min. Helium was used as the carrier gas, at a flow rate of 1 ml/min by using electronic pressure control. The GC-MS interface temperature was maintained at 280 ºC.

The MS detector was operated in electron impact (EI) ionization mode with electron energy of 70 eV and the scan to determine appropriate masses for selected ion monitoring ranged from 50 to 500 amu (atom to mass unit). The injection volumes were 0.5 µl. Injector and detector temperatures were 270 ºC and 280 ºC, respectively (Hesham *et al.,* 2012). GC-MS library search was used to confirm the metabolites without standards.

RESULTS AND DISCUSSION

Physicochemical parameters and microbial loads of the soil sample: Results of the physico-chemical parameters and microbial loads of the soil sample are presented in Table 1. pH of the soil sample was 6.56 while the moisture content was 9.28%. The Total Organic Carbon, nitrate and phosphate content were 3.60%, 0.82mg/kg and 0.73 mg/kg respectively. However, the total heterotrophic bacterial count, fungal count and hydrocarbon utilizing bacterial count were 3.60×10^6 cfu/g, 2.2×10^4 cfu/g and 7.90×10^4 cfu/ml respectively. Determination of physico-chemical properties of soil samples used was very crucial in order to determine the physical factors, limiting nutrients, and pollutants that could be used as an indicator to determine the activity and type of microbial population that inhabit the soil (Haytham, 2016). The pH of the soil sample was weakly acidic (6.56). This result is similar to the report presented by Obayori *et al.* (2008). Unamended soil sample gave hydrocarbon utilizing bacteria population value of 7.9 $x10^4$ cfu/g, which is in line with the findings of Ijah and Antai (2003). It was reported by Haytham, 2016, that when the population of microorganisms capable of degrading the target contaminant is less than $1.05x10^2$ colony-forming units (cfu/g of soil), bioremediation will not occur at a significant rate. However, the microbial population present in soil samples were significantly about the right population that will support natural attenuation of polluted soil in the presence of the right amount organic and inorganic nutrients (Table 1).

Table 1: Physicochemical parameters and microbial loads of the soil sample

Parameters	Values
pH	6.56 ± 0.2
Phosphate (mg/kg)	0.73 ± 2.0
Nitrate (mg/kg)	0.82± 0.6
Moisture content (%)	9.28± 1.4
Total organic carbon (%)	3.60± 2.2
Total heterotrophic bacterial (cfu/g)	$3.6x10^6$
Total heterotrophic fungi (cfu/g)	$2.2x10^4$
Total hydrocarbon utilizing bacteria (cfu/g)	$7.9x10^4$

Biodegradation study in soil microcosm and residual oil concentration: The residual oil concentration (ROC) decreased with increasing microbial counts from week 1 to week 4 for all the treatments and Control. However, a decrease in microbial population was observed from week 5. The highest hydrocarbon utilizer counts were obtained at week 4 for all the set-ups (Figures 1-4). However, treatment 2 (T2) have the highest total hydrocarbon utilizing bacteria count compared to treatment 1 (T1), treatment (T3) and the control that was not amended with cow dung.

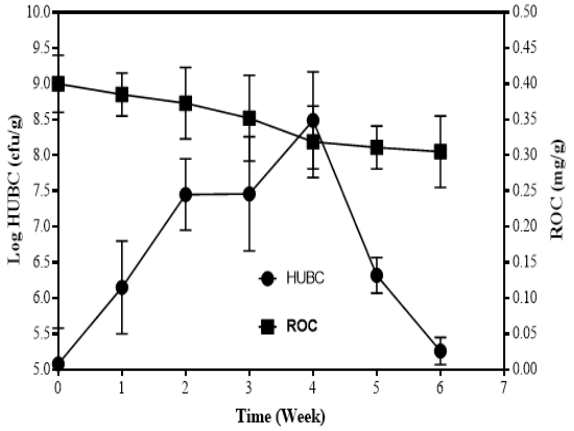

Fig 1: Residual oil content recovered and total hydrocarbon utilizing bacteria enumerated in control (CON) over a period of six weeks.

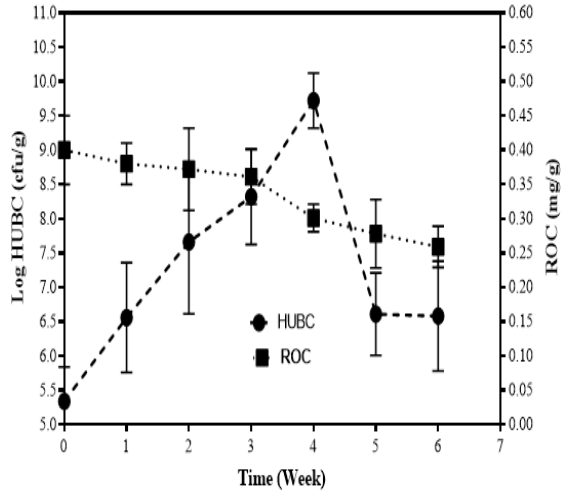

Fig 2: Residual oil content recovered and total hydrocarbon utilizing bacteria enumerated in treatment 1 (T1) over a period of six weeks experiment.

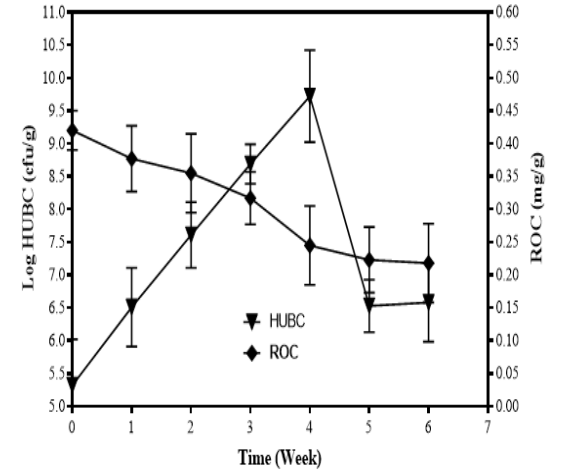

Fig 3: Residual oil content recovered and total hydrocarbon utilizing bacteria enumerated in treatment 2 (T2) over a period of six weeks experiment

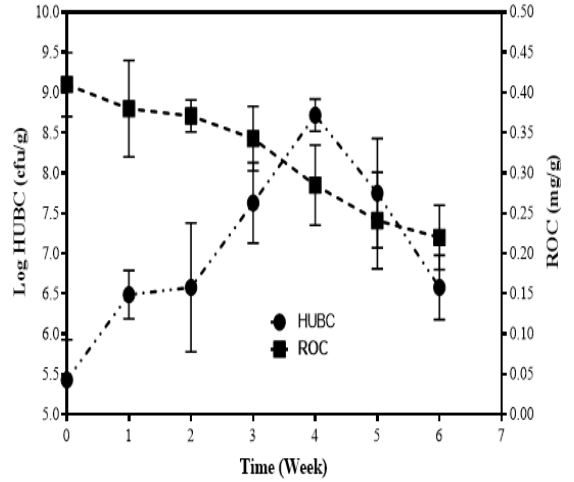

Fig 4: Residual oil content recovered and total hydrocarbon utilizing bacteria enumerated in treatment 3 (T3) over a period of six weeks experiment.

Table 2: Percentage degradation of spent engine oil after six weeks of incubation

Sample	% degradation
Treatment 1 (T1)	35.29
Treatment 2 (T2)	45.45
Treatment 3 (T3)	44.94
Control (CON)	23.81

Many bacterial species have been identified in organic compound degradation by different researchers (Abioye *et al.,* 2009; Bento *et al.,* 2005; Margesin *et al.,* 2007) and these are known to be the active degraders of these pollutants. Reports by previous researchers shows that limiting organic nutrients like phosphate and nitrate is the major factor that affects the rate at which indigenous microbial population remediate soils that are contaminated with spent engine and other hydrocarbons (Abioye *et al.,* 2009; Bento *et al.,* 2005; Margesin *et al.,* 2007). Residual oil concentration showed a remarkable decrease throughout the incubation period (0.400-0.259mg/g, 0.420-0.218mg/g and 0.410-0.220mg/g for treatments 1, 2 and 3 respectively) when compared to that of control which ranged from 0.400-0.304mg/g. The percentages of spent engine oil degraded in T1, T2, T3 and the control after 6 weeks of incubation were 35.29%, 45.45%, 44.94% and 23.81% respectively (Table 2). Ogunjobi and co-worker reported a similar result after they observed a reduction (50.7% and 14 %) for amended and unamended spent lubricating oil contaminated soils respectively after 42 days of incubation (Ogunjobi and Ekanem, 2017). However, it is noteworthy that the observed decrease in ROC of spent engine oil during the experiment may not only be attributed to microbial degradation process induced by biostimulation but due some other abiotic factors such as volatilization, sorption to soil particles, or photodegradation.

Gas chromatography-Mass Spectrometry (GC-MS) analysis of spent engine oil: Results obtained from the GC-MS analysis of the spent engine oil at week zero (before bioremediation treatment) showed that the spent engine oil contained both aromatic and aliphatic hydrocarbons.

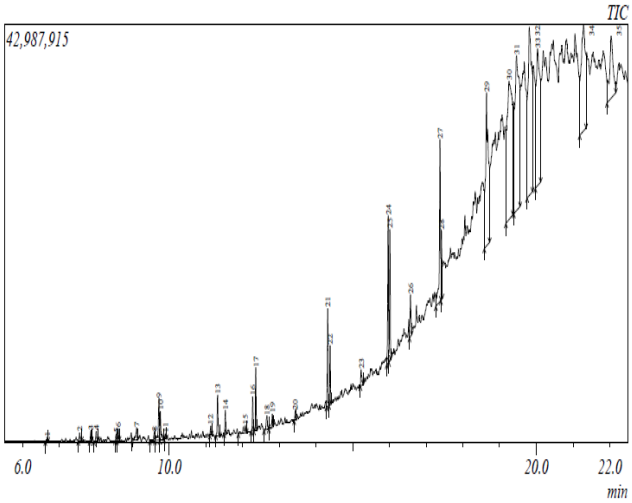

Fig 5: Gas chromatography profiles of spent engine oil recovered from the soil sample at week zero before the bioremediation treatments.

Table 3: The identified hydrocarbons and their abundance or intensities in spent automobile engine oil before bioremediation treatment

Peak	Retention time (min)	Identified compound	Abundance/ Intensity
1	6.674	1,2,4-Trimethylbenzene	461652
2	7.551	1,4-Diethylbenzene	864023
3	7.856	2,6-Dimethyl-1,3,5,7-octatetraene	1049577
4	8.005	1-Ethyl-2,3-dimethylbenzene	1016766
5	8.547	1,2,4,5-Tetramethylbenzene	723947
6	8.608	1,2,4,5-Tetramethylbenzene	1185260
7	9.108	1-Methyl-2-(2-propenyl)-benzene	1256983
8	9.591	1-Dodecene	901724
9	9.713	Dodecane	4300323
10	9.754	Azulene	3001614
11	9.894	3-Eicosene	536675
12	11.123	Dodecane	926548
13	11.313	2-Methylnaphthalene	4219866
14	11.524	1-Methylnaphthalene	2457730
15	12.066	17-Pentatriacontene	781728
16	12.269	9-Octadecene	3563759
17	12.358	Dodecane	6532097
18	12.659	2,6,10-Trimethylundecanoic acid	1442511
19	12.801	1,3-Dimethylnaphthalene	1363798
20	13.429	2-Methyltetracosane	1011941
21	14.317	1-Nonadecene	10042537
22	14.379	Tetratetracontane	6087533
23	15.227	2-Methyltetracosane	1682234
24	15.963	1-Nonadecene	15154897
25	16.010	Tetratetracontane	13536132
26	16.567	1,54-Dibromotetrapentacontane	4309860
27	17.376	Octacosanol	16654201
28	17.417	2-Methyltetracosane	7077370
29	18.641	17-Pentatriacontene	15791452
30	19.259	1,54-Dibromotetrapentacontane	14225917
31	19.463	1,54-Dibromotetrapentacontane	15910687
32	19.807	Hexacosyl pentafluoropropionate	17299911
33	20.034	1,54-Dibromotetrapentacontane	14059205
34	21.284	1,54-Dibromotetrapentacontane	10927048
35	22.042	1,54-Dibromotetrapentacontane	6400853

The 35 peaks of hydrocarbons detected in the spent engine oil before the bioremediation treatments were of high intensities (Figure 5 and Table 3). The GC-MS analysis of the residual spent engine oil recovered from T1, T2, T3 and the control after 6 weeks of incubation showed that there was a decrease in the number of peaks of hydrocarbons detected in all treatments and control (Figures 6, 7, 8 and 9 respectively) when compared to number of peaks of hydrocarbons detected in the spent engine oil before the bioremediation treatments. However, the reduction in the hydrocarbon contents was more remarkable in soil samples amended with cow dung compared to the control soil sample without cow dung fortification.

In Tables 4, 5, 6 and 7, the identified hydrocarbons in the residual spent engine oil recovered from T1, T2, T3 and the control respectively after 6 weeks of incubation are presented. The GC-MS result indicated that by end of week 6, there was a total disappearance of aromatic hydrocarbons from the residual engine oil recovered from all treatments and control (Tables 4 – 7).

However, the intensities and the number of hydrocarbons in the unamended control soil sample were higher than that of the cow dung amended soil samples. It is noteworthy that T2 amended with 100g of cow dung had the highest reduction of spent engine oil contents compared to T1, T3 and the control.

It could be observed that intensity of chromatographic peaks was more pronounced in control compared to T1, T2 and T3.

The least among the studied was the control (CON) where the peaks were much more pronounced.

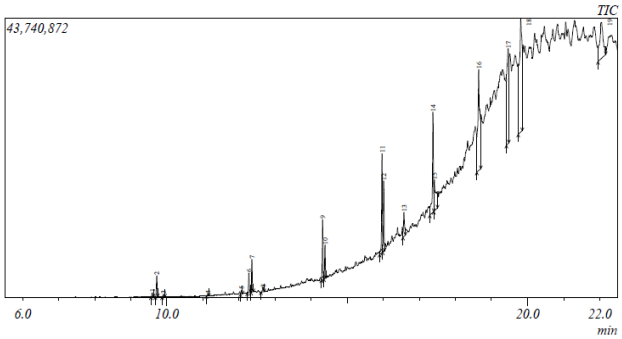

Fig 6: Gas chromatography profiles of residual spent engine oil recovered from the soil sample after 6 weeks of bioremediation treatment with 50g of cow dung (T1).

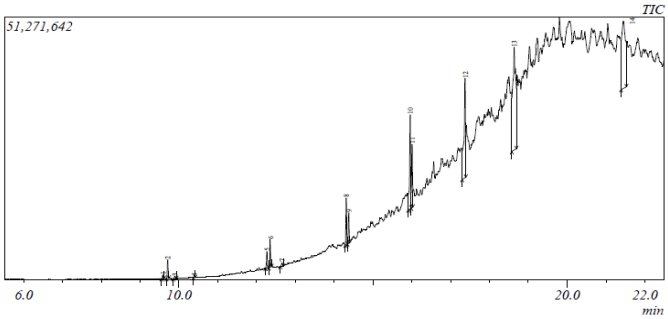

Fig 7: Gas chromatography profiles of residual spent engine oil recovered from the soil sample after 6 weeks of bioremediation treatment with 100g of cow dung (T2).

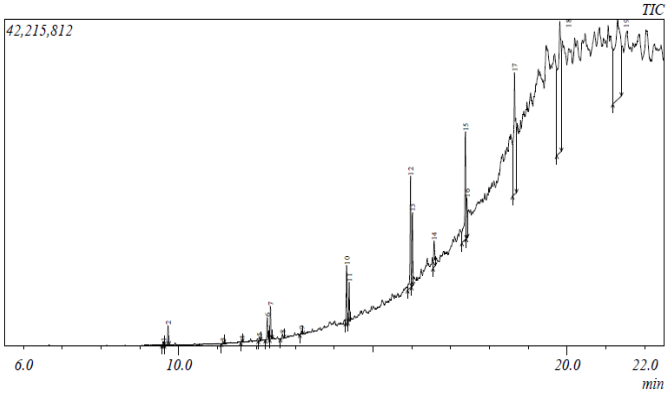

Fig 8: Gas chromatography profiles of residual spent engine oil recovered from the soil sample after 6 weeks of bioremediation treatment with 150g of cow dung (T3).

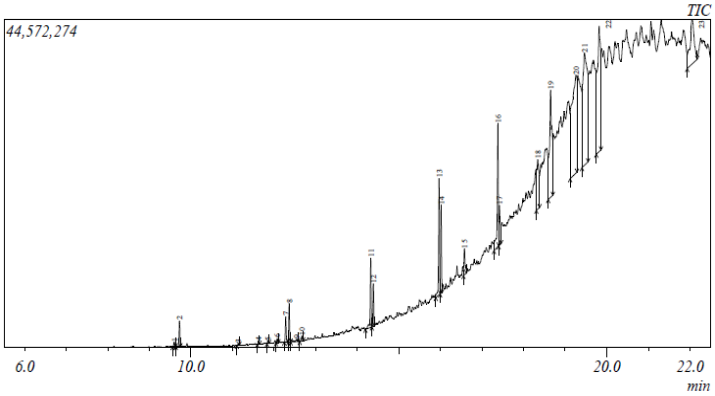

Fig 9: Gas chromatography profiles of residual spent engine oil recovered from the soil sample after 6 weeks of incubation without cow dung amendment (CON).

These observations could be attributed to the increase in degradation of spent engine oil due to supply of more organic nutrients present in cow dung. Cow dung has been reported to be rich in nitrogen (3%), phosphorus (2%) and potassium (1%), which are major limiting nutrients in soils contaminated with petroleum hydrocarbons (Girija *et al.*, 2013; Ogboghodo *et al.*, 2005; Onifade *et al.*, 2007). It increases moisture holding capacity and also improve aeration, thereby helping to breakup compacted soils. Biostimulation using organic waste has been reported previously to increase microbial population (bioaugumentation) and available nutrients (Edmo *et al*., 2020; Abioye *et al.*, 2012; Obayori, 2008). Residual oil concentration was used to determine the extent of the hydrocarbon degradation in the contaminated soil; the results obtained showed a decreasing trends as depicted in Figures 6-9.

Conclusion: Bacterial removal of spent engine oil from polluted soil microcosms occurred in all the soil microcosms samples studied. The constant decrease of spent engine oil in the microcosm indicates the presence of indigenous bacterial population in both the soil and cow dung that are capable of hydrocarbon degradation in soil. Thus, biostimulation using cow dung enhanced the biodegradation ability of indigenous microorganisms by increasing available essential nutrients that were limiting in the soil samples.

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Table 4: The identified hydrocarbons and their abundance or intensities in spent automobile engine oil after 6 weeks of bioremediation treatment with 50g of cow dung (T1)

Peak	Retention time (min)	Identified compound	Abundance/ Intensity
1	9.592	3-Tetradecene	643292
2	9.714	Dodecane	3268490
3	9.897	1-Pentyl-2-propylcyclopentane	365499
4	11.125	2-Methyltetracosane	262326
5	12.067	7-(Bromomethyl)-7-pentadecene	571441
6	12.270	Cetene	3061663
7	12.359	Dodecane	5162756
8	12.662	1-Docosanol	656930
9	14.318	1-Nonadecene	9371143
10	14.380	Tetratetracontane	5305568
11	15.964	1-Nonadecene	15513953
12	16.011	Tetratetracontane	11074583
13	16.568	1,54-Dibromotetrapentacontane	3839628
14	17.377	Octacosanol	15684069
15	17.415	1,54-Dibromotetrapentacontane	4882056
16	18.642	17-Pentatriacontene	15852810
17	19.464	1,54-Dibromotetrapentacontane	14873418
18	19.810	17-Pentatriacontene	17779288
19	22.055	1,54-Dibromotetrapentacontane	5629023

Table 5: The identified hydrocarbons and their abundance or intensities in spent automobile engine oil after 6 weeks of bioremediation treatment with 100g of cow dung (T2)

Peak	Retention time (min)	Identified compound	Abundance/ Intensity
1	9.584	7-Hexadecene	744065
2	9.706	Dodecane	3692291
3	9.888	7-(Bromomethyl)-7-pentadecene	417239
4	10.383	E,E-2,13-Octadecadien-1-ol	163789
5	12.263	1-Docosene	3202803
6	12.351	Octacosane	5358247
7	12.655	17-Pentatriacontene	693629
8	14.311	1-Nonadecene	9128901
9	14.373	Tetratetracontane	5987986
10	15.957	1-Eicosene	18330819
11	16.004	2-Methyltetracosane	12377503
12	17.371	Octacosanol	19426470
13	18.634	17-Pentatriacontene	20114866
14	21.449	17-Pentatriacontene	13154393

Table 6: The identified hydrocarbons and their abundance or intensities in spent automobile engine oil after 6 weeks of bioremediation treatment with 150g of cow dung (T3)

Peak	Retention time (min)	Identified compound	Abundance/ Intensity
1	9.595	7-Hexadecene	414198
2	9.717	Dodecane	2498570
3	11.127	2-Methyltetracosane	220898
4	11.616	7-(Bromomethyl)-7-pentadecene	310553
5	12.071	7-(Bromomethyl)-7-pentadecene	411072
6	12.274	9-Octadecene	2847511
7	12.361	Octacosane	4186992
8	12.666	1-Docosanol	587584
9	13.158	E-11-Hexadecenal	506630
10	14.320	1-Nonadecene	7363519
11	14.383	Tetratetracontane	5090669
12	15.966	1-Nonadecene	14388023
13	16.013	Tetratetracontane	9482543
14	16.569	1,54-Dibromotetrapentacontane	3376838
15	17.380	Octacosanol	13897751
16	17.416	1,54-Dibromotetrapentacontane	5159621
17	18.642	Tricosyl pentafluoropropionate	15726126
18	19.809	17-Pentatriacontene	16988609
19	21.297	1,54-Dibromotetrapentacontane	10393241

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Table 7: The intensity or abundance of identified hydrocarbons in spent automobile engine oil after 6 weeks of biodegradation without cow dung fortification (CON)

Peak	Retention time (min)	Identified compound	Abundance/ Intensity
1	9.598	cis-3-Tetradecene	667350
2	9.719	Dodecane	3514731
3	11.131	n-Tetratetracontane	273459
4	11.618	7-(Bromomethyl)-7-pentadecene	415505
5	11.848	Dodecane	376049
6	12.073	7-(Bromomethyl)-7-pentadecene	566748
7	12.275	Cetene	3440615
8	12.364	Dodecane	5273097
9	12.539	17-Pentatriacontene	293137
10	12.667	1-Docosanol	710128
11	14.323	1-Nonadecene	9498786
12	14.384	Tetratetracontane	5878126
13	15.968	1-Nonadecene	15843601
14	16.015	Tetratetracontane	12049468
15	16.572	1,54-Dibromotetrapentacontane	3560896
16	17.382	Octacosanol	16792934
17	17.418	1,54-Dibromotetrapentacontane	5416562
18	18.341	1,54-Dibromotetrapentacontane	6750285
19	18.647	17-Pentatriacontene	14579104
20	19.260	1,54-Dibromotetrapentacontane	13331340
21	19.467	1,54-Dibromotetrapentacontane	15300104
22	19.815	1,54-Dibromotetrapentacontane	17013009
23	22.054	1,54-Dibromotetrapentacontane	5977014

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