



Potential of Chicken Droppings in Reclaiming Diesel-Contaminated Soil from a Farmland situated at Gonin-gora, Chikun Local Government Area (L.G.A), Kaduna State, Nigeria

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ABSTRACT: Diesel pollution of soil is widespread and adversely affects soil fertility, plant growth and soil microflora population. This study determined the potential of chicken droppings in reclaiming diesel-contaminated soil from a farmland situated at Gonin-gora, Chikun Local Government Area (L.G.A), Kaduna State, Nigeria using standard methods. Three earthen pots containing 1kg of diesel-contaminated soil each were amended with chicken droppings. Sample A, B and C were amended with 100g, 200g and 300g of pulverized chicken droppings respectively. A fourth sample D was maintained as the control (contained diesel-contaminated soil but was not amended). The duration of the experiment was 12 weeks and sampling was carried out monthly for changes in diesel-utilizing bacteria, physicochemical properties and diesel degradation. The diesel-utilizing bacteria ranged from 1.86×10^6 CFU/g to 7.36×10^6 CFU/g. Samples amended with chicken droppings had higher bacterial growth than the control sample. The diesel-utilizing bacteria identified in this study belonged to the genera *Bacillus*, *Acinetobacter*, *Pseudomonas*, *Micrococcus* and *Staphylococcus*. The degradation of diesel was monitored using the weight loss method and there was significant degradation in the diesel content after the study period. The highest degradation of diesel was recorded in Sample C (polluted soil + 30% CD) (30.1%); followed by sample B (polluted soil + 20% CD) (28.3%); then sample A (polluted soil + 10% CD) (22.6%). Sample D (polluted soil without amendment) which was the control had the lowest (17.2%) degradation of diesel. GC-MS showed a significant decrease in carbon compounds of the residual diesel in all samples after the study period. Thus, the results obtained demonstrated the potential of chicken droppings for enhanced bioremediation of diesel-contaminated soil.

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The major sources of energy for daily life and for the industries are petroleum-based products (Agarry and Latinwo, 2015). However, when these products are leaked or spilled on the environment either during exploration, production, refining, transport and storage, they cause contamination (Agarry and Latinwo, 2015). This contamination poses a global threat to the ecosystem, and thereby affecting human health. Among petroleum products, diesel oil is a complex mixture of alkanes and aromatic compounds that are frequently reported as soil contaminants

leaking from storage tanks and pipelines or released in accidental spills (Gallego *et al.*, 2001). Diesel is produced by the fractional distillation of crude oil between 200°C and 350°C at atmospheric pressure, resulting in a mixture of carbon chains that typically contain between 8 and 21 carbon atoms per molecule (Demirel, 2012). Diesel oil contains low molecular weight compounds that are usually more toxic than long-chained hydrocarbons, because long-chained ones are less soluble and less bioavailable (Dorn *et al.*, 2000). Light oils contain a relatively high proportion

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of saturated hydrocarbons, hence these can be more toxic than heavy oils (Kauppi, 2011). Diesel oil contamination of the soil can create an anaerobic condition in the soil, coupled with water logging and acidic metabolites; the result is high accumulation of aluminum and manganese ions, which are toxic to plant growth. Soil contaminated with diesel can induce several pathologies including encephalopathy, arrhythmia, acidosis and dermatitis (Tormoehlen *et al.*, 2014). When there is ingestion of this hydrocarbon, it can lead to pneumonitis (Mickiewicz and Gomez, 2006). Technologies such as Mechanical, burying, evaporation, dispersion, and washing are the common technologies employed in remediating diesel-contaminated soil. However, these technologies are expensive and can lead to incomplete decomposition of contaminants (Das and Chandra, 2011). Therefore there is need for utilizing technologies that are less expensive and eco-friendly. Bioremediation, a technology which involves the use of microorganisms to detoxify or remove pollutants through the mechanisms of biodegradation is environmentally-friendly, non-invasive and relatively cost-effective (April *et al.*, 2000). This process is based on the ability of certain microorganisms to convert, modify and utilize toxic pollutants in order to obtain energy and biomass in the process (Tang *et al.*, 2007). When compared to chemical technologies used in degrading hydrocarbon, bioremediation is simple, cheap and less labour intensive due to their role in the environment (Sharma, 2012). Also upon completion, the residue from bioremediation are harmless products such as water, carbon dioxide and cell biomass (Abatenh *et al.*, 2017). Bioremediation promotes natural clean-up of dangerous toxins by the environment and can either be done *in-situ* or *ex-situ*. *In-situ* bioremediation is carried out at the site of interest whereas in *ex-situ* bioremediation, the contaminated soil is collected and processed at an offsite area such as a laboratory for cleansing (Adelana *et al.*, 2011). There are strategies involved in bioremediation such as bioventing, biostimulation, bioaugmentation, biopiles and bioattenuation. Lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon by microorganisms in soil and water environment (Abioye *et al.*, 2012). However, biostimulation is a type of natural remediation that can improve pollutant degradation by optimizing conditions such as aeration, addition of nutrients, pH and temperature control (Margesin *et al.*, 2000). This kind of strategy injects specific nutrients at the contaminated site (soil/ground water) to stimulate the activity of indigenous microorganisms. It focuses on the stimulation of indigenous microbial community present in the contaminated environment (Kumar *et*

al., 2011; Adams *et al.*, 2015). Part of the stimulation is the addition of nutrients and oxygen to the contaminated site which helps indigenous microorganisms to degrade the contaminant properly. These nutrients are the basic building blocks of life and allow microbes to create the basic requirement for example, energy, cell biomass and enzymes to degrade the pollutant. All of them will need nitrogen, phosphorous and carbon (Madhavi and Mohini, 2012). Nutrients can be gotten from plant residues, animal wastes for the purpose of stimulating hydrocarbon-contaminated soil.

Ogboghodo *et al.* (2004) reported that the addition of poultry manure to crude oil-polluted soil not only increased the growth of autochthonous microorganisms but also enhanced plant height and this proposed the adoption of poultry manure for the stimulation of hydrocarbon in the soil as a good technique of battling petroleum contamination in the natural environment. Another study by Okafor *et al.* (2016) has proven that poultry manure is rich in organic matter and therefore encourages the growth of a substantial amount of microorganisms. According to Hamid *et al.* (2005), the addition of chicken manure as a nitrogen source may be necessary to increase microorganism populations at a hydrocarbon contaminated site. An examination of chicken-dropping for oil spill remediation was carried out (Ijah and Antai, 2003) and the results indicated that chicken droppings enhanced degradation of the crude oil in the soil environment. A study showed that bacteria in chicken manure were able to break down 50 percent more crude oil than soil lacking the amendment (Bello *et al.*, 2009). The aim of this study was to determine the potential of chicken droppings in reclaiming diesel-contaminated soil from a farmland situated at Gonin-gora, Chikun Local Government Area (L.G.A), Kaduna State, Nigeria.

MATERIALS AND METHODS

Collection and Processing of Samples: Pristine soil samples (20 Kg) used for this study was collected at a depth of 0-20 cm, from a farmland situated at Gonin-gora, Chikun Local Government Area (L.G.A), Kaduna State, Nigeria using a shovel at two different points and mixed together to obtain a composite sample (Nwogu *et al.*, 2015). The soil sample was prepared for bioremediation by first removing sticks and stones (Feyisayo *et al.*, 2018) and then air dried, sieved through 2mm standard mesh sieve (Ugochukwu *et al.*, 2016). The diesel (5 L) used in this study was purchased from a commercial petroleum filling station using a clean plastic can and transported to the laboratory at the Department of Microbiology, Kaduna State University, Kaduna, Nigeria. Chicken

droppings (4 Kg) were obtained from a local poultry house in Federal Housing Estate Gonin-gora, Kaduna State, Nigeria. The chicken droppings were air-dried for two weeks and then pulverized into powder using a mortar and pestle. The pulverized chicken droppings were then passed through a 2 mm standard mesh sieve (Ugochukwu *et al.*, 2016).

Enumeration of diesel-utilizing bacteria (DUB): The media used for the enumeration of diesel-utilizing bacteria (DUB) in the two samples was mineral salt medium of Zajic and Supplisson (1972) with the following composition (1.8 g K_2HPO_4 , 4.0 g NH_4Cl , 0.2 g $MgSO_4 \cdot 7H_2O$, 1.2 g KH_2PO_4 , 0.01 g $FeSO_4 \cdot 7H_2O$, 0.1 g NaCl, 20 g agar agar, 1% diesel oil in 1,000 mL distilled water, pH7.4 with 50 g/L Nystatin added to medium to inhibit fungi growth). The diesel oil agar plates were incubated at 30°C for 5 days (Tyabo *et al.*, 2019). Pure isolates were obtained by repeated sub-culturing on nutrient agar.

Characterization and Identification of diesel-utilizing bacteria: The bacterial isolates were characterized using colonial morphology, gram staining and standard biochemical tests (Cheesebrough, 2006) and identified by comparing their characteristics with those of known taxa using Bergey's Manual of Systemic Bacteriology (Vos *et al.*, 2009).

Determination of Physicochemical Properties of Soil: HANNA Instrument (Multiparameter Photometer with COD model HIB3399) was used for the determination of nitrogen, phosphorus and potassium. In brief, 5g of every sample was weighed in three test tubes each. Each test tube was introduced into the sample chamber and the reagents (nitrogen, phosphorus and potassium) were added to each of the sample. The reaction time of 20 min. was set and device was allowed to run.

pH Determination: The pH was determined using HANNA instruments model HI 9813-6 on 1:2.5 (w/v) soil/water mixture, after 10 min. equilibration.

Bioremediation studies: One kilogram (1 Kg) of soil sample was introduced into four different earthen pots (EP) labeled A to D. The EP A to D were polluted with 200 g (w/w) of diesel oil. EP A, B and C were amended with 10g, 20g and 30g of chicken droppings respectively while EP D served as control which was not amended with chicken droppings. 100 mL of distilled water was introduced into each EP and the contents were mixed thoroughly and incubated at room temperature for 12 weeks. Sampling was carried out monthly for a period of 12 weeks. The samples

were analyzed for changes in nitrogen, phosphorus, potassium, bacterial counts and degradation of diesel.

Biodegradation of diesel: The Environmental Protection Agency (EPA) 418.1 method of Kulkarni (2014) was used to determine the extent of diesel biodegradation in the contaminated soil. Five grams (5 g) of the sample was introduced into a beaker containing 50 mL of carbon tetrachloride (CCL_4) and then mixed properly using a stirrer for 10 min. before decanting the solvent containing dissolved hydrocarbons. The solution was weighed and the percentage increment in the solution was the total petroleum hydrocarbon present in the sample.

Gas chromatography-Mass spectrometry (GC-MS) of Residual Diesel (recovered from soil after bioremediation): Residual diesel samples were analyzed using gas chromatography/mass spectrometry (GC/MS), using Agilent-Technologies (Little Falls, CA, USA) 6890N Network GC system, equipped with an Agilent-Technologies 5975 inert XL Mass selective detector and Agilent- Technologies 7683B series auto injector.

Statistical Analysis: Individual statistics were used to obtain mean and standard error (SE). One-way Analysis of Variance (ANOVA) was carried out to determine significant difference ($p < 0.05$) or otherwise among treatment options. Correlation analysis was carried out to establish a relationship between hydrocarbon degradation and bacterial counts. The data obtained were analyzed using Statistical Package for Social Science (SPSS) 20.0 and averages compared with Duncan Multiple Range Test (DMRT) (Tyabo *et al.*, 2019).

RESULTS AND DISCUSSION

Bacterial Counts: The total diesel-utilizing bacterial (TDUB) count of all experimental samples is presented in Table 1. Samples treated with chicken droppings exhibited increase in TDUB counts than in the control sample throughout the study period. The control sample showed gradual decrease in TDUB counts from the beginning to the end of the study. At the end of the study, sample C (polluted soil + 30% CD) had the highest bacterial count of 7.36×10^6 CFU/g while sample D (polluted soil without amendment) had the least bacterial count of 1.86×10^6 CFU/g. However, there was no significant difference ($p > 0.05$) in TDUB counts among the samples but TDUB counts with respect to time (days) showed significant differences ($p < 0.05$).

Identification of Bacterial Isolates: A total of twelve (12) bacterial isolates were obtained from all the

samples. The probable diesel-utilizing bacteria isolated during the study belonged to five genera. The genus, *Bacillus* had the highest frequency of occurrence (33.3%), followed by *Pseudomonas* (25%), *Acinetobacter*, *Micrococcus* (16.6% each) and *Staphylococcus* (8.3%).

Table 1: Total Diesel-Utilizing Bacterial (TDUB) Counts in Diesel-Polluted Soil Amended with Chicken Droppings

Time (days)	TDUB counts ($\times 10^6$ CFU/g) Treatments			
	A	B	C	D
0	4.63 \pm 2.6 ^a	5.33 \pm 2.5 ^a	5.96 \pm 1.5 ^a	3.23 \pm 5.13 ^c
30	4.69 \pm 2.8 ^a	5.53 \pm 3.5 ^a	6.33 \pm 2.1 ^a	2.53 \pm 2.1 ^d
60	5.02 \pm 2.6 ^a	5.73 \pm 3.1 ^a	7.13 \pm 3.1 ^a	2.0 \pm 1.7 ^d
90	5.11 \pm 3.1 ^a	5.93 \pm 7.7 ^a	7.36 \pm 3.2 ^a	1.86 \pm 3.7 ^d

Values are mean of duplicates \pm SD, Values with different alphabets across a row are significantly different ($p < 0.05$)
 Key: A: (polluted soil + 10% CD); B: (polluted soil + 20% CD); C: (polluted soil + 30% CD); D: (polluted soil without amendment).

Physicochemical parameters of Samples: The nitrogen, phosphorus and potassium content were observed in all samples throughout the entire study period. The nitrogen content in all the samples ranged from 0.1% to 2.02% throughout the study. It was observed that in all samples, the nitrogen content increased steadily throughout the study period with Sample C (polluted soil + 30% CD) having the highest nitrogen content (2.02%) at the end of the study. The nitrogen contents showed significant differences ($p < 0.05$) among the treatments. Figure 1 shows the changes in nitrogen content from the initial day to the final day of the remediation study. It was observed that the phosphorus content in all samples increased throughout the study period. The phosphorus content ranged from 0.08% to 1.24%. There were significant differences ($p < 0.05$) in phosphorus contents among the samples. Figure 2 shows the changes in the phosphorus content from the initial day and the final day of remediation. Figure 3 shows the changes in the potassium content on the initial day of remediation and the final day of remediation. Potassium content was observed to increase in the samples. The control sample D (polluted soil without amendment) showed the least potassium content (0.085%) at the beginning of the study while sample C (polluted soil + 30% CD) showed the highest potassium content (0.88%) at the end of the study. There was no significant difference in the potassium content among the samples.

pH Determination: The pH of the samples ranged from pH 7 to 9. The control sample D (polluted soil without amendment) had the lowest pH (7.8) while sample B had the highest pH (9.9). There were no significant differences ($p > 0.05$) in pH values among

the samples. Table 2 shows the changes in pH in all the samples.

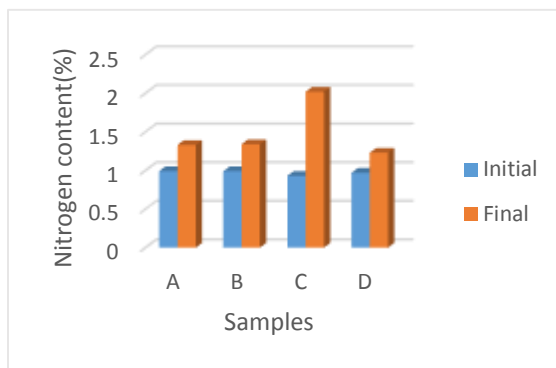


Fig 1: Changes in nitrogen content of diesel-polluted soil remediated with chicken droppings

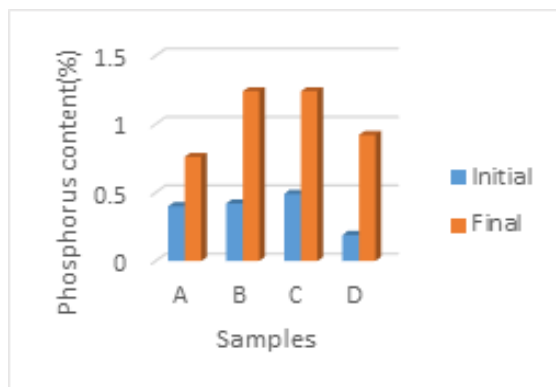


Figure 2: Changes in phosphorus content of diesel-polluted soil remediated with chicken droppings

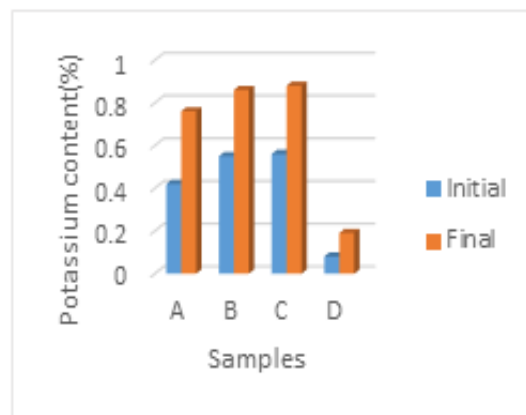


Figure 3: Changes in potassium content of diesel-polluted soil remediated with chicken droppings

Diesel biodegradation: The extent of diesel degradation is showed in Table 3. The highest diesel degradation was recorded in sample C (polluted soil + 30% CD) (30.1%) while the control sample D (polluted soil without amendment) had the lowest diesel degradation (17.2%).

Table 2: Changes in pH values in samples throughout the study

Sampling days	pH Values			
	A	B	C	D
0	8.2±0.07 _a	8.7±0.14 ^{ab}	8.95±0.07 _a	7.85±0.07 _b
30	8.6±0.07 _a	8.75±0.21 ^a	8.9±0.00 ^a	7.85±0.07 _b
60	8.7±0.07 _a	9.05±0.07 ^a	9.45±0.07 _a	8.5±0.14 ^{ab}
90	8.9±0.07 _a	9.9±0.00 ^a	9.85±0.07 _a	8.9±0.00 ^a

Values are mean of duplicates ± SD, Values with different alphabets across a row are significantly different (p<0.05)
 Key: Key: A: (polluted soil + 10% CD); B: (polluted soil + 20% CD); C: (polluted soil + 30% CD); D: (polluted soil without amendment).

Table 3: Percentage weight loss of diesel during the study

Samples	Initial weight	Final weight	Weight loss of Diesel (%)
A	5.3	4.1	22.6
B	5.3	3.8	28.3
C	5.3	3.7	30.1
D	5.8	4.8	17.2

Gas chromatography-Mass spectrometry: Gas chromatography mass spectrometry (GC-MS) is the method used for identification of compounds, its application allows the detection of carbon compounds

in the samples. (Lawal *et al.*, 2015). Figure 4 shows the chromatogram of the original diesel used for the remediation study. It consisted of 59 carbon compounds and the chromatogram shows varying peaks. The major compounds present in the original diesel were aliphatic, cyclohexanes, dodecane, octane and nonane. All the treatments were able to degrade diesel to certain extents in the different samples. After the study period, the carbon compounds present in sample A (polluted soil + 10% CD) were reduced to 7 as compared 59 first observed in the original diesel. The compounds present were majorly naphthalene and benzene compounds. Figure 5 shows the chromatogram of the residual diesel after remediation. Sample B (polluted soil + 20% CD) had 8 carbon compounds at the end of the study with naphthalene as its major compound. Figure 6 shows the chromatogram of the residual diesel after remediation. Sample C (polluted soil + 30% CD) shows the highest reduction in carbon compounds (3) at the end of the study. Pentane compounds were major in this sample. Figure 7 shows the chromatogram of the residual diesel after remediation. The control sample D (polluted soil without amendment) had 22 carbon compounds at the end of the study. Figure 8 shows the chromatogram of the residual diesel after remediation.



Fig 4: Chromatogram of Diesel



Fig 5: Chromatogram of treatment A (polluted soil + 10% CD) after remediation

This study revealed that diesel-utilizing bacterial count was higher in samples amended with chicken

droppings than the control sample which was not amended. The organisms increased with time

gradually as diesel degraders adapted and began to breakdown diesel to other components that can be utilized by other indigenous bacterial cells. Proliferation of bacteria may be due to the addition of nutrients from the biostimulants (especially nitrogen and phosphorus) into the treated soil samples (Ijah and Antai, 2003; Adesodun and Mbagwu, 2008). Macro elements and micro elements serve as nutrients for the proliferation and maintenance of microorganisms (Lee *et al.*, 2003). The diesel-utilizing bacterial counts in this study ranged from 4.63×10^6 CFU/g to 7.36×10^6 CFU/g; this result is comparable with that of Ibiene *et al.* (2011) who reported that the total culturable hydrocarbon utilizing bacterial counts in crude oil contaminated soil ranged between 1.8×10^3 CFU/g and 5.4×10^6 CFU/g. Differences in bacterial counts could be due to the different experimental soils used or diverse microbial ecology (Tyabo *et al.*, 2019). Increase in hydrocarbon-utilizing bacteria as a result of addition of animal wastes have been reported by other researchers (Nwogu *et al.*, 2015; Williams and

Amaechi, 2017). Lower diesel-utilizing bacterial counts in the unamended sample could be as a result of depletion of limiting nutrients (Adebusoye *et al.*, 2007). In this study, bacteria isolated were from five genera with *Bacillus* having the highest occurrence. *Bacillus* has also been isolated by other researchers (Ijah and Antai, 2003; Nwogu *et al.*, 2015; Tyabo *et al.*, 2019). Its high occurrence in hydrocarbon-contaminated soils could be as a result of its ability to survive harsh condition by the formation of spores. Also, its hydrocarbon degrading enzyme system and ability to emulsify petroleum hydrocarbon is another reason for its high occurrence in Nigerian soils (Ijah and Antai, 2003). *Pseudomonas*, *Acinetobacter*, *Micrococcus* and *Staphylococcus* were also isolated in this study which corresponds with the work of Tyabo *et al.* (2019) with exception of *E. coli*. Presence of certain bacteria in a hydrocarbon degraded soil suggests that they are dynamic degraders of hydrocarbon.

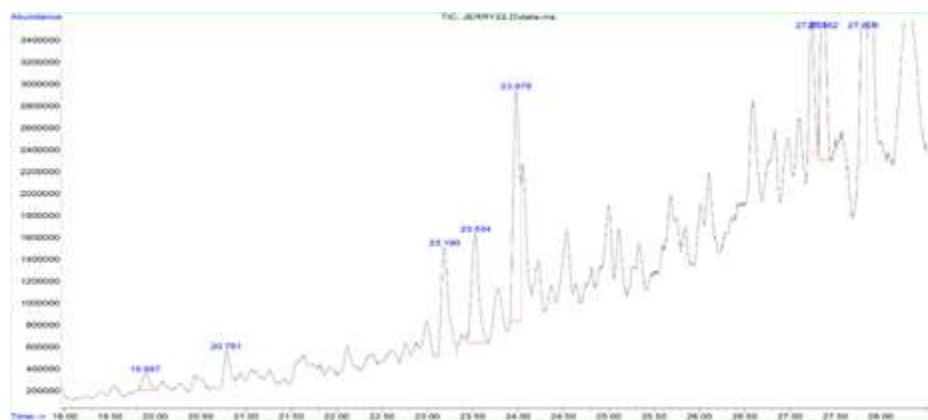


Fig 6: Chromatogram of treatment B (polluted soil + 20% CD) after remediation

Nitrogen, phosphorus and potassium contents increased throughout the study in amended samples. Unamended samples showed decrease. The increase in these nutrients could be as a result of the addition of chicken droppings which increased the nutrients in the amended samples. Nitrogenous compound and other necessary nutrients present in chicken droppings were reasons for this increase. This result differs with that of Nwogu *et al.* (2015), who observed decrease in nitrogen, phosphorus and potassium levels in amended samples for a period of 42 days. Nwogu *et al.* (2015) treated 1 Kg of contaminated soil with 20g of organic waste. However, 1 Kg of contaminated soil was treated with 100/200/300g of chicken droppings in this present study. The ratio of organic waste to soil used by Nwogu *et al.* (2015) could have been the reason for

the decrease in nitrogen, phosphorus, potassium contents of the soil. This experiment revealed that the pH of all the amended samples increased with time. This is similar to the report of Adams *et al.* (2015) who also observed increase in pH with time after amending soil with organic manure. Bacterial growth and activity are readily affected by pH and in this study, the pH ranged from 6.8 to 9.9. This observation slightly differs from the pH range (6.0 to 8.9) that was reported by Boonchan (2000) as the best pH range for bioremediation of hydrocarbon polluted soils and that these changes in pH level could be due to the release of acidic and alkaline intermediates and final products during biodegradation of hydrocarbons, which has an effect on the pH.

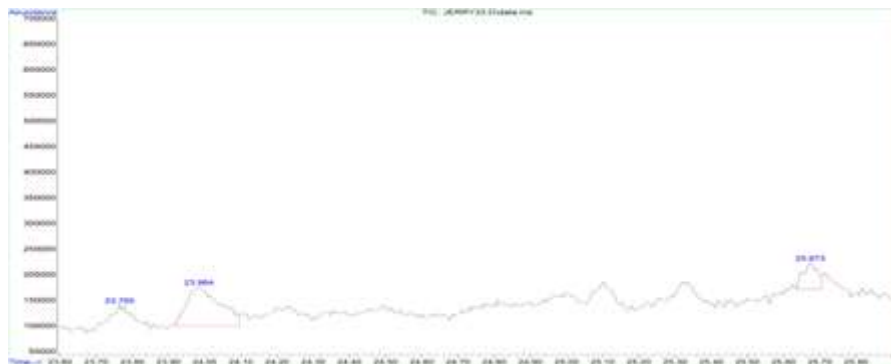


Fig 7: Chromatogram of treatment C (polluted soil + 30% CD) after remediation



Fig 8: Chromatogram of sample D (polluted soil without amendment) after remediation

The results from this study showed that chicken droppings aided in the degradation of diesel. Therefore all the treatments exhibited ability to enhance hydrocarbon bacteria degradation. A similar observation has been reported for crude oil degradation using poultry manure (Ijah and Antai, 2003; Okolo *et al.*, 2005). The samples amended with chicken droppings degraded diesel more than the sample that was not amended. This may possibly be due to a higher nutrient level present in chicken droppings. Also chicken droppings could be carriers of hydrocarbon-utilizing bacteria (Agarry *et al.*, 2010). The Biodegradation recorded in the un-amended soil sample (17.2%) could be due to non-biological factors such as evaporation, photo-degradation (Williams and Amaechi, 2017); volatilization, adsorption, abiotic factors (temperature and pH) (Onouha, 2013). Reduction of petroleum hydrocarbon in un-amended sample has also been reported by other researchers (Nwogu *et al.*, 2015; Obiakalaje *et al.*, 2015; Idowu and Ijah, 2017). The GC-MS result in this study showed that chicken droppings enhanced degradation of diesel by reducing carbon compounds. The carbon compounds dodecane, benzene, cyclohexane, eicosane were similar to those reported by other researchers (Feyisayo *et al.*, 2018, Tyabo *et al.*, 2019) with few differences. The differences in carbon compounds after the remediation study could be due

to difference in degradative enzyme system of the bacteria present in the soil (Auta *et al.*, 2014). The unamended sample at the end of the study had 22 carbon compounds; this could be due to absence of bacteria. In contrast, amended samples at the end of the study had low carbon compounds (7, 8, 3). This could be due to the attenuation of fractions in amended samples thus indicating hydrocarbon degradation by organisms (Tyabo *et al.*, 2019).

Conclusion: Diesel pollution of soil is widespread and has adverse effects, it is therefore necessary to remediate diesel-polluted soils using cost effective, environmental friendly and more reliable techniques such as bioremediation. Chicken droppings used in this study for the remediation of diesel polluted soil proved to be effective. Chicken dropping is an abundant source of nitrogen, phosphorus and potassium, which have great potential for enhanced bioremediation of diesel-polluted soils and it's therefore recommended for reclaiming hydrocarbon-contaminated soil.

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