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The Effects and Efficacy of Chicken Manure Digestates on Bioremediation of Petroleum Hydrocarbons Polluted Soils

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ABSTRACT

The efficacy of chicken manure digestates for the bioremediation of hydrocarbons polluted soils (HPS) was studied. Two levels (5 and 10%) of spiked HPS were studied using three levels (0, 10 and 20%) of nutrient stimulation with chicken manure digestates (CMD) and observed at different time intervals (0, 14, 28, 56, 84, 168 and 336 days). Analysis of samples was carried out using a GC-FID according to standard procedures. Results obtained indicate that the 10% CMD treated samples gave the highest degradation of Total petroleum hydrocarbons (TPH) with values of 59% and 39% TPH removal respectively in the 5 and 10% polluted soils. While the 20% CMD treatments gave values of 52% and 35% remediation respectively for 5 and 10% polluted soils. Time was also observed to influence the rate of remediation with day 168 of the 20% CMD stimulation with remediation values of 84% and 80% TPH removal in the 5 and 10% HPS respectively. The 10% CMD stimulation presented better results for short–term remediation while 20% CMD was better for long term bioremediation with applications for highly hydrocarbon polluted soils. The study revealed that CMD could be a good alternative organic stimulation for the remediation of organic pollutants in soils.

Keywords: Bioremediation, Chicken Manure Digestates, Petroleum Hydrocarbons, Polluted Soils

INTRODUCTION

Bioremediation technology has gained several applications in the remediation of numerous environmental pollutants including heavy metals and petroleum products due to its environmental compatibility, friendliness and cost effectiveness [1-3]. Basically, the drivers of bioremediation are micro-organisms present in the environment. Consequently, conditions that are detrimental to growth, diversity and activities of microbes are to be ameliorated and those that would promote microbial population and diversity as well as increase their metabolism are to be provided during bioremediation processes. This has led researchers to investigate various ways of enhancing bioremediation methods.

Remediation by enhanced natural attenuation (RENA) is one of the evolved bioremediation technologies in recent time [1-3]. The three basic approaches to RENA are biostimulation involving provision of sufficient nutrient (particularly nitrogen (N) and phosphorus (P)); bio-augmentation which involves microbial inoculation to increase microbes' population and diversity in the polluted environment and bio-facilitation (such as Landfarming) which aims on improving soil physicochemical conditions and microbial accessibility to pollutants, as well as increasing oxygen supply to the microbes in the pollutants' impacted environment. One of the conventional RENA techniques is landfarming which basically involves the tilling of polluted soils and spreading the excavated soils for increased pollutants' oxidative potentials [4].

Landfarming is environmentally compatible and cost effective. Therefore, a combination of landfarming with nutrient stimulation or microbial inoculation or both would be a better enhancement protocol for the remediation of hydrocarbons pollutants in soils. The use of inorganic fertilizers and composted animal dung and poultry droppings in enhancement of landfarming of petroleum polluted environment has been well documented [3, 5]. However, there are scanty reports on the use of organic bio-digestates such as chicken layers droppings digestates in the remediation of petroleum polluted soils. Besides, it has been reported that bioremediation technique would not be effective when the level of petroleum pollution in soils is above 5% [5].

Therefore, the aim of this study is to seek better form of nutrient stimulation during bioremediation particularly for higher levels of hydrocarbons pollution in soils. The objectives include: (1) To evaluate the nutrient content of locally generated chicken manure digestates (2) To evaluate the inoculate potentials of locally bio-digested chicken droppings, (3) To determine the effectiveness and efficacy of CMD in the remediation of petroleum hydrocarbon pollutants in soils. In this study two levels of 5 and 10% of soil pollution by hydrocarbon products were investigated. The level of removal of the total petroleum hydrocarbons (TPH) was used as the indices for the assessment. The results obtained would contribute to the existing data on the use of various composts in the bioremediation of organic and inorganic pollutants from soils

MATERIALS AND METHODS

Petroleum Products and Analytical Chemicals

BTEX, naphthalene and other analytical regents were purchased from Sigma-Aldrich, UK through Pyrex-IG Scientific Company, Benin City, Nigeria. All the chemicals and equipment used for the study were of analytical grade.

Location of Study and Chicken Manures Digestate Preparation

This work was carried out at Department of Chemistry, Faculty of Physical Sciences, University of Benin, Benin City, Nigeria, under a screen house environment, having average day and night temperatures of 38.5 ^oC ±4 ^oC and 26.5 ^oC ±1.5 ^oC respectively. Bulk soil samples were collected at depth of 0 – 30 cm using spade from the University Oil Palm Estate, Igue, Edo State, Nigeria. The soil was air dried, ground and sieved through a 2 mm mesh. Diesel was collected from a local commercial filling station in Benin City, Nigeria, and was weathered for 3 weeks with regular daily stirring for about 5 minutes each [3]. Chicken manure digestates was locally prepared. The chicken layers droppings (from a battery cage system) was collected from the dump site (after about 2 hours of dumping) at Songhai Delta Farm PLC, Amuokpe, Delta State, Nigeria, and brought to the screen house in a plastic container. At the greenhouse, the droppings were homogenized after removing large matter like broken eggs shells, feathers, twigs, and wooden shaven etc. The digestates were made by mixing the homogenized droppings with tap water at 1:2.5 (droppings to water ratio) and were stirred daily for about 5 minutes each for the first-two weeks and then twice weekly till the end of digestion which lasted 8 weeks.

Soil Spiking

The diesel, BTEX and Naphthalene were dissolved in petroleum spirit to form the spiking mixture hereafter referred to as HCM. The soil spiking with the HCM was done sequentially, a method adopted from Okieimen and Okieimen [3] and the samples were arranged in a Randomized Complete Block Design (RCBD) after the spiking. The total amount of the pollutants used is shown in table 1 representing total of 5% pollution (composing of 4, 0.8% and 0.2% of diesel, BTEX and naphthalene respectively). At day 1, 10% of the intended HCM was added to the soil. This was increased to 20, 30 and 40% of the intended pollution value at days 7, 14 and 21 respectively. For the 10% HCM polluted samples, the concentrations were doubled with regards to the values used for the 5% pollution. Therefore, the total pollution level was

50,000 mg and 100,000 mg/kg soil for the 5 and 10% HCM pollution respectively (Table 1). After the final spiking, the samples were left undisturbed for four weeks for stabilization [3] prior to the nutrients stimulation. The floor of the greenhouse was fortified with 5 L diesel during the soil spiking to attract hydrocarbons utilizing microbes to the environment [6].

Table 1: Sequential spiking of soils with 5% HCM (adopted from Okieimen and Okieimen [3]

Remediation Indices (mg/kg)	Conc. (mg) and % of intended HCM added to the soils			
	Days of spiking			
	1 (10%	7 (20%)	14 (30%)	21 (40%)
Diesel (DROs)	4000	8000	12000	16000
Benzene	200	400	600	800
Toluene	200	400	600	800
Ethyl Benzene	200	400	600	800
Xylene	200	400	600	800
Naphthalene	200	400	600	800
Sub-total	5000	10000	15,000	20000
Gross total	50,000			

Nutrient Stimulation

The CMD were used as nutrients sources for the bioremediation of the hydrocarbons polluted soils. Appropriate amount of the fertilizers (as explained below) were added to the samples on weekly basis for the first four weeks according to a method adopted from Okieimen and Okieimen [3] and were properly mixed using a plastic turner to mimic land farming. Two levels of nutrient stimulation of 10 and 20% (with respect to the level of pollution) were applied. For the 10% treatment (for 5% HCM polluted samples), 5 g of CMD was added weekly up to the 4th week and for the 20% treatments, 10 g of the manures weekly were applied for the same period. Therefore, the total amount of manures added to the samples was 20 and 40 g/kg of soil for the 10 and 20% treatments respectively. And for the 10% HCM polluted samples, the same procedure of nutrient stimulation was adopted except that the concentration of the digestates used was doubled. That is, the total concentration of the digestates applied in this case were 40 and 80 g/kg soil respectively for the 10 and 20% treatments.

Sampling and chemical analyses

About 50 g of each sample were collected at the following periods: day 1 (i.e. just before the nutrient stimulation), 14, 28, 56, 84, 168, and 136. Each time the samples were properly protected and preserved for analyses at EarthQuest International Laboratory, Warri, Nigeria. The physicochemical properties of the soil and the digestates were determined according to standard methods [6]. Hydrocarbons analyses were done according to USEPA method 8015B using a GC-FID (HP6890 US) [7]. For the digestates, Ammonium nitrogen (NH4+– N) was determined by Direct Nesslerization cum colorimetric method as prescribed by APHA [8], reading was done at 425nm using Hach 2010 UV - VIS Spectrometer, (Germany)

The concentration of NH_4^+ – N was calculated as:

$$NH_4^+ - N(mg/kg = C \text{ x } {}^{V}/_{W} \qquad (1)\ [6]$$

Where C is the concentration of NH_4^+- N in the sample in mg/l (as calculated from the calibration curve regression equation), v = the final volume of the digest (litres) and w is the weight of the digestate used (kg)

Similarly, Nitrate nitrogen (NO_3^- – N) was determined by colorimetric method as described by Okalebo *et al* [6] and (Freney and Wetselaar [9]. The procedure for NO_3^- -N extraction followed the same way as described for NH_4^+ - N above except that 1M K_2SO_4 was used instead of KCl solutions for the samples extractions. The total nitrate – N in the samples was calculated as:

$$NO_3^- - N = C \text{ x } {}^{V}/_{W} \qquad (2)\ [6].$$

Where C is the concentration of NO_3^--N in the sample in mg/l (as calculated from the calibration curve regression equation), v = the final volume of the digestates (litre) and w is the weight of manure digestates used (kg).

Total nutrient nitrogen (TNN) was calculated as the sum of the ammonium-N and the nitrate-N. While the available Phosphorus (AP) in the digestates was determined by the Olsen [10] method as described by Royland and Haygarth [11].

Microbial Count

The microbial inoculation potent analysis of the nutrients supplements was carried out according to standard methods and procedures. Heterotrophic microbes were evaluated using bacteriological agar and Rose Bengal agar for bacteria and fungi populations respectively [15]. Hydrocarbon-degrading microorganisms were determined on solid noble agar plates using diesel fuel as carbon source [12]. A soil suspension was made by mixing 0.50 g of the manure in 9.50 ml of distilled water. A 10-fold serial dilution was carried out to enumerate the number of colonies. Samples were prepared in triplicate and were cultured for 8 days at 27^{o}C and the number of colony forming units (CFU) were counted in each sample [17].

Statistical Analysis

Data obtained were further evaluated using statistical averaging, Analysis of variance (ANOVA) and regression analysis using SPSS statistical software. Post Hoc interpretation using Harmonic mean for the level of significance between the different nutrients' stimulation with regards to TPH degradation were also evaluated.

RESULTS AND DISCUSSION

The physicochemical characters of the soil used for the study

Table 2 presents the physicochemical properties of the soil used for the study. The results showed that the soil has a pH of 5.42 ±0.03 which was slightly acidic in nature typical of agricultural soils within the Niger Delta area. Ekebafe and Oviasogie [13] reported a pH range of 4.20 – 5.90 for Oil palm fields at Nigeria Institute of Oil Palm Research, (NIFOR), Benin City, Edo State, which is of similar geophysical features with source of the soils used for this study. A similar pH value (5.03) was also obtained from an Oil Palm field at Eko-Iyobhebhe, Irrua, Edo State [14]. However, pH of 5.26 has been reported previously for the University of Benin Research farms and environs [15]. Soils pH varies from one location to another depending so much on their inorganic and organic compositions. Udo and Dambo [16] has reported the pH of most agricultural soils in the tropics particularly in Southern Nigeria to range from about 5.00 to 6.80. Soil pH is a master factor in its chemistry and biochemistry. It influences the availability of nutrients and life of soil living organism [17, 18]. Thus, soil pH do affect soil microbial growth and bio-diversity which could hinder or reduce bioremediation of oil contaminants in soils. For a better growth and performance of soil microbes, less acidic or slight alkaline soil pH is recommended. Dibble and Bartha [19] reported a pH range of 6.50 - 8.00 as optimal for hydrocarbon degradation. Similarly, Atlas reported a neutral pH value for optimal bioremediation of hydrocarbon contaminated soils [20]. The pH of the soil used in this study is less than the recommended pH for optimal microbial activity and biodegradation. However, Table 3 showed that the nutrient supplements used in the study was basic in nature with a pH of 7.58 (Table 3), and therefore provided liming effects on the soils to which it was applied for remediation enhancement [21].

The soil was loamy sand having about 85, 4 and 11% of sand, silt and clay respectively which depicts the textural properties of most Agricultural soils in the rainforest of Nigeria [22]. The soil organic matter (SOM) content was about 6% which was relatively higher than the values of 1.18 and 1.80% reported by Ekebafe and Oviasogie [23] and Oshomoh and Ikhajiagbe [24] respectively for similar oil palm estate soils. The high SOM may be attributed to the regular clearing, no burning and the use of organic fertilizers which the site is known for.

Correspondingly, the macronutrients contents: N, P, and base elements, were also high in comparison to similar reports. [14]. In the absence of inorganic fertilizer application to soil, the nutrients contents relate positively and arithmetically to the organic matter content (OMC) of the soil.

As expected of loamy sand soils, the water retention capacity (WRC) of the soil was relatively low having a value of about 18%. This value is within the range of WRC reported for sandy loam soils by Onwudide *et al.* [25].

This soil was considered suitable for this study as it depicts the characteristics of typical agricultural soils in the Niger Delta region of Nigeria where oil pollution has been very challenging.

Table 2: Selected Physicochemical Properties of Soil used in this study

Parameters	Units	Quantification/Qualification	Remarks
pH		5.42 ±0.03	Acidic soil
Sand	%	85 ±06	High sand content
Silt	%	4.40 ±0.70	Low content
Clay	%	10.60 ±1.51	Low content
WRC	%	18.20 ±1.80	Low WRC

OMC	%	5.95 ±1.11	-
Total P	%	0.14 ±0.06	-
Total N	%	0.23 ±0.03	-
TOC	%	3.44 ±0.11	-
Textural class		loamy Sand	-

Physicochemical and microbial properties of the digestates

Livestock droppings and dungs are composted with green, and or food wastes or singly prior to agricultural applications. These composting methods are common and their usages depend on the available spaces. Sometimes, farmers keep these organic materials in perforated bags or apply to the farms directly. The type of composting method used affects the nutrients content [26]. The physicochemical and microbial properties of the digestates used for this study are presented in Table 3.

Table 3: Selected physicochemical and microbial properties of the nutrient supplements used in this study

Parameters	pH	TOC (%)	TNN (mg/kg)	AP (mg/kg)	THB (CFU)	THF (CFU)	THUB (CFU)	THUF (CFU)
Quantity	7.58 ±0.30	5.02 ±0.04	1421.59 ±63	957.73 ±47	1.5 x 10^4	1.4 x 10^4	1.6 x10^4	1.3 x 10^4

TNN = Total nutrient nitrogen (NO3- - N plus NH4+ - N)
THB = Total Heterotrophic bacteria;
THF = Total Heterotrophic Fungi
HUB = Hydrocarbons Utilizing bacteria
HUF = Hydrocarbons Utilizing Fungi
TOC = Total organic carbon

The results showed that the total nutrient nitrogen and available phosphorus of the digestates (CMD) were about 1,422 mg/l and 957.73 mg/l respectively which makes it suitable as nutrient supplement for microbial growth. Besides, the organic manure was alkaline in nature with pH values of 7.58. The alkaline nature of the digestate would be favorable for bioremediation since the pH falls within pH range of 6.50 – 8.00, which is the range of pH favorable for microbial growth and bioremediation activities [20, 27]. Furthermore, the digestate has relatively low available phosphorus value which means it would have less eutrophication tendency and be more environmental friendly. Also, the TOC of the digestate was relatively low depicting suitable organic substance for soil conditioning and enhancement of biodecomposition of petroleum pollutants in soils.

Inoculant status of digested CMD

The major drivers of soil contaminants or pollutants bio-decomposition processes are the microbes which are mainly the bacteria and the fungi [15]. The heterotrophic bacteria and fungi are capable of feeding and oxidizing all available organic carbon source but they have preference for non-hydrocarbons substances which have potentials for easy oxidation. In other words the heterotrophic bacteria or fungi would only go for the hydrocarbons in absence or deficiency of other organic carbon sources. On the other hand, the hydrocarbon utilizing bacteria and fungi are lovers of hydrocarbons and are often attracted to hydrocarbon pollutants in soils [28, 29]. Thus in a hydrocarbon polluted soils, the hydrocarbon utilizing microbes play the major role in the decomposition of such contaminants. Consequently, in bio-augmentation studies, the hydrocarbon utilizing microbes were introduced into the environment to boost their population for optimal bioremediation [30]. Table 4 presents the microbial status of the soils and the nutrients supplements prior to the various treatments for bio-remediation. The results showed the inherent total heterotrophic microbes as well as the THUB and THUF of the nutrients supplements.

Tables 4: Microbial Counts of soils and the nutrients supplements prior to treatments

Samples	THB	THUB	THF	THUF
Unpolluted soil (UPS)	7.0×10^3	3.0×10^3	1.2×10^4	5.0×10^3
HC Polluted soil (PSC)	1.4×10^4	7.0×10^3	1.5×10^4	1.2×10^4
Layers manure Digestates	1.5×10^4	1.6×10^4	1.4×10^4	1.3×10^4

The results showed that the digestates would be a good source of microbial population for bio-remediation. The THB and THF were 1.5×10^4 and 1.4×10^4 respectively. It has been mentioned that the HUB and HUF are the major drivers of hydrocarbon degradation and soil bio-remediation. Therefore, the manure digestates should have high bioremediation potentials as a source of nutrients stimulation. Besides, the TOC of the CMD is relatively low. Consequently, the effects of heterotrophic bacteria and fungi on hydrocarbon degradation would be high. The presences of HUB and HUF in similar organic wastes have been reported by previous researchers[31]. These findings have indicated that chicken manure digestates could be better nutrient supplement and microbial inoculant in landfarming of petroleum hydrocarbons polluted soils.

Bioremediation of petroleum hydrocarbon polluted soils

Table 5 represents the base concentration of the total hydrocarbons (TPH) after stabilization and prior to nutrient supplementation for remediation.

Table 5: Selected hydrocarbons conc.(mg/kg) of samples at day 1

Samples	Remediation indices		
	TDROs	TPAHS	TPH
PSC5	25302 ±244	2398.20 ± 209	34003 ±1501
CMD510	24507 ±241	2457.58 ± 243	33389 ±1560
CMD520	25317 ±257	1952.28 ±179	34295 ±844

One of the ways of assessing the level of bioremediation of petroleum polluted environment is the analyses of the TPH. The TPH comprises mainly of the aliphatic, cyclic and aromatic hydrocarbons including the DROs, BTEX and the PAHs (Table 5). The initial or base concentration of the TPH in the samples was presented in table 5. Figure 1 presents the percentage removal of TPH in the samples during the remediation. The results showed that the values of TPH degraded across the 5% HCM polluted samples at the early days were minimal ranging from about 4.07 (in PCS5) to 8% (in CMD 520) at day 14 and from about13 (in PCS 5) to about 32% in CMD 510 as at day 28. Similar low values of remediation were also recorded for the 10% HCM polluted samples with values ranging from about 6% in PCS 10 to about 7% in CMD 510 as at day 14 and from about 10% in PCS 10 to about 22% in CMD 1010 at day 28.

The low values of remediation of contaminants at the beginning of remediation protocol could be attributed to the period required by microbes to acclimatized [32, 33] to treatments made on the samples, especially when the use of inorganic fertilizers or easily oxidized carbon sources are involved.. As the remediation time increased the values of percentage removal of TPH increased correspondingly. For instance, about 59 and 87% of TPH were degraded at day 56 and 336 respectively in the 10% CMD treated samples. Similarly, about 52 and 97% of the TPH were removed in the aforementioned dates respectively for the 20% CMD treatments. At this level of HCM pollution (i.e. 5% HCM pollution), the use of 10% CMD was more effective up to day 56. For the 10% HCM polluted samples, the use of 10% CMD treatment led to the degradation of TPH by about 39 and 69% at day 56 and day 336 respectively while 20% CMD treatments gave percentage removal of the TPH as 72 and 89% at day 84 and day 336 respectively. Again the 10% CMD treatment was more effective at day 56 compared to the 20% treatments even at this high level of HCM pollution. But as the bioremediation period increased, the 20% manure digestates treatments led to higher TPH degradation. For instance at day 84 and 168, CMD 520 degraded about 77 and 84% of TPH as against about 76 \and 82% TPH degraded in samples CMD 510 (Figure 1).

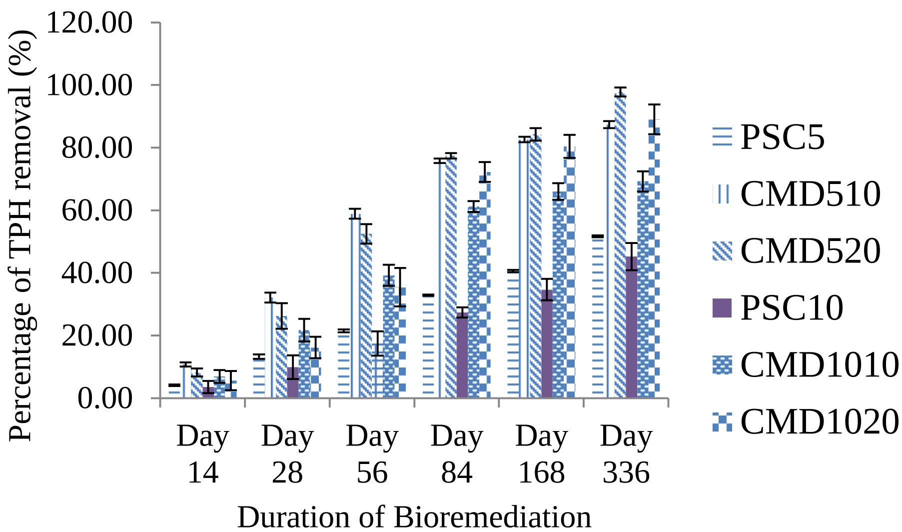

Figure 1: Percentage removal of TPH at different periods of remediation

Similar remediation trend was observed in the 10% HCM polluted samples. Generally, the percentages of TPH removal across the samples were relatively low up to day 56 compared to the amount of TPH degraded at day 84 or day 168. Figure 2 presents the percentage amount of TPH removed at the remediation intervals.

The results showed that for the CMD 510 and CMD 520 samples, greater amounts of TPH were degraded between day 28 and 56 in the 5% HCM polluted samples. But for the 10% HCM polluted samples greater amounts of TPH was removed in both CMD 1010 and CMD 1020 samples between days 56 and 84. For instance between days 56 and 84, about 22 and 37% of TPH were degraded in the CMD 1010 and CMD 1020 respectively as against 18 and 19%

TPH degradation in CMD 1010 and CMD 1020 samples respectively between days 28 and 56. Similar observation was made by [3]. The researchers reported higher values of degradation of TPH at the mid-period of bioremediation of crude oil polluted soils using poultry droppings and rubber processing sludge for stimulation. This observation may be attributed to sufficient period of acclimatization of the soil microbes and adequate nutrient in the soil which facilitated microbial population and diversity and consequential increase in oil degradation [34]. As the nutrient content of the soil depletes coupled with already reduced level HCM pollution, the smaller amount of TPH degradation in the CMD 1010 and CMD 1020 samples could be envisaged. It has been emphasized that soil microbes in oil polluted need sufficient time for acclimatization as well as adequate nutrients and favorable environmental conditions for optimal biodegradation of soil pollutants.

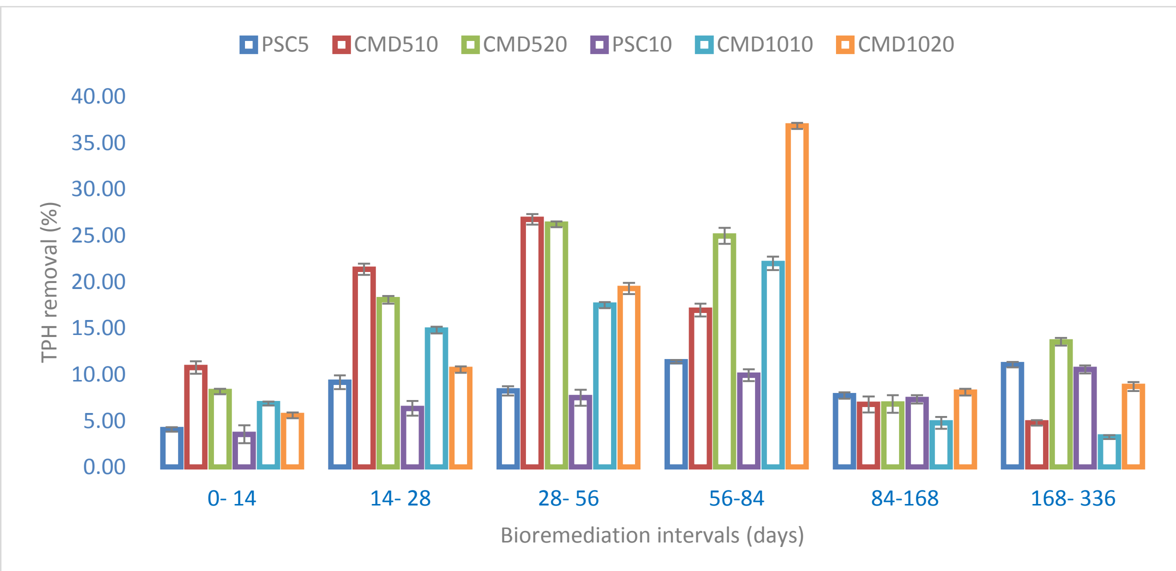

Figure 2: Percentage amount of TPH removed at the remediation intervals.

It is expedient to evaluate if the treatments significantly removed these hydrocarbons with regards to the control samples. Table 6 presents a statistical (Post Hoc one way Anova) of the percentages of TPH removal across the 5% HCM polluted samples at day 84. The results showed that there was no significant difference between the use of 10 and 20% of CMD (i.e. the two levels of treatment) stimulation at day 84 though arithmetical difference existed between them with CMD 520 showing greater degradation of TPH. However, both levels of treatments with the CMD showed significant differences when compared to the control treatment (Table 6).

But at day 168, there was significant difference in TPH removal between the control (PSC 5) and the CMD treated samples as well as between the two levels of treatments made Tables 7. It should be noted that treatments whose harmonic means appeared in the same column in the tables had no significant differences.

Table 6: Post Hoc interpretation of TPH percentage removal from 5% HCM polluted soils at day 84

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
PSC5	3	32.870			
CLD510	3				75.810
CLD520	3				77.440
Sig.		1.000	0.774	0.862	0.994

Means for groups in homogeneous subsets are displayed.
Harmonic Mean Sample Size = 3.000

Table 7: Post Hoc interpretation of TPH percentage removal from 5% HCM polluted soils at day 168

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
PSC5	3	40.600			
CLD510	3			72.580	
CLD520	3				84.270
Sig.		0.124	0.642	.943	1.000

Means for groups in homogeneous subsets are displayed.
Harmonic Mean Sample Size = 3.000.

Similarly, at the end of the remediation period (i.e. day 336), the CMD 510 and CMD 520 treatments showed significant difference in TPH degradation with the control treatment and between themselves with the later leading to greater removal of TPH. The efficiency of the CMD 520 here may be attributed to its richer content of nutrient as against the nutrient depletion in CMD 510 (Table 8).

Table 8: Post Hoc interpretation of TPH percentage removal from 5% HCM polluted soils at day 336

Treatment	N	Subset for alpha = 0.05			
s		1	2	3	4
PSC 5	3	51.670			
CM/D 510	3	57.370	57.370		
CMD 520	3				87.810
Sig.		0.117	0.405	0.053	0.082

Means for groups in homogeneous subsets are displayed.
Harmonic Mean Sample Size = 3.000

For the 10% HCM polluted soils, the degradation of the TPH followed similar trend to what was observed in the case of 5% HCM pollution in that there were no significant differences observed in use of 10 or 20% of the nutrients stimulation at the earlier stages of the bioremediation. No significant differences were observed in TPH removal between CMD 1010 and CMD 1020 (as at day 84) though both of them showed significance differences with the control samples (Table 9). But all the treatments were significantly different from each other) including the control (as at day 168, Table 10).

Table 9: Post Hoc interpretation of TPH percentage removal from 10% HCM polluted soils at day 84

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC 10	3	27.330			
CLD 1010	3				69.180
CLD 1020	3				72.270
Sig.		1.000	0.975	0.990	0.899

Means for groups in homogeneous subsets are displayed.
Harmonic Mean Sample Size = 3.000

Table 10: Post Hoc interpretation of TPH percentage removal from 10% HCM polluted soils at day 168

Treatments	N	Subset for alpha = 0.05		
		1	2	3
PSC 10	3	34.650		
CLD 1010	3		65.980	
CLD 1020	3			80.380
Sig.		0.059	0.997	0.065

Means for groups in homogeneous subsets are displayed.
Harmonic Mean Sample Size = 3.000.

Similarly, at day 336, there were great significant d

CMD stimulated samples and the control and betwe

This observation was reflected by the percentage of TPH degraded in the three treatments, PSC10, CMD 1010 and CMD 1020 at days 84 - 336 (Figure 1).

Table 11: Post Hoc interpretation of TPH percentage removal from 10% HCM polluted soils at day 336

Treatments	N	Subset for alpha = 0.05			
		1	2	3	4
PSC10	3	45.210			
CMD1010	3		61.230	61.230	
CMD1020	3				79.090
Sig.		.130	.966	.057	.605

Means for groups in homogeneous subsets are displayed.
Harmonic Mean Sample Size = 3.000.

CONCLUSION

The bioremediation technology for the decontamination of petroleum polluted soils and waters have been well reported. Approaches that could lead to short throughput, environment friendly and cost effective as well as less technicality are being sought day in and out. The quest for enhancing bioremediation of petroleum hydrocarbon polluted soils necessitated this study. The findings from the work indicated that locally produced chicken manure digestates was a good source of hydrocarbons utilizing microbes hence it could serve as microbial inoculant as well as nutrient supplement for bioremediation of petroleum hydrocarbon polluted soils. . It was observed that the chicken manures digestates led to significant removal of TPH from petroleum polluted soils up to 10% pollution. This study has revealed that with organic manure digestates, higher petroleum soil pollution levels than 5% can be effectively remediated. It was further observed that level of treatments with the organic digestates depends on the level of pollution, intended duration of remediation and the rate of application of the manure. While 20% of organic stimulation is ideal for petroleum polluted soil above 5% intended to be remediated for 84 days or more, 10% treatment would be sufficient if 56 days of remediation is intended or repeated application of the said amount when remediation prolong beyond 56 days

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