The Effects of Chicken Manure Digestates on the Removal of Diesel Range Organics from Petroleum Products Polluted Soils

S. U. Oghoje*, J. E. Ukpebor, E. E. Ukpebor****

*Department of Chemistry, Faculty of Physical Sciences,
Delta State University, PMB 1, Abraka, Delta State, Nigeria.

**Department of Chemistry, Faculty of Physical Sciences,
University of Benin, PMB 1154, Benin City, Edo State Nigeria.

Corresponding Author: S. U. Oghoje, e-mail: oghojesteve@yahoo.com

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Abstract

This study was designed to investigate the efficiency of using locally produced chicken manure digestates (CMD) as nutrient supplement for the removal of diesel range organics (DRO) from petroleum products polluted soils by land farming process. Soils samples spiked with hydrocarbon mixture (HCM), 5 % and 10 %) were treated with three rates of the CMD (0, 10 or 20 %) for a 336 days period. Soil samples were collected at day 1, 14, 28, 56, 84, 168 and 336 time interval and the samples were extracted using standard methods. The concentrations of DRO at various sampling periods were determined using a gas chromatography equipped with a flame ionization detector (GC – FID). The percentage removal of the DRO and the significance differences of the different treatments were evaluated using statistical tools.

Results obtained showed that the use of 10 and 20 % CMD led to the removal of about 50 and 58 % of the DRO respectively from the 5 % HCM polluted soils and about 41 and 35 % respectively for the 10 % HCM polluted samples at day 56 and by day 84, the percentage removal of the DRO increased to about 60, 76 and about 46, 52 respectively using the two rates of treatments; in the 5 and 10 % HCM polluted samples respectively. These values were reasonable considering the level and toxicity of HCM pollution.

Significant differences ($P < 0.05$) between the treatments and the various control samples were observed in the removal of the DRO from day 56 to 168. And at the end of 336 days, the 10 and 20 % CMD treatments enhanced the removal of about 81, 96 and 56, 67 % from the 5 and 10 % HCM polluted soils respectively but no significant differences were observed between the control and the treatment samples particularly in the 10 % HCM polluted soils at this date.

This study has revealed that: the use of chicken manure digestates could facilitate the removal of reasonable proportion of DRO in a short remediation time of 56 days from high and toxic petroleum polluted soils. The efficacy of the CMD was observed to decrease as the remediation period increased. It was also observed that 10 % CMD treatment was optimal for the removal of DRO at a short remediation period and repeated application of the nutrient supplement would be required if remediation period exceeded 56 days. Furthermore, it could be concluded that CMD is an effective inoculant and nutrient supplement during

bioremediation of the DRO from toxic petroleum polluted soils. These findings are contributions to the existing data on organic stimulation for removal of diesel and by extension petroleum hydrocarbons from soils.

Key Words: chicken manure digestates, diesel range organics, bioremediation, petroleum polluted soils

Introduction

The need to clean up and reclaim petroleum polluted lands for agricultural and environmental health reasons (Okieimen and Okieimen, 2005) has been intensified in recent decades and these demands had called for several soils remediation strategies (Raskin et al., 1997; Ijah et al., 2008). Among the various soil remediation techniques developed in recent time, bioremediation techniques have gained wider acceptance and applications (Abatenh et al., 2017). This is because; the methods and materials involved are environment friendly and compactable (Mmom and Deekor 2010). Also, the technology can be localized and it is cost effective (USEPA 1994.).

Basically, the drivers of the bioremediation technology are the soil or water microbes (Bing and Kenneth, 2019). Therefore, for any bioremediation technique to work optimally there must be sufficient microbial population and diversity. Besides, the soil or water environment must be favorable for microbial growth and metabolic activity (Radwan et al., 2000, Phillip and Atlas, 2005). Naturally, there are several soil indigenous microbes that are capable of degrading soil pollutants. Soil macro and microorganisms can degrade soil contaminants in various ways including biodegradation, bioaccumulation, bio-assimilation and bio-transformation. During biodegradation the microbes use the petroleum carbon as sources of energy and end up breaking down the compounds into nontoxic compounds, water and carbon dioxide (H_2O and CO_2) (Bing and Kenneth, 2019). In bioaccumulation or bio-assimilation the soil organism such as earth worms are able to accumulate certain amount of hydrocarbon compounds into certain organs in their bodies and these compounds are metabolized or immobilized unless the organism dies while in biotransformation, certain enzymatic compounds are produces by the soil microbes which are capable of combining with the petroleum compounds making them far less bioavailable and immobile in soils. In all these ways, the population and diversity of the soil organisms as well as the soil physicochemical properties are. paramount (Radwan et al. 2000; Anoliefo 2016) The toxicity and intensity or volume of pollutants including petroleum hydrocarbons in soil is detrimental to soil microorganisms as well as the entire ecosystem (Adedokun and Ataga, 2007; Adoki and Orugbani 2007; Akpoveta et al., 2011). Consequently, a polluted land with petroleum hydrocarbon could take several years to redeem its vitality by natural attenuation. This has led several researchers exploiting various ways by which the bioremediation process can be enhanced (Adoki and Orugbani, 2007; Mmom and Deekor 2010; Akpoveta et al., 2011).

Remediation by enhanced natural attenuation (RENA) is a general term used to embrace the various approaches used to achieve the desired enhancement for the purpose of optimizing and fasten the bioremediation process. The RENA approaches or techniques include biostimulation, bioaugmentation and biofacilitation. Land farming is an example of biofacilitation for the remediation of petroleum contaminated or polluted soils. According to

USEPA, (1994) 'Land farming is a surface-soil remediation technology for petroleum contaminated soils that reduces concentration of hydrocarbon through biodegradation to a level that is safe for human health and the environment'. It specifically involves the tilling and spreading of excavated soils to enhance the biodegradation of petroleum products (Jorgensen et al., 2000; Mmom and Deekor 2010; Silvia- Castro et al., 201; Cerqueira et al., 2014,;SPDC 2017). In some recent researches, two or more of RENA techniques were combined for more effects and efficiency (Jorgensen et al., 2000, Semple et al., 2001; Okieimen and Okieimen 2005; Oghoje et al., 2018). Such as, Phytoremediation plus phytooxidation plus land farming (Xiao-Dong et al., 2005), biostimulation and biopile (Dias et al., 2015), inoculation, composting and biopile (Gomez and Sartaj 2014), Chemo – land farming (Millioli et al., 2009); bioaugmentation plus semi-permeable reactive barrier (Xin et al., 2013) etc. Diesel is the medium distillates from crude of petroleum oils. The major constituent of diesel are the C10 – C28 and for some diesel, up to C32). It is one of the major petroleum products distributed via trucks and pipeline in Nigeria. The several usage of this product couple with its spillage in the environment either due to accidental discharge, faulty installation and storage facilities, transportation pipelines defects or sabotage has led to pollution of several agricultural land in Niger Delta region of Nigeria that calls for urgent and effective remediation protocols (Sojinu et al., 2009; Oyem and Oyem, 2013). Over the year, various inorganic and organic supplements have been used to enhance the bioremediation of diesel and other petroleum products polluted soils (Dadrasnia and Agumuthu, 2013). To the best of our knowledge, the reports on the use of chicken manure digestates (particularly generated locally) in the bioremediation of diesel from toxic petroleum products polluted soils are scanty.

Organic digestate contains high proportions of organic nitrogen (N) especially in the form of ammonium nitrogen (NH_4^+ - N) which could be available for plants and soil microbes. It also contains other macro and micro elements necessary for biochemistry and metabolism of microorganisms. Therefore, organic digestates could be a useful source of plant nutrients, an effective fertilizer for crops and microbial growth; the organic fractions of the digestate can contribute to soil organic matter turnover, thereby influencing the biological, chemical and physical properties of the soil. It is envisaged that this biostimulation supplement could use its microbial content as an additional enhancement potential for efficient and optimal degradation of the (DRO). In this study, soils spiked with HCM comprising of various percentages of diesels, BTEX and PAH were used as petroleum polluted soils to investigate the effect of CMD in optimizing the removal of the DRO. The aim is to seek a better and quicker method of degrading the DRO in the presence of other higher toxic pollutants. Specifically, the objectives of the study include to: 1) determine the physicochemical and biological properties of the chicken manure digestates 2) evaluate the rate and level of DRO removal due to the CMD stimulation 3) assess the amount of CMD required for optimal DRO removal and 4) evaluate the level of significance differences ($P < 0.05$) between the CMD treated and untreated samples

Materials and Methods

Soil, CMD and HCM sourcing

Soil collection and processing

This study was carried out at the Analytical Laboratory of the Department of Chemistry, University of Benin, Benin City, Edo State, Nigeria, in a greenhouse environment with average day and night temperatures of 38.5^{o}C ±4^{o}C and 26.5^{o}C ±1.5^{o}C) respectively (Oghoje and Ukpebor, 2020). A composite soil was collected (at depth of 0 – 30 cm) from the University Oil Palm Estate Igue, Edo State using a spade. The bulk soil was packed into large polyethylene bags for transportation to the greenhouse for processing. The soil samples were air dried, ground and sieved through 2 mm mesh. The dried and sieved soil was stored till further use.

Chicken Manure Digestate Processing

The chicken manure digestates was locally prepared; chicken layers droppings (from a battery cage system) was collected from Sorghai Delta Farm PLC, Amuokpe, Delta State, Nigeria. Large debris (broken eggs shells, feathers, twigs, and wooden shavings etc) were removed and then the droppings were homogenized. Tap water was then added to the droppings and treated for maturity as previously reported (Oghoje and Ukpebor, 2020).

HCM procurement and Treatment

Commercially available diesel was obtained from local retailers in Benin City, Nigeria and was weathered for 3 weeks with daily stirring for about 5 minutes (Okieimen and Okieimen, 2005).

Laboratory analyses and Chemicals used

The major chemicals used in the study including; Benzene, toluene, ethyl benzene, xylene (BTEX), naphthalene, petroleum spirit and trichlorobenzene were purchased from Sigma-Aldrich, (UK). All the chemicals used for the study were of analytical grade.

Table 1. *Sequential spiking of soils with hydrocarbon mixture; HCM (adopted from Okieimen and Okieimen (Okieimen and Okieimen, 2005)).*

Remediation Indices (mg/kg soil)	5% HCM Pollution				10% HCM Pollution			
	Weeks				Weeks			
	1 (10%)	2 (20%)	3 (30%)	4 (40%)	1 (10%)	2 (20%)	3 (30%)	4 (40%)
Diesel (DROs)	4000	8000	12000	16000	8000	16000	24000	32000
Benzene	200	400	600	800	400	800	1200	1600
Toluene	200	400	600	800	400	800	1200	1600
Ethyl Benzene	200	400	600	800	400	800	1200	1600
Xylene	200	400	600	800	400	800	1200	1600
Naphthalene	200	400	600	800	400	800	1200	1600
Sub-total (mg)	5000	10000	15,000	20000	10000	20000	30000	40000
Gross total (mg)	50,000				100,000			

Source - (Oghoje et al., 2020)

Soil Spiking

The diesel, BTEX and naphthalene were dissolved in petroleum spirit to form the spiking mixture being referred to as the HCM. The soil spiking with the HCM was done sequentially, a method adopted from Okieimen and Okieimen (2005) 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 levels of pollution with 0.8, 0.2 % or 1.6, 0.4 % being BTEX and naphthalene respectively for the 5 and 10 % HCM pollution respectively (excluding their concentrations in the diesel). That is, 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 per week. Therefore, the total pollution levels were 50 g or 100 g/kg soil for the 5 and10 % HCM pollution respectively. After the final spiking, the samples were left undisturbed for four weeks for stabilization prior to the nutrients stimulation. The floor of the greenhouse was fortified with 5 liters diesel (during the soil spiking) prior to the nutrient treatments to attract hydrocarbons utilizing microbes to the environment (Anoliefo, 2016).

Table 2, presents the description and base concentrations of the total DRO in the soil samples after stabilization and prior to nutrient supplementation for remediation.

Table 2. *Samples description and DRO concentration (conc.) at day 1 after stabilization and final treatment.*

Samples	HCM (%)	CMD (%)	CMD (g/kg soil)	Base conc. of DRO (mg/kg soil)
PSC 5	5	0	0	25302 ±244
CMD5/10	5	10	20	24507 ±241
CMD5/20	5	20	40	25317 ±257
PSC10	10	0	0	50206 ±488
CMD10/10	10	10	40	50342 ±296
CMD10/20	10	20	80	49797 ±407

Nutrient Stimulation

The CMD was used as nutrients supplement for the bioremediation of the hydrocarbons polluted soils. Appropriate amounts of the digestates were added to the samples on weekly basis for the first-four weeks according to a method adopted from Okieimen and Okieimen (2005) and were thoroughly mixed using a plastic turner to mimic landfarming. Three levels of the organic stimulation of, 0, 10 and 20 % (with respect to the level of pollution) were applied to both levels of HCM pollution. For the 5 % HCM pollution and 10 % treatment, 5 g CMD was added weekly up to the 4th week and for the 20 % treatments, 10 g of the digestates was applied weekly for the said periods while for 10 % HCM pollution the treatments were doubled (Okieimen and Okieimen 2005; Oghoje et al. 2020). Therefore, for the 5 % HCM polluted soils, the amount of CMD added to the samples were 0, 20 and 40 g/kg for PCS5, CMD5/10, and CMD5/20 respectively and for the 10 % HCM pollution, the CMD amount was doubled accordingly for PCS10, CMD10/10 and CMD10/20 (Okieimen and Okieimen 2005; Oghoje et al., 2020).

Sampling and Chemical Analysis

About 50 g of each sample was 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, Delta State Nigeria. The soil pH was determined at soil to water ratio of 1:2.5; the compost pH was determined at compost to water ratio of 1:5 while the digestates' pH were read directly after adequate stirring for about 5 minutes using Orion model 420A pH meter, UK, (Okalebo et al. 2002). The moisture content of the soil and the composted manures were determined by the gravimetric method, the textural analysis of the soil was by Bouyoucos hydrometer method (Okalebo et al., 2002). The Total Organic Carbon content (TOC) of the soil and the manures was determined using the Walkey-Black 1934 Method as described by Okalebo et al., (2002). Soil Organic Matter (SOM) content was calculated by multiplying the average TOC value by 1.72. Okalebo et al., (2002). The total phosphorus and total nitrogen was by Murphy and Riley method and by acid digestion-colorimetric method respectively (Okalebo et al,. 2002). Hydrocarbons analyses were done according to USEPA method 8015B using GC-FID (HP6890 USA) (USEPA 1996). The analyses for sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg) were carried out as recommended by ASTM (ASTM, 1982), using a Flame Photometer (KRUSS FP8000, Germany) for K and Na and Atomic Absorption Spectrophotometer (AAS) (GBC 904AA, Australia), for Ca and Mg. And for the digestates, the physicochemical characterization as well as nutrients analyses were carried out as previously reported (Oghoje and Ukpebor, 2020).

Microbial Count

The microbial inoculation potent of the nutrients supplements were 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 (Anoliefo 2016). Hydrocarbon-degrading microorganisms were determined on solid noble agar plates using diesel fuel as carbon source (Margesin et al., 2000). A soil suspension was made by mixing 0.50 g of the manure in 9.50 ml of distilled water. A ten-fold serial dilution was carried out to enumerate the number of colonies. Samples were prepared in triplicates and were cultured for 8 days at 27 oC and the number of colony forming units (cfu) were counted in each sample (Colores et al., 2000). This analysis has been previously reported (Oghoje and Ukpebor 2020).

Quality Control and Statistical Analysis

Standard methods and procedures were used in samples treatments and analyses. Triplicate samples were made and the average determined. Statistical averaging, analysis of variance (ANOVA) and regression analysis were carried using appropriate statistical software. Post Hoc interpretation using Harmonic mean for the level of significance between the different nutrients stimulation with regards to DRO removal were evaluated (Oghoje and Ukpebor, 2020).

Results and Discussion

The Physicochemical characters of the Soil used for the study

The physicochemical properties of the soil used for the study has been previously reported (Oghoje and Ukpebor, 2020). The results showed that the soil was acidic with a pH of 5.42 ±0.03 which was slightly acidic in nature and typical of agricultural soils within the Niger Delta area. Similar soil pH have been reported for soils in the region (Ekebafe and Oviasogie, 2015; Falodun et al., 2015). Soil pH is a master factor in its chemistry and biochemistry. It influences the availability of nutrients and life of soil living organism

(Osodeke and Osondu, 2006; Oelkers and Valsami-Jones, 2008). Therefore, soil pH could affect soil microbial growth and bio-diversity which could hinder or reduce bioremediation of oil contaminants in soils (Oghoje and Ukpebor, 2020). For a better microbial growth and performance Dibble and Bartha (Dibble and Bartha, 1979) recommended soil pH range of 6.50 - 8.00 as optimal for hydrocarbon degradation. Similarly, Atlas (1981) reported a neutral pH value for optimal bioremediation of hydrocarbon contaminated soil. The pH of the soil used in this study is less than the recommended pH for optimal microbial activity and biodegradation. However, the results obtained showed that the CDM supplements was basic in nature and would have liming effects on the soils to which they were applied for remediation enhancement (Egwunatum, 2015; Oghoje and Ukpebor, 2020).

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 (Iren et al., 2014; Oghoje and Ukpebor, 2020). The soil organic matter (SOM) content was relatively higher than the values of 1.18 and 1.80 % reported by Ekebafe and Oviasogie (2015) and Oshomoh and Ikhajiagbe (2015) respectively for similar oil palm estate soils. The high SOM was attributed to the regular clearing, no burning and the use of organic fertilizers which the site is known for (Oghoje and Ukpebor, 2020). Correspondingly, the macronutrients contents; N, P, and base elements were also high in comparison to similar reports (Oshomoh and Ikhajiagbe, 2015). 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 (Oghoje and Ukpebor, 2020). The soil texture was loamy sand; therefore its water retention capacity (WRC) was relatively low having a value of about 18 % (Oghoje and Ukpebor, 2020). However, this value was within the range of WRC reported for loamy sand soils by Abdulkadir (2015) and Onwudide et al.,(2016). The soil was considered suitable for this study as it depicts the characteristics of typical agricultural soils within the Niger Delta region (of Nigeria, where oil pollution has been very challenging (Oghoje and Ukpebor, 2020).

Physicochemical, Nutrient and Microbial Potential of the CMD Supplement

In this study, the chicken layers droppings were locally fermented to generate digestate under certain abiotic conditions. The results showed that the total nutrient nitrogen for the CMD was about 1,422 mg/l while the available phosphorus was 958 mg/l as previously reported (Oghoje and Ukpebor, 2020). It has slightly alkaline pH value, 7.58 and TOC values of 5.02 %. 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 favorable for microbial growth and bioremediation activities (Atlas, 1981; Bossert and Bartha, 1984). Furthermore, the digestate has the least value of available phosphorus which means it would have less eutrophication tendency and be more environmental friendly compared to the other form of nutrient stimulation (Oghoje et al., 2020).

Beside the favorable physicochemical properties and good nutrient potential of the CMD, it contained adequate hydrocarbons degrading microbes. Our previous results showed that the total hydrocarbons degrading bacteria (THUB) and fungi (THUF) were as much as 1.6×10^4 and 1.3×10^4 cfu respectively. Similarly, the total heterotrophic bacteria and fungi were 1.5×10^4 and 1.4×10^4 cfu respectively (Oghoje and Ukpebor, 2020). This implies that the CMD has both nutrient supplementing and microbial inoculating potentials. It has been asserted that the major drivers of soil contaminants or pollutants bio-decomposition processes are the microbes which are mainly the bacteria and the fungi (Molina-Barahona et al. 2004; Oghoje et al. 2020). 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 others words, the heterotrophic bacteria or fungi would only go for the hydrocarbons in absence or deficiency of other organic carbon sources (Oghoje et al., 2020). But, the HUB and HUF are often attracted to hydrocarbon pollutants in soils as a result of their affinity for hydrocarbon (Akoachere et al., 2008; Okoro, 2010; Anoliefo, 2016). This behavior was observed weeks after the soil spiking.

A drastic increase in the microbial content of the polluted soils prior to the nutrient supplementation with the CMD was observed. Therefore, in a diesel or hydrocarbon polluted soils, the HUB and HUF would increase in population and diversity (provided the level of pollution is not high enough to be detrimental to their growth) and they play the major role in the bioremediation of such contaminants (Oghoje and Ukpebor, 2020). Consequently, in several bio-augmentation studies, the hydrocarbon utilizing microbes were isolated, cultured and introduced into the oil polluted environment to boost their population for optimal bioremediation (Abdulsalam and Omale, 2009; Anoliefo, 2016). For instance, the THUB and THUF of the unpolluted soil (UPS) were 3.0×10^3 and 5.0×10^3cfu respectively but were increased to 7.0×10^3 and 1.2×10^4 cfu respectively in HCM polluted soil (PSC). Similarly, the THB and THF in the UPS were 7.0×10^3 and 1.2×10^4 cfu respectively but the values increased to 1.4×10^4 and 1.5×10^4 cfu in the oil polluted soils (PCS) respectively. Also the values of all the microbes were higher in the CMD supplements soils than their corresponding values in both the UPS and PSC with the values 1.5×10^4, 1.6×10^4, 1.4×10^4 and 1.3×10^4 cfu for the THB, THUB, THF and THUF respectively.

Therefore, the manure digestates should have higher bioremediation potentials as compared to the other sources of nutrients stimulation as observed in previous studies (Oghoje and Ukpebor, 2020). The presences of HUB and HUF in similar organic wastes have been reported by previous researchers (Adekunle, 2011). These findings have indicated that chicken manures digestates could be better as alternative nutrients supplement and microbial inoculants in land farming of petroleum hydrocarbons polluted soils.

Removal of the DRO from the HCM polluted soils

The effects of three levels of CMD stimulations on the removal of DRO from soils polluted by petroleum products mixture (HCM) was determined using the analyses of total diesel range organics (TDRO) as the remediation indices. The results showed that for the 5 % HCM pollution treated with 10 (i.e. CMD5/10) or 20 % (i.e. CMD5/20) of CMD, the base concentration of the DRO were about 24507 and 25317 mg/kg soil respectively while for the 10 % HCM pollution, the total DRO concentration were 50342 mg/kg for those treated with 10 % CMD (CMD10/10) and about 49797 mg/kg for the soils treated with 20 % CMD (CMD10/20). The base concentrations of the control soils were similar with values of 25302 and 50206 mg/kg for the 5 and 10 % HCM pollution respectively (Table 2).

The percentage removal of the DRO as remediation proceeded is presented in figure 1. A general increase in the percentage removal of the DRO in both nutrients stimulated and un-stimulated samples were observed. Figure 1 showed, that the percentages remediation of the

DRO in the soils was minimal in the first two weeks especially with the untreated and the 10 % HCM polluted soils. For instance, the DRO in PCS5, PSC10, CMD10/10 and CMD10/20 were reduced by about 3.5, 0.53, 3.75 and 3.32 % respectively at day14

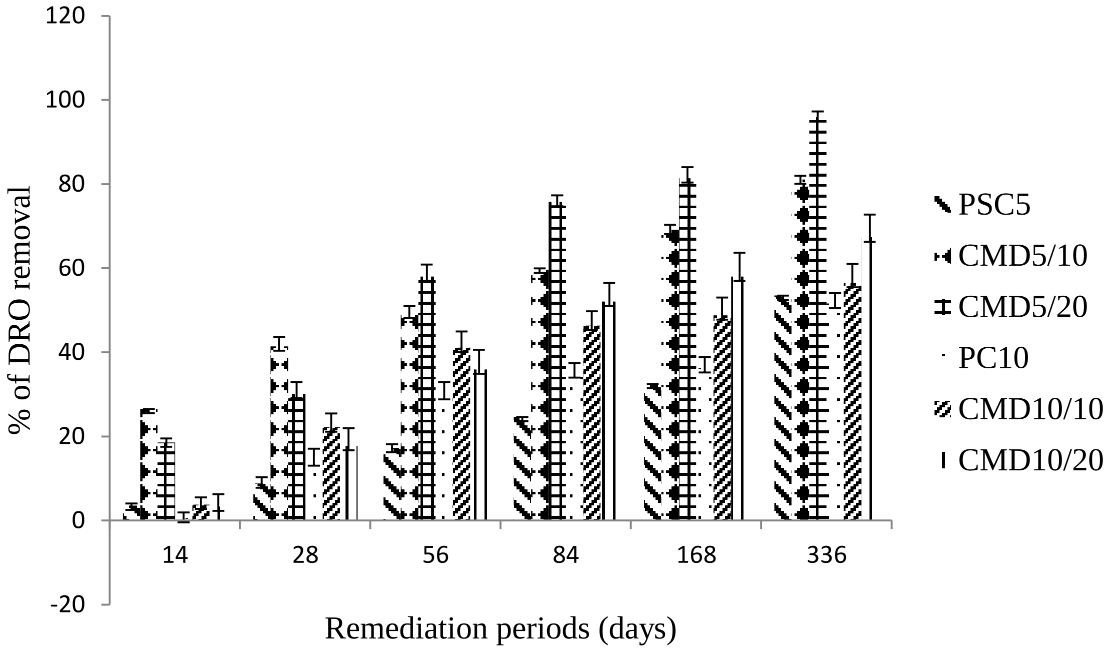

Fig. 1. *Percentage removal of DRO from the HCM polluted soils*

It has been reported that the rate and degree of bioremediation is determined by many factors including the prevailing chemical (such as pH and pollutants' concentrations), physical (moisture and temperature and biotic (microbial population and diversity) conditions (Amadi et al., 1993; Adoki and Orugbani, 2007; Mmom and Deekor, 2010). Therefore, the low remediation values at the early weeks of the process may be attributed to the high volume and toxic petroleum products such as the BTEX and PAHs present in the HCM particularly in the 10 % HCM polluted samples which could be inimical to microbial activities as reported by Adekunle (Adekunle 2011). Besides, microbes need time to acclimatize to major changes in the soil environment (Okieimen and Okieimen, 2005; Mmom and Deekor, 2010, Adekunle, 2011). However, for the 5 % HCM pollution organic nutrient stimulated samples, the values of the DRO removal were relatively higher when compared to those of the controls (PSC5 and PSC10)) and the 10 % HCM polluted samples (i.e. CMD10/10 and CMD10/20).with CMD5/10 and CMD5/20 having DRO removal values of about 27, and 19 % respectively at day 14. This improvement may be attributed not only to the organic nutrient contents of these treated samples but the microbial inoculation potentials of the organic digestates used in their stimulation. After about three months, precisely at day 84, about 60 and 76 % of the DRO were degraded from the samples treated with CMD at 10 (i.e. CMD5/10) and 20 % (i.e. CMD5/20) respectively and about 46 and 52 % for the 10 % HCM polluted samples stimulated with 10 (C10/10) and 20 % CMD. (C10/20) respectively. For the control soils, PSC5 and PSC10, the percentage DRO removal were quite low when compared to the nutrient stimulated samples with corresponding percentage removal values of 25 and 35 % respectively at the mentioned day. The results obtained from the nutrients stimulation using 10 or 20 % manure digestates were in close range with the values of 71 and 84 % DRO removal from diesel polluted soils reported by Jorgensen et al.,(2000), and Van Gestel et al., (2001), who used spruce bark and food waste for composting diesel polluted soils respectively.

These results conformed to several reports on organic stimulation of oil polluted soils. However, other environmental determinants may lead to wide variation in the values of remediation obtained from similar organic composting (Jorgensen et al., 2000; Van Gestel et al. 2001; Okieimen and Okieimen 2005; Oghoje and Ukpebor, 2020). It was observed that at short duration the use of 10 % CMD gave better remediation of the DRO as compared to the use of 20 % of the nutrient supplement. For instance at day 14, 10 % CMD enhanced the removal of about 27 % as against the 20 % CMD treatment which degraded just about 19 % of the DRO from the 5 % HCM polluted samples at this same period of remediation. Similarly, for the 10 % HCM pollution, 10 and 20 % CMD treatments led to DRO removal valued about 4 and 3 % respectively at day 14. However, beyond day 28, the 20 % CMD stimulation led to better DRO removal in both 5 and 10 % HCM polluted soils. It had earlier been documented that bioremediation required certain amount of nutrient stimulation and that the rate and/or degree of soil contaminants bio-removal are not always proportional to the amount of nutrient supplied (Okieimen and Okieimen 2005, Chorom et al. 2010, Oghoje and Ukpebor 2020). For the 5 % HCM pollution, the use of 10 % CMD was adequate at shorter periods of remediation. Okieimen and Okieimen (2005) also found the use of 10 % of rubber processing sludge (RPS) and Poultry droppings to be adequate for optimal biodegradation of the aliphatic hydrocarbons at 5 % petroleum pollution level. But for longer period of remediation particularly with high levels of petroleum pollution 20 % or repeat application of 10 % CMD would be ideal.

It was observed that the percentage of DRO degradation in all the samples up to day 56 were all less than 50 % except for the CMD5/20 samples with average value of 58 %. Therefore, it can be said that at these periods, the samples were still too toxic to support optimal microbial activities. However, reasonable percentages of DRO degradation were recorded in the samples after day 56. For example, in the CMD 5/10 and CMD5/20, the percentages of DRO removed were, 60, 69, 81 and 76, 81 and 96 % respectively at days 84, 168 and 336 respectively. Similarly, for the CMD10/10 and CMD10/20 the DRO degraded values were about, 46, 49, 56 and 52, 58 and 67 % respectively at days 84, 168 and 336 respectively. The untreated samples gave the least percentage removal of DRO as expected (in each level of pollution) when compared to their counterpart stimulated samples. It was also observed that the proportion percentage removal of the DRO were higher at the early bioremediation intervals. Figure 2 presents the percentage removal of the DRO at the remediation intervals. The results showed that for the CMD5/10 and CMD5/20 samples, greater amounts of DRO were degraded between day 0 and 14 in the 5 % HCM polluted samples. But for the 10 % HCM polluted samples greater amounts of DRO was removed in CMD10/10 and CMD10/20 samples between days 28 and 56. For instance between days 28 and 56, about 28, 19 and 18 % of DRO were degraded in the CMD5/20, CMD10/10 and

CMD10/20 respectively as against 18, 5 and 16 % degradation in these samples respectively between days 56 and 84 (Figure 2).

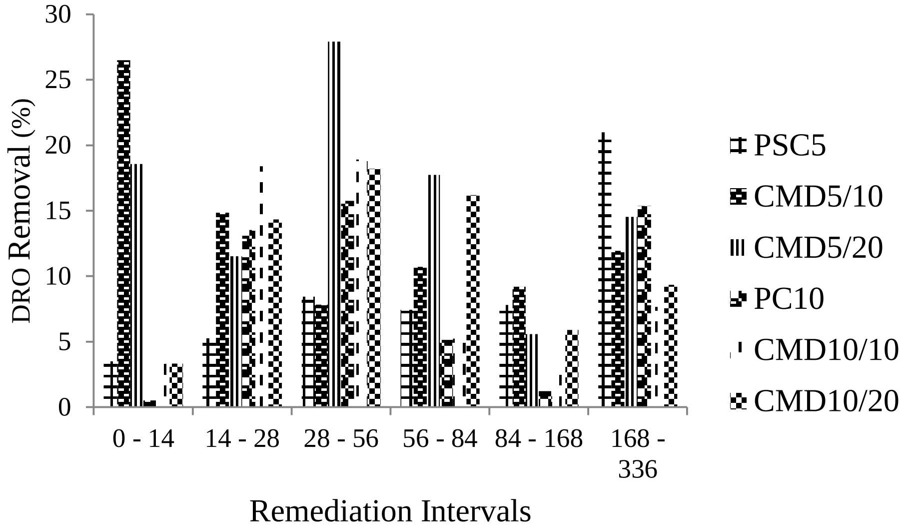

Fig. 2. *Percentage amount of DRO removed at the remediation intervals*

Similar observation was made by Okieimen and Okieimen (2005). The researchers reported higher values of degradation of saturated alkanes (DRO) at the middle phase of bioremediation of crude oil polluted soils using poultry dropping and rubber processing sludge for stimulation. This observation may be attributed to relatively ease of breaking down of the aliphatic alkanes in comparison to other families of hydrocarbons such as the aromatics. The dynamism in microbial growth, diversity and metabolisms may also be another contributing factor as well as the nutrient availability which may be favored at the early phase of the bioremediation protocol.

Adequate nutrients in the soils facilitate microbial population and diversity and consequentially increase oil degradation (Van Gestel et al., 2001). As the nutrient content of the soil depletes coupled with already reduced level of HCM pollution, the smaller amount of DRO degradation in the CMD5/10, CMD10/10 and CMD10/20 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 concerned (Okieimen and Okieimen, 2005; Chorom et al., 2010, Oghoje and Ukpebor, 2020). These may be the reasons why the untreated samples PSC5 and PSC10 (i.e. the controls) have their higher proportion of DRO removal, about 21 and 16 % respectively between days 168 and 336.

Significance effects of CMD on DRO removal from HCM polluted soils

It is necessary to evaluate if the treatments significantly removed these hydrocarbons with regards to the control samples. Tables 3a and b presents statistical Post Hoc analysis of percentage DRO degradation at day 84 (3 months). The results showed that there was significant difference ($P < 0.05$) between the control (PSC5) and all the nutrient stimulated samples. There was also such significance difference between the two nutrient treatments in the 5 % HCM polluted soils (Table 3a). But for the 10 % HCM polluted samples, no significant difference between percentage DRO degradation in the 10 (i.e. CMD10/20) and 20 % (i.e. CMD10/20) CMD treatments though the CMD10/20 gave higher arithmetic value of DRO removal at Day 84 than CMD10/10 (Figure 1). However, both treatments led to DRO removal significantly difference from that of the corresponding control samples (Table 3b) at day 84. It should be noted that treatments whose harmonic means appeared in the same column in the tables had no Significance differences.

Table 3a. *Post Hoc interpretation of DRO percentage removal from 5 % HCM polluted soils at day 84.*

Treatment	N	Subset for alpha = 0.05				
		1	2	3	4	5
PSC5	3	24.650				
CLD5/10	3				59.910	
CLD5/20	3					75.750
Sig.		0.080	0.360	0.950	1.000	1.000
Means for groups in homogeneous subsets are displayed						
Harmonic Mean Sample Size = 3.000						

Table 3b. *Post Hoc interpretation of DRO percentage removal from 10 % HCM polluted soils at day 84.*

Treatment	N	Subset for alpha = 0.05			
		1	2	3	4
PSC10	2		39.825		
CMD10/10	2			71.405	71.405
CMD10/20	2				74.385
Sig.		1.00	1.000	0.070	0.050
Means for groups in homogeneous subsets are displayed.					
Harmonic Mean Sample Size = 2.000.					

Table 4a. *Post Hoc interpretation of DRO percentage removal from 5 % HCM polluted soils at day 168*

Treatment	N	Subset for alpha = 0.05		
		1	2	3
PSC5	2	41.665		
CMD510	2	52.145		
CMD520	2			79.345
Sig.		0.620	1.000	0.190
Means for groups in homogeneous subsets are displayed.				
Harmonic Mean Sample Size = 2.000.				

Table 4b. *Post Hoc interpretation of DRO percentage removal from 10 % HCM polluted soils at day 168*

Treatment	N	Subset for alpha = 0.05	
		1	2
PSC10	4	38.463	
CMD1010	4	55.118	55.118
CMD1020	4		64.693
Sig.		0.380	0.060
Means for groups in homogeneous subsets are displayed.			
Harmonic Mean Sample Size = 4.000.			

Similarly, at day 168 (6 months), significance differences were observed in the percentage removal of the DRO between the control and the nutrient stimulated samples and between the two rates of nutrients stimulation (I.e. CMD5/10 and CMD5/20) for the 5 % HCM polluted samples (Table 4a). But in the 10 % HCM polluted samples; there was no significance difference between the control samples and the 10 % nutrient stimulated samples. Also there was no significance difference between the two nutrients stimulated samples except the observed significance difference between the control and the 20 % nutrient stimulated samples (Table 4b).

Similar trends were observed at day 336 in which there were significance differences between the percentage removal of DRO in the control samples and the two levels of nutrient stimulation and between the two nutrient stimulation levels (i.e. CMD5/10 and CMD5/20) in terms of the percentage removal of DRO at this period (Table 5a). But for the 10 % HCM polluted samples, there were no such significance differences between the control samples (PSC10) and the nutrient simulated samples (Table 5b).

Table 5a. *Post Hoc interpretation of DROs percentage removal from 5 % HCM polluted soils at day336*

Treatment	N	Subset for alpha = 0.05		
		1	2	3
PSC5	2	61.950		
CMD5/10	2		79.635	
CMD5/20	2			93.985
Sig.		0.08	0.39	1.00
Means for groups in homogeneous subsets are displayed.				
Harmonic Mean Sample Size = 2.000.				

Table 5b. *Post Hoc interpretation of DROs percentage removal from 10 % HCM polluted soils at day 336*

Treatment	N	Subset for alpha = 0.05
		1
PSC	4	55.610
CLD10	4	63.383
CLD20	4	75.433
Sig.		0.090
Means for groups in homogeneous subsets are displayed.		
Harmonic Mean Sample Size = 4.000.		

This implies that at this long period of remediation the two levels of treatments no longer produce significant effects in terms of percentage removal of the DRO even though their arithmetic percentage removal of the contaminants were higher than that of the control in the order of; CMD10/20 > CMD10/10 > PSC10 (Figure 1).

The observation may be attributed to gross depletion in the nutrient of the CMD as the bioremediation period prolong with a consequential drop in microbial population and metabolism and reduction in the degradation of the DRO (Figure 2). The results obtained from this study showed that at different stages of the remediation up to a throughput of 56, the use of 10 % CMD would be sufficient but beyond this period, the treatment no longer has significant effects on DRO removal from the HCM polluted soils particularly for the higher level of the petroleum products pollution. The use of 20 % of the CMD treatment produced significant removal of the DRO to about 168 days (6 months) even in the 10 % HCM polluted samples but as the remediation was extended to day 336, its nutrient supplement and inoculates effects became minimal. In other words, there would be drastic drop in the nutrient supply as well as gross drop in the microbial population and diversity due to the use of the manure if the bioremediation period is extended beyond days 84 or 168. Similarly, Okieimen and Okieimen (2005) reported the drop in the bioremediation potentials and effectiveness of rubber processing sludge (used for the enhancement of bioremediation of crude oil polluted soil) as the period of remediation prolongs. The researchers observed that 10 % of this organic supplement could produce optimal effects up to day 84.

The higher remediation effect of 20 % CMD treatment in comparison to the 10 % treatment may be attributed to its higher value or volume of microbes and water. In favorable conditions, the magnitude of bioremediation of soil pollutants could be directly correlated with the values of microbial population and diversity. Soil water is another important determinant of microbial growth and metabolic activities. Hence, it has direct effects on the degree and rate of pollutants degradation in soils (Molina-Barahona et al., 2004). Specifically, Molina-Barahona et al., (2004) reported 30 % soil moisture content for optimal biodegradation of hydrocarbons contaminated soils. Generally, the remediation periods by bioaugmentation are usually short which may be attributed to the short life span of microbes especially in unfavorable conditions to them. Therefore as the CMD loses it's inoculate potency due to prolong remediation periods, decrease in their bioremediation potential could be envisaged.

Conclusion

The need to seek for environmental friendly, cost effective and a short throughput for the remediation of petroleum products polluted soils cannot be overemphasized. This is for economic, environmental health and safety and adequate food production reasons. In this study, the use of locally produced chicken manure digestates for the enhancement of natural attenuation of petroleum products polluted soils was investigated. Specifically, the effects and efficacy of this organic stimulation on the removal of the DRO fractions from petroleum polluted soils were evaluated

Previous researchers have shown that when the level of petroleum soil pollution is higher than 5 %, effects of bioremediation would be insignificant particularly at short duration. But this study has shown that chicken manure digestate could enhance substantial removal of petroleum contaminants from pollution levels higher that the 5 % limit. Specifically, this study has revealed that: the use of chicken manure digestates could optimally enhance the removal of petroleum contaminants in petroleum polluted soils up to 10 %. The use of chicken manure digestates led to shorter remediation throughput during the removal of the DRO from petroleum polluted soils and was efficient at short remediation periods. When the remediation period is above 84 or 168 days, repeat application of the CMD is recommended.

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