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Studies on the Removal of Petroleum Hydrocarbons (PHCs) from a Crude Oil Impacted Soil Amended with Cow Dung, Poultry Manure and NPK Fertilizer

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Abstract The potential effects of animal-derived organic wastes and commercially available NPK fertilizer as nutrient supplements to biostimulate soil indigenous microorganisms for hydrocarbons degradation were investigated. 150 g each of soil samples were spiked with Bonny light crude oil (BLCO) (10 % w/w) and one week after contamination, about 30 g each, of the amendment agents (cow dung (CD), poultry manure (PM) and NPK (15:15:15) fertilizer) was added to the soil samples equivalent to 2000 kg/hectare. The soil samples were tilled daily to ensure homogenization and adequate aeration. The rates of degradation of the crude oil were studied for a remediation period of 8 weeks under laboratory conditions. The results showed that there was a positive relationship between the rate of petroleum hydrocarbons degradation and presence of the animal-derived organic wastes and NPK fertilizer in soil microcosms contaminated with crude oil, having achieved the reduction of PHCs from 25450 mg/kg to 6910 mg/kg, 3820 mg/kg and 6770 mg/kg for cow dung, NPK fertilizer and poultry manure respectively, which is above 70% removal of PHCs within the remediation period. The BLCO degradation data fitted well to the first-order kinetic model. The model revealed that BLCO contaminated-soil microcosms amended with animal-derived organic wastes had higher biodegradation rate constants (K) as well as lower half-life times ($t_{1/2}$) than unamended soil (natural attenuation) remediation system. The biodegradation rate constant and calculated removal efficiency values showed that NPK offered the best biostimulation performance, which was closely followed by poultry manure and then cow dung. The data, subjected to a two- way analysis of variance for significance at $p < 0.05$, indicated that time and the material used are significant, it was also noted that time had more effect on the remediation process than the amendment agents used. The system proposed here is inexpensive, efficient, and environmentally friendly and may thus offer a viable choice for petroleum hydrocarbons contaminated, soil remediation.

Keywords Degradation, Remediation, Amendment agents, Biostimulation

Introduction

Crude oil is the parent material for petroleum-based products used as major sources of energy for industry and daily life. Leakages and accidental spill occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. The contamination of soil by crude oil and petroleum products has become a serious problem that represents a global concern for its potential consequences on ecosystem and human health [1]. These spills cause soil pollution which is a major issue in Nigeria, especially in the Niger Delta states leading to huge environmental degradation [2]. The technology commonly used for soil remediation includes evaporation, dispersion, and washing. However, these technologies are expensive and can lead to incomplete decomposition of contaminants [3]. For this reason an increasing attention has been directed towards developing new strategies and environmental-friendly technologies for the remediation of soil contaminated by petroleum hydrocarbons. Among these, bioremediation technology which involves the use of microorganisms to detoxify or remove pollutants through the mechanisms of biodegradation has been found to be an environmentally-friendly, non-invasive and relatively cost-effective option [4]. Crude oil bioremediation in soil can be promoted by stimulation of the indigenous microbial population, by introducing nutrients and oxygen into the soil (biostimulation) [5].

Following oil pollution, nutrients are rapidly assimilated by soil microorganisms thus depleting the nutrient reserves [6]. Therefore, apart from the environmental problems caused by oil pollution, the agronomic and economic aspects are significant [7-8]. The objective of using soil amendments is to augment the native fertility status of such soil and to enhance the rate of oil degradation, thus minimizing the contamination of scarce groundwater sources and to improve crop production [9]. The addition of inorganic or organic nitrogen-rich nutrients (biostimulation) is an effective approach to enhance the bioremediation process. Positive effects of nitrogen amendment using nitrogenous fertilizer on microbial activity and/or petroleum hydrocarbon degradation have been widely demonstrated [10-12].

The search for cheaper and environmentally-friendly options of enhancing petroleum hydrocarbon degradation through biostimulation has been the focus of research in recent times [12-14]. One of such option is the use of organic wastes derived from plants and animals. Few workers have investigated the potential use of plant organic wastes such as rice husk and coconut shell [14], plantain peels and cocoa pod husk [15], *Moringa oleifera* and soya beans [13] and animal organic waste like cow dung, pig dung, and goat dung contaminated with petroleum hydrocarbons and were found to show positive influence on petroleum hydrocarbon biodegradation in a polluted environment. Cow dung, poultry droppings and NPK fertilizer are cheap, readily available manure for soil.

Materials and Methods

Sample Collection

The soil sample used for the study was collected from the top surface soil (0-15cm) of the botanical garden, Abia State University, Uturu, Abia State, Nigeria. The soil sample was air dried for two weeks, homogenized, passed through a 2-mm (pore size) sieve, stored in a polyethylene bag and kept in the laboratory prior to use. The cow dung was obtained from the cow market in Lopka, Abia State, Nigeria, the poultry droppings from a local poultry farm in Okigwe Imo State, Nigeria and NPK fertilizer obtained from Eke market in Okigwe, Imo State, Nigeria. All the different amendment agents were each air dried for two weeks, ground and sieved to obtain uniform sized particles. The crude oil used for the study is Bonny light crude obtained from the core analysis laboratory of the Nigeria National Petroleum Corporation (NNPC), Moscow Rd., Port Harcourt, Nigeria. Each amendment agent was stored in a polyethylene bag and kept in the laboratory prior to use.

Sample Preparation and Soil Treatment

The method of Agarry et al., (2013) [16] with slight modification was adopted. About I50 g each, of the oil sample was measured out, placed in plastic containers, labeled A-P respectively. Sample A was used as the control while sample B-P were divided into five (5) groups to represent the different time intervals of 1,2,4,6 and 8 weeks respectively. The soil in each plastic container was spiked with 10 % (w/w) bonny light crude oil and thoroughly mixed together to achieve complete artificial contamination. 10 % spiking was adopted in order to achieve severe contamination because above 3 % concentration, oil has been reported to be increasingly destructive to soil biota and crop growth [17]. One week after contamination, the different remediation treatments were applied. About 30 g each, of the amendment agents which is equivalent to 2000 kg/hectare, was measured out and added, one to each container in a group. The same was repeated for all five (5) groups representing each time interval. Each container was made up to 50% volume by distilled water for proper percolation. The contents of each container was tilled daily to ensure homogenization and adequate aeration. The contaminated soil in plastic container A, was without amendment agents and thus served as control.

Characterization of Soil and amendment agents

The soil sample and amendment agents were characterized for Total organic carbon (TOC), Total Nitrogen (N), Total Phosphorus (P), Moisture Content (MC), Electrical Conductivity, Cation exchange capacity and pH according to standard methods. Nitrogen in the sample was estimated following the micro Kjeldhal method as outlined by Bremner and Mulvaney (1982) [18], moisture content was evaluated according to ASTMD2216,

Analysis of Petroleum Hydrocarbons

About 10 g of the soil sample was weighed into an extraction bottle and 20 cm^3 of extraction mixture (DCM: n-Hexane: acetone) in the ratio 2:2:1 was added. The mixture was sonicated for 1 h and the organic layer was decanted. The extracted organic phase was dried using anhydrous sodium sulphate and concentrated using a rotary evaporator to about 10 cm^3. About 10 cm^3 of the final extract was injected into already calibrated Gas Chromatography (HP 5890, USA) equipped with a capillary column. The peak areas were used in the quantifications. The organic extract was fractionated by using column packing. The column was packed by placing 1 g of glass wool into the column and gently packed. About 1 cm^3 of silica gel was placed on it and 1 cm^3 of sodium sulphate was added on top of the silica gel. The column was pre-conditioned by running 10 cm^3 of n-Hexane through the column. 1 cm^3 of the concentrated extract was placed on the column and eluted with 10 cm^3 n-hexane.

Analysis of Total Bacterial Count

The total heterotrophic bacterial count was enumerated by the method described by Chikere*et al.,* (2009). 1 g of the soil sample was aseptically transferred into a 100 cm^3 of peptone water medium and incubated for 2 h. 1 cm^3 of the solution was used to carry out 10-40 cm^3 serial dilution separately using peptone water as the diluents. 1 cm^3 of the aliquot from the 10^6 dilution tube of the sample was used to inoculate a prepared nutrient agar plate by spread plate method. The inoculated plates were incubated at 37^0 C for 24 h and 48 h and the colonies were counted.

$$CFu/g = \frac{\text{No. of colony counted}}{\text{Dilution factor x volume of inoculums}} \quad (1)$$

Dilution factor = 10^6, volume of inoculums = 1 cm^3

Results and Discussion

The result of the physicochemical analysis of the soil sample and amendment agents is as shown in Table 1.

Table 1: Physicochemical Characterization of the Soil and Amendment Agents.

	Soil Samples	Cow Dung	NPK Fertilizer	Poultry Manure
pH	5.68	7.11	7.69	7.55
Electrical conductivity (EC) (µS/cm)	98.78	24800	180000	7000
Moisture content (%)	4.33	17.67	21.93	6.04
Total Organic Carbon (TOC) (%)	0.2	6.22	9.18	5.98
Total Nitrogen (mg/kg)	20.14	4658	10250	2140
Total phosphate (mg/kg)	7.19	ND	18.6	ND
Potassium (%)	43.59	2450	6580	996.87
Cation Exchange Capacity (CEC) (meq/100g)	2.14	ND	ND	ND

The amount of PHC removed by the different amendment agents is as shown in Fig. 1

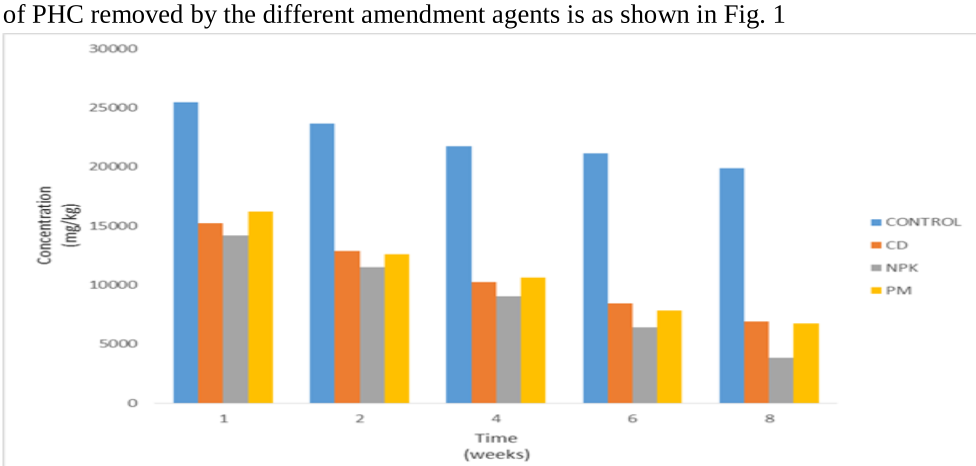

Figure 1: PHC removal from Crude Oil Contaminated Soil Using Cow Dung (CD), NPK Fertilizer (NPK) and Poultry Manure (PM).

The biodegradation efficiency of the removal of PHCs by the different amendment agents is shown in Fig. 2

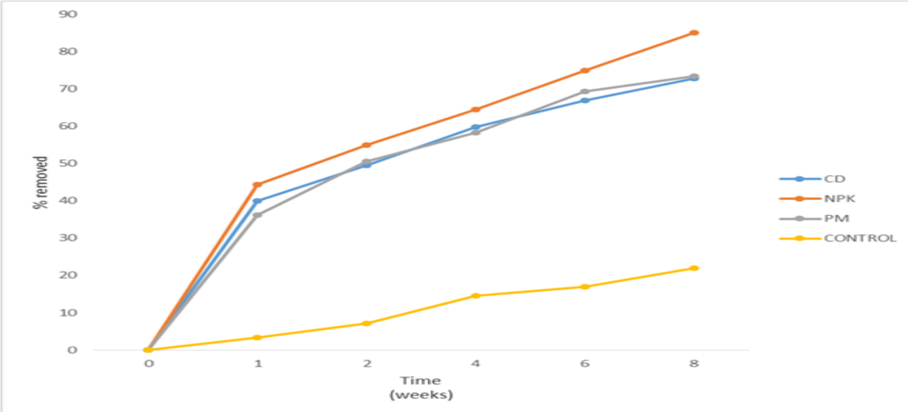

Figure 2: Removal Efficiency of PHC from Crude Oil Contaminated Soil by Cow Dung (CD), NPK Fertilizer (NPK) and Poultry Manure (PM)

The result for the microbial analysis of the hydrocarbon degrading bacteria is as shown in Table 2.

Table 2: Total Hydrocarbon Degrading Bacteria Count

	Total Heterotrophic Bacterial count (Cfu/g)			
Time (weeks)	**Soil sample (Unamended)**	**Soil +NPK**	**Soil + Poultry manure**	**Soil + Cow dung**
1	$3.0 \times 10^6 \pm 0.3$	$3.7 \times 10^7 \pm 0.3$	$13.6 \times 10^7 \pm 0.5$	$19.9 \times 10^7 \pm 0.3$
2	$3.2 \times 10^6 \pm 0.5$	$4.5 \times 10^7 \pm 0.5$	$14.9 \times 10^7 \pm 0.4$	$19.3 \times 10^7 \pm 0.5$
4	$3.8 \times 10^6 \pm 0.5$	$7.4 \times 10^7 \pm 0.5$	$19.6 \times 10^7 \pm 0.5$	$21.3 \times 10^7 \pm 0.4$
6	$4.6 \times 10^6 \pm 0.4$	$10.87 \times 10^8 \pm 0.4$	$25.67 \times 10^8 \pm 0.3$	$23.3 \times 10^7 \pm 0.5$
8	$5.4 \times 10^6 \pm 0.4$	$10.97 \times 10^8 \pm 0.6$	$26.07 \times 10^8 \pm 0.4$	$23.5 \times 10^7 \pm 0.5$

From Fig. 1, it can be observed that each of the amendment agents significantly reduced the Hydrocarbons, with an initial concentration of 25450 mg/kg, it was reduced to 6910 mg/kg, 3820 mg/kg and 6770 mg/kg by cow dung, NPK fertilizer and poultry manure respectively, after eight (8) weeks of treatment. Fig. 2 showed that NPK has the best removal efficiency after eight (8) weeks of remediation with 85 % removal of PHCs. This was closely followed by poultry manure which has 73.4 % and cow dung was also good with above 70% removal efficiency. The performance of the materials (CD, PM and NPK) are very good compared to the control (natural attenuation).

Gogoi*et al*. (2003) [19], observed that addition of commercial oleophilic fertilizers containing nitrogen and phosphorus to hydrocarbon contaminated soil increased the hydrocarbon degrading microbial abundance and total petroleum hydrocarbon degradation and also reported 77 to 90 % loss of total alkanes and 80% of PAH in a hydrocarbon contaminated soil after a remediation period of 180 days. In another study using poultry manure as organic fertilizer in contaminated soil, biodegradation was reported to be enhanced in the presence of poultry manure alone, but the extent of biodegradation was influenced by the addition of alternate carbon substrates. From Table. 2, it can be observed that on the application of the amendment agent, there was a significant increase in the total heterotrophic bacterial count for all the soil samples with amendment agents compared to the sample without amendment agent, which steadily increased throughout the period of soil treatment. It can also be observed that within the first four (4) weeks of soil treatment there was a rapid increase in the bacterial load of the samples which tapered off as it approached the final weeks of the treatment. This confirms that there was an increase in soil microbial load following biostimulation by the different amendment agents [10-12].

Kinetic Modelling

Kinetic models can be a useful tool for the prediction of residual contaminant concentrations during bioremediation. Kinetic analysis is a key factor for understanding biodegradation process, bioremediation speed measurement and development of efficient clean up for a petroleum hydrocarbon contaminated environment. The information on the kinetics of soil bioremediation is of great importance because it characterizes the concentration of the contaminant remaining at any time and permit prediction of the level likely to be present at some future time. Biodegradation kinetic data for the biodegradation of TPH, PAH and heavy metals was fitted to zeroth and first order kinetic equations. These kinetic equations are:

Zeroth order: $[A]_t = [A]_0 - k_0t$ (2)

First order: $\ln[A]_t = \ln[A]_o - k_1t$ (3)

Where $[A]_0$ and $[A]_t$ are amounts of contaminants present at the beginning of the experiment and at various time intervals, k is the ith order rate constant and t stands for the various time intervals. The kinetic plots using equations 2 and 3 are shown in Figs 3 to 6.

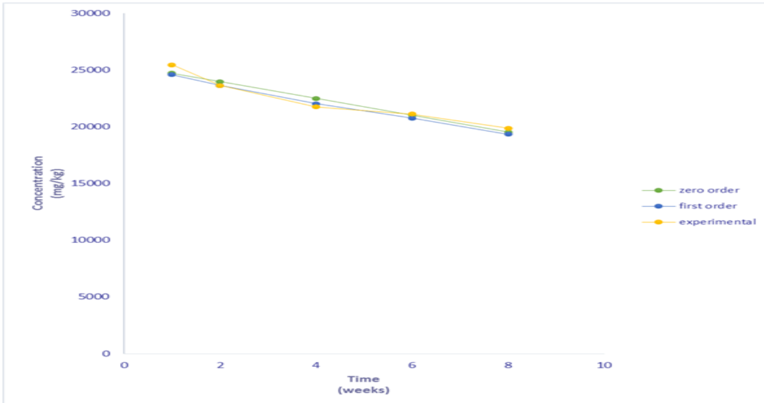

Figure 3: Kinetic models for PHC degradation in crude oil contaminated soil (Control)

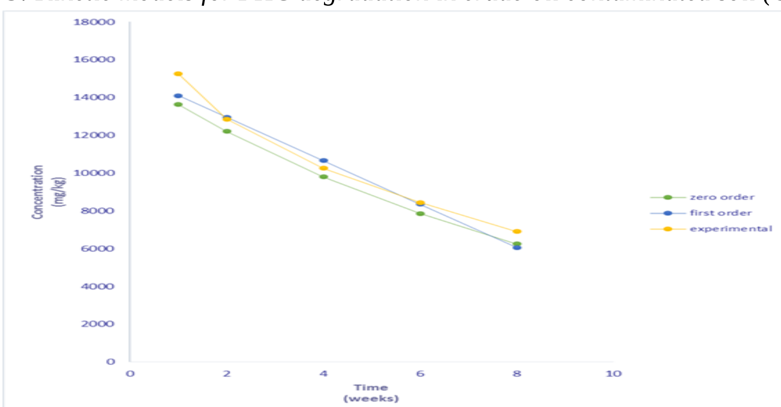

Figure 4: Kinetic models for PHC degradation in crude oil contaminated soil using cow dung

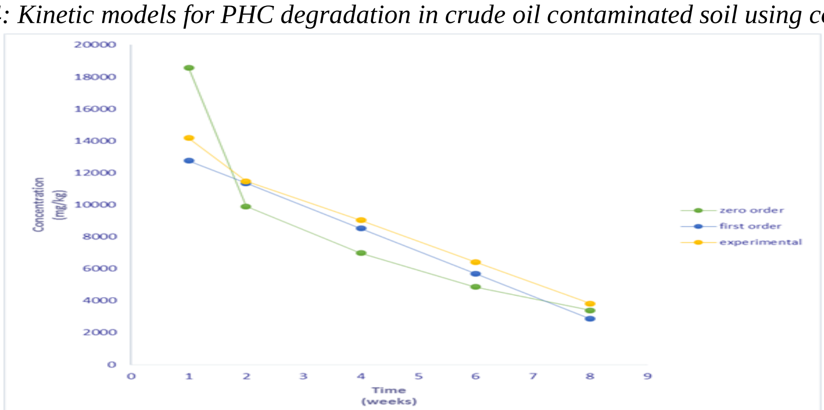

Figure 5: Kinetic models for PHC degradation in crude oil contaminated soil using NPK fertilizer

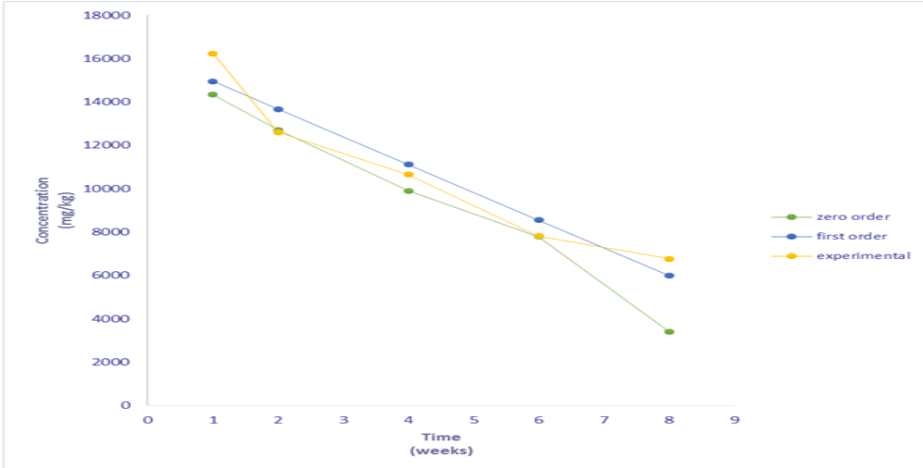

Figure 6: Kinetic models for PHC degradation in crude oil contaminated soil using poultry manure

From Fig. 3 to 6, it can be noted that the degradation of PHC, followed a first order kinetics reaction pathway. The data obtained by the first order model is closest to the experimental value. We can conclude that the reaction followed the first order kinetics more than the zero order models.

Degradation of hydrocarbons in soil has been observed to follow a first order path [20-22]. This indicates that the rate of degradation is directly proportional to the concentration of amendment agents added. This means that an increase in the amount of amendment agents would cause an increase in the rate of degradation. This also means that regardless of the concentration of the contaminants, the degradation rate would remain constant.

The biological half-life is the time taken for a substance to lose half of its amount. Biodegradation half-lives are needed for many applications such as chemical screening [23], environmental fate modelling [24], and describing the transformation of pollutants [25-26]. Biodegradation half-life times, $t_{1/2}$ are calculated by Eq. (4) [11, 22].

$$t_{1/2} = \frac{\ln 2}{k} \quad (4)$$

Where k is the biodegradation constant. The half-life model is based on the assumption that the biodegradation rate positively correlated with the pollutant pool size in soil [26].

From the kinetic plots above, the rate constants, the half-life and the correlation coefficient was deduced and is shown in Table 3.

Table 3: First Order biodegradation rate constant, half-life times and correlation coefficient (R^2) values of pollutants with different amendment agents

Parameters	**Rate (k) (day^{-1})**	**Half-life ($t_{1/2}$)(days)**	**R^2**
CT	0.005	138.6	0.9583
CD	0.016	43.3	0.9932
NPK	0.025	27.7	0.9806
PM	0.017	40.8	0.9724

*CT=Control, CD=Cow Dung, PM= Poultry Manure.

From Table 3, it can be noted that the biodegradation rate reveals a positive correlation coefficient R^2 for the reduction in level of PHC. It can also be observed that the soil sample amended with NPK, reveals a higher rate (k) and a lower half-life $t_{1/2}$ for all the parameters, this was closely followed by the soil amended with poultry manure and then the soil amended with cow dung which has the lowest rate constant and highest half-life for all the parameters analysed.

The degradation data obtained for all the pollutants was analysed for significant differences at the level of $p< 0.05$ between treatments using two-way analysis of variance (ANOVA) for time and materials (CD, NPK and PM) and the results are presented in Table 4.

Table 4: ANOVA results for PHC showing significance between time and materials.

Dependent Variable: PHC

	Sum of Squares	Df	Mean Square	F-ratio	Significance
Time	167208825.600	4	41802206.400	157.626	0.000
Material	10683212.133	2	5341606.067	20.142	0.001
Error	2121595.200	8	265199.400		
Total	1734723601.000	15			

Values of the significance for time and materials in Table 4 for PHC is 0.000 and 0.001 respectively. These values are less than 0.05 suggesting that the use of different materials (CD, NPK and PM) and the time interval of the experiment has significant effects on the degradation of PHCs. In addition, the values of the F-ratio are 157.626 and 20.142 respectively for time and materials, likewise the mean square (MS) values are 41802206.400 and 5341606.067 respectively. Higher F-ratio and MS values for time indicates that time had more effects than materials.

Conclusion

The present study confirms that the use of cow dung, poultry manure and NPK fertilizer, improved the rate of PHC degradation in a crude oil contaminated soil. The biodegradation rate constant obtained from the application of first order kinetics described the rate of degradation with the biostimulants. It is also noteworthy, from the calculated removal efficiency that all the amendment agents performed creditably well in removal of the contaminants. It can be concluded that the order of the removal of contaminants is NPK>PM>CD. The results of the kinetic modelling indicates that the remediation process fitted well to first order kinetics more than zero order. It was also observed that the length of time of the remediation process had more impact than the amendment agents used, that is to say that the effectiveness of the remediation process is more dependent on the length of time of the process rather than the amendment agents used for the remediation process.

The bioremediation technique employed in this study for soils contaminated with hydrocarbons could be suitable in field, because of its low cost, and low environmental risks associated with volatile hydrocarbon losses. However the success and efficiency of these bioremediation techniques may vary considerably from one site to another; since, there is no universal soil treatment regimen for the remediation of all crude oil contaminated soil. The effectiveness of any soil treatment applied for such purpose has to be evaluated on a case-specific basis.

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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