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Laboratory Design Criteria for Monitoring Biostimulated Bioremediation of a Crude Oil Contaminated Soil in Niger Delta Using Total Petroleum Hydrocarbon

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Abstract

The remediation of crude oil-impacted soil has always been a challenge in different soil environments and climatic conditions. Bioremediation technology has offered a breakthrough in restoring crude oil-impacted soil/sediment in muddy, dry soil and wetlands. Though, there have been varied environmental conditions that have hampered the success of the bioremediation process. This study has evaluated the effectiveness of a biostimulated bioremediation of crude oil-impacted soil using some design criteria—nutrient amendment (NPK fertilizer) and moisture content. Soil sample sets—A, B, C, D, E, F, and G were impacted with crude oil at a ratio of 10 g/kg and amended with varying amounts of nutrient 30, 60, and 80 g of N.P.K fertilizer. The medium for the inoculation of the nutrient was water and the volume of water applied varied from 30% to 80% saturation. The soil sample sets were harvested at an interval of 3 months for 180 days to determine the concentration of total petroleum hydrocarbon left in the soil. The analysis of the total petroleum hydrocarbon was achieved using a GC-FID with a capillary column and autosampler. Soil samples were extracted with mixed solvent dichloromethane and acetone at a 1:1 ratio. The total petroleum hydrocarbon results show that biostimulated bioremediation achieved better results in soil sample sets with low moisture content (30% water saturation) and moderate nutrient amendment. The biodegradation of the sample sets with high water saturation and a high nutrient amendment was slow with a higher amount of total hydrocarbon content at the end of the 180 days. The variability in the hydrocarbon degradation pattern of contaminated soil shows that biostimulated bioremediation achieved better results in soils with low moisture content than in soil environments with high water content (saturation). More so,

nutrient overdosing of the substrate hampered the effectiveness of the remediation process.

Keywords

Bioremediation, Soil, Nutrient, Moisture Content, Total Petroleum Hydrocarbon, Crude Oil

1. Introduction

The Niger Delta is known to be one of the ten most important wetlands and marine ecosystems in the world. The presence of oil industries located within this area has contributed immensely to the growth and development of the Nigerian economy but unsustainable oil exploration activities have caused the Niger Delta region to be described as one of the five most severely petroleum-damaged ecosystems of the world (Kadafa, 2012). There have been quite a lot of remediation technologies for oil spill-impacted soil in the Niger Delta but most of these technologies have not yielded the much-desired results as the methods used were either unsuitable for the environment or ineffective for the different soil types in the Niger Delta. Crude oil is a common environmental contaminant, and its occurrence has produced many analytical and remediation technologies (Smith et al., 2015). The conventional technologies for the remediation of crude oil-impacted soils incorporate many technologies which include the ex-situ traditional removal of contaminated soil to a landfill (M'rassi et al., 2015), on-site incineration of pollutants, soil washing, pump-and-treat operations, and the in-situ thermal treatment, chemical oxidation, and the use of reactive barriers. These methods have so many drawbacks; such as the secondary release of contaminants/pollution. However, negative public opinion and perception towards them have resulted in the development of other treatment options. Consequently, other better technologies to destroy the pollutant or transform it into a harmless product have been widely adopted in many countries. Bioremediation provides a good clean-up strategy for crude oil-polluted soils. According to Beškoski et al. (2012), to increase the rate of biodegradation of hydrocarbons in the ecosystem and maximize the process in bioremediation technologies, these main approaches are applied: biostimulation in which nutrients are added to stimulate the natural hydrocarbon degraders; bioventilation which ensues the required quantity of the molecular oxygen-aeration; bioaugmentation in which microbial strains with specific degrading abilities are added to work cooperatively with normal indigenous soil microorganisms (Alvarez & Illman, 2006). Osuji and Raji (2007) used chemical augmentation as the regenerative capacity of soil macronutrients by the addition of inorganic fertilizers to remediate hydrocarbon polluted soil. This study has considered the different soil environments in the Niger Delta such as muddy environments, and dry and wetlands using biostimulated bioremediation in its design of a bioremediation process, unlike previous stu-

dies.

2. Materials and Method

2.1. Laboratory Experiment

A bulked clean soil sample was impacted with crude oil at a ratio of 10 g/kg (10% w/w) as described as reported by Okorondu et al. (2017). One hundred and fifty grams (150 g) of the contaminated soil was placed in each of the seven microcosms (bioreactors) in an aerobic condition at an average temperature of 30˚C (Okorondu et al., 2017).

The laboratory samples were exposed to different conditions that foster biodegradation of the hydrocarbon compounds inherent in the matrix of the soil (substrate). The substrate in this context is the medium in which the microorganism lives. The conditions were constrained to the variableness of the concentration of nutrients inoculated into the media (bioreactors) and the medium of presentation of the nutrient inoculants. The nutrient was specifically NPK (Nitrogen−Phosphate−Potassium) fertilizer, the medium for the presentation of the nutrient was water and the volume of water used varied from 30% to 80% saturation while the mass of the nutrient was constant (30 g) over a variable volume of water. No cultured series and specific microorganisms were introduced into the media (substrate) for all samples, the biodegradation media, which was soil, implies that only the microorganisms present in the substrate were allowed to act on the substrate (Okorondu et al., 2017). The samples were coded as shown in Table 1.

Periodically, soil samples from each of the sample sets were taken to determine the amount of hydrocarbon in the soil at intervals of 3 months for 180 days.

2.2. GC Analysis

Ten grams (10 g) of the soil sample was blended with 10 g of anhydrous sodium sulfate and extracted by sonication using a Dichloromethane acetone mixture (1:1 ratio). The sample extract was later concentrated to about 2 ml in a rotary

Table 1. Sample codes and composition.

SAMPLE CODES	COMPOSITION
SET A	Crude oil + 30 g NPK + Soil
SET B	Crude oil + 60 g NPK + Soil
SET C	Crude oil + Soil (Control)
SET D	Crude oil + 80 g NPK + Soil
SET E	Crude oil + 30 g NPK + Soil + 30% H_2O saturation
SET F	Crude oil + 30 g NPK + Soil + 50% H_2O saturation
SET G	Crude oil + 30 g NPK + Soil + 80% H_2O saturation

evaporator (USEPA 355˚C). The total petroleum hydrocarbons were determined using a GC-FID (Agilent 6890 N) with an HP-5 fused silica column of dimensions 30 m × 250 μm × 250 μm film thickness and 5% phenyl methyl siloxane capillary column. The oven temperature program was maintained at 40˚C for 2 min and then increased at a rate of 10˚C/min until a final temperature of 320˚C was reached. The final temperature was held for 2 min with Helium carrier gas held at a constant flow rate of 2.6 ml/min and pressure of 10.4 psi.

The calibration standard was obtained from AccuStandard (USA). A hydrocarbon window-defining calibration mixture was used to perform a 5-point calibration of the TPH method using o-Terphenyl as an internal standard surrogate. Calibration verification was done by running a solvent (Dichloromethane) blank, mid-concentration standard and o-Terphenyl surrogate QC standard (8 mg/l) every day as a minimum requirement before the start of work with a recovery of 80% - 120% (i.e. ±20%).

3. Result and Discussion

3.1. Result Presentation

The amount of hydrocarbon remaining in the soil after exposure to bioremediation, which could be regarded as the target quantity is used to evaluate the degree of remediation of a spill. The laboratory samples were subjected to a biostimulated biodegradation process at controlled conditions as shown in Table 1.

Table 2 shows the initial concentration (36441.9 mg/kg) of the crude in soil samples after spiking in March, by June, three months after the start of the laboratory process of biostimulated biodegradation, the substrates could have performed differently in the enhancement of biodegradation given the different compositions of the substrates to evaluate the efficiency of the substrate/media. At the end of three months of exposure, sets A and E had the lowest concentrations (2725 mg/kg, 2353 mg/kg) of total petroleum hydrocarbon in the soil as shown in the June harvest on Table 2, while sets G and F had the highest amount of total petroleum hydrocarbon in the soil (8215 mg/kg, 7404 mg/kg). It is pertinent to understand that the inoculant is an NPK fertilizer, and it is a

Table 2. Hydrocarbon concentrations in soil sample sets June, August, and November.

SETS	MARCH (mg/kg)	JUNE (mg/kg)	AUGUST (mg/kg)	NOVEMBER (mg/kg)
A	36441.9	2725	871	466
B	36441.9	4079	1167	423
C	36441.9	3664	1810	277
D	36441.9	4393	778	955
E	36441.9	2353	1229	416
F	36441.9	8215	5970	3899
G	36441.9	7404	3076	1972

slow-releasing fertilizer. The number and species of microorganism present in the soil effectively determine the efficiency of remediation, however, the rate of bioremediation could be adversely affected, if the release of nutrient in the inoculants is slower or faster in relation to retarding the growth of the microorganism colony in the soil. The F and G sets had the highest concentration of hydrocarbons for all the samples harvested in June, August, and November as shown in **Figure 1**.

The F and G samples had very watery substrate with 50% to 80% water saturation (**Table 2**), this is confirmed by the observation of Xia et al. (2006), who observed that the degradation of hydrocarbon component, which was aromatic was less effective in water/watery media relative to media with high sediment/soil content. This is because the survival and degrading ability of the microorganism in the media highly depends on environmental conditions (Vogel, 1996). Potentially, degrading strains in one site may not apply to another site since the objective of the method is to provide the microorganism colony with the most favorable environment in which they can degrade effectively (Mohan et al., 2006; Ueno et al., 2007). Sarkar et al. (2005) found that the microorganisms in a stimulated biodegradation substrate can decrease and remain ineffective due to the toxicity of the media as a result of over-dose of the nutrient from the inoculants. The nutrient-based biostimulation was observed to prove that the application of 60% of NPK is the best treatment option resulting in the degradation/removal of 50% of crude in the soil, while the control for which no nutrients were added removed 25% (Ubochi et al., 2006).

By the end of August harvest and analysis, sample sets A and D had the lowest amount of hydrocarbon (871 mg/kg, 778 mg/kg) in the substrate, while sample sets F and G had the highest amount of hydrocarbon (5970 mg/kg, 3076 mg/kg) in the substrate as shown in **Figure 1**. The biodegradation profiles of sample set A, D, F, and G are shown in **Figure 2** and **Figure 4** and **Figure 5** and **Figure 7** respectively. The consistency of sample sets F and G with the highest hydrocarbon

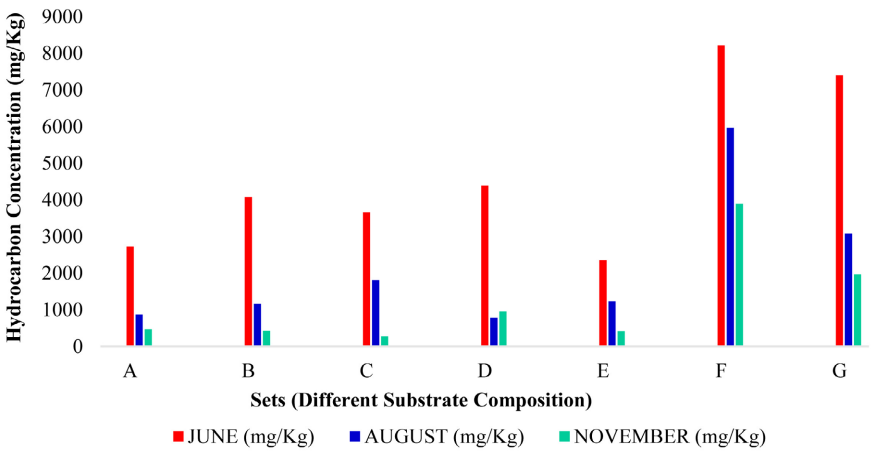

Figure 1. Clustered column plot for hydrocarbon composition in different soil sample sets derived from the sample harvest for June, August, and November.

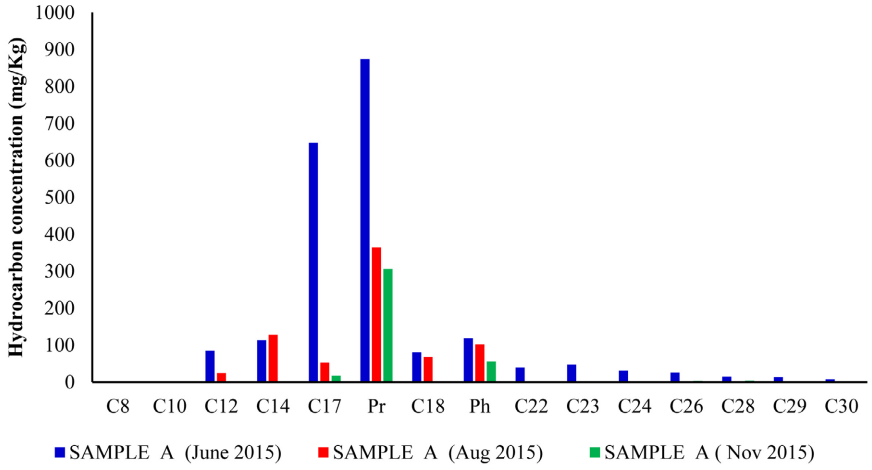

Figure 2. Plot representing hydrocarbon distribution profile in sample sets A.

content had been established as shown in **Table 2** because biodegradation is slow and inefficient in systems with high water content. However, the inconsistency of the pair of sample sets A and E in the June harvest and analysis and changing to A and D in the August harvest and analysis, could be explained by the fact that sample E has a high water content relative to the other sample sets and may not degrade efficiently in its substrate Xia et al. (2006). The November harvest and analysis showed appreciable changes in trends as shown in **Figure 1**. The controlled set, which is sample set "C", had the lowest total petroleum hydrocarbon components in the substrate, followed by sets E, B, and A, but sample sets F and G remain the highest in their concentrations of the total petroleum hydrocarbon components in the substrate. The change in trend could be attributed to the fact that NPK is a slow-releasing fertilizer; hence, constant inoculation could result in overdosing the substrate since the inoculant is slow releasing, the substrate could become toxic to the microorganism (Sarkar et al., 2005). This will result in rendering the microorganism colony inefficient in degrading the petroleum hydrocarbon components in the substrate at that stage of the processes compared to the initial capability at the start. The toxicity of the substrate could also result from the fertilizer-induced acidity of the substrate, which has a high tendency of originating from the nitrates, which is the form in which most or all fertilizers supply nitrogen into the soil when applied (Sarkar et al., 2005). Chaillan et al. (2006) also observed that overdosing the soil with nutrients will result in the inhibition of the degradation of less degradable hydrocarbon compounds.

The hydrocarbon distribution profile of the sample sets A, B, C, D, E, F, and G as shown in **Figures 2-8** respectively shows C_{17} and Pristane (Pr) were slow to biodegradation and does not show a consistent distribution profile. Sample set E had in addition isoprenoid Phytane (Ph) as a recalcitrant compound. These recalcitrant compounds contributed largely to the amount of hydrocarbon left in the soil at each of the sample harvest periods and could be used to monitor the degradation pattern.

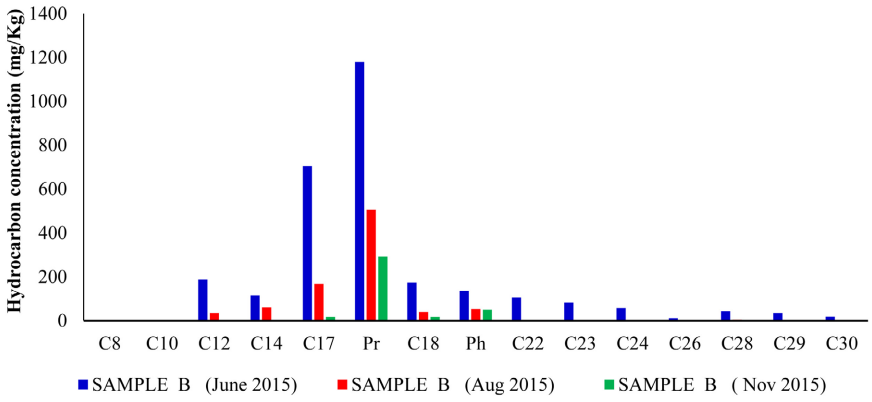

Figure 3. Plot representing hydrocarbon distribution profile in sample set B.

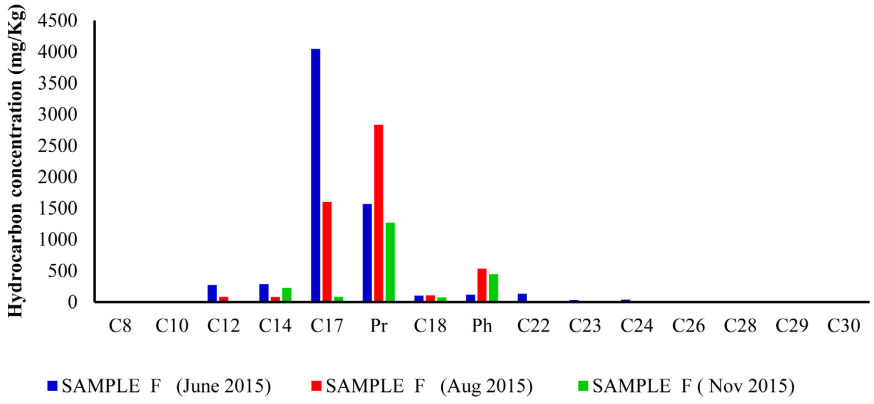

Figure 4. Plot representing hydrocarbon distribution profile in sample set F.

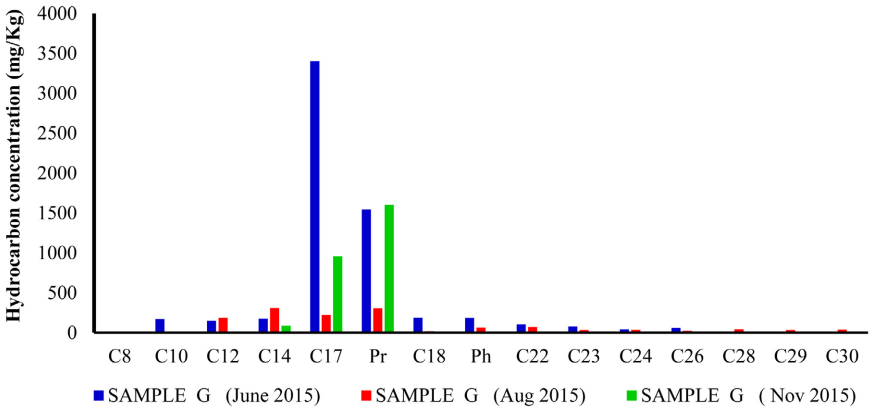

Figure 5. Plot representing hydrocarbon distribution profile in sample set G.

Relating the Hydrocarbon in the Soil to Substrate Composition

The bioremediation design/composition of the substrate could play a vital role in the degree of degradation of hydrocarbon in the soil. However, bioremediation depends on a variety of factors including microorganisms present, concentrations of hydrocarbons, and environmental conditions (pH, temperature, nutrients, oxygen, and moisture content) suitable for microbial degradation (Betancur-Galvis et al., 2006; Gouda et al., 2008; Leahy & Colwell, 1990; Perfumo

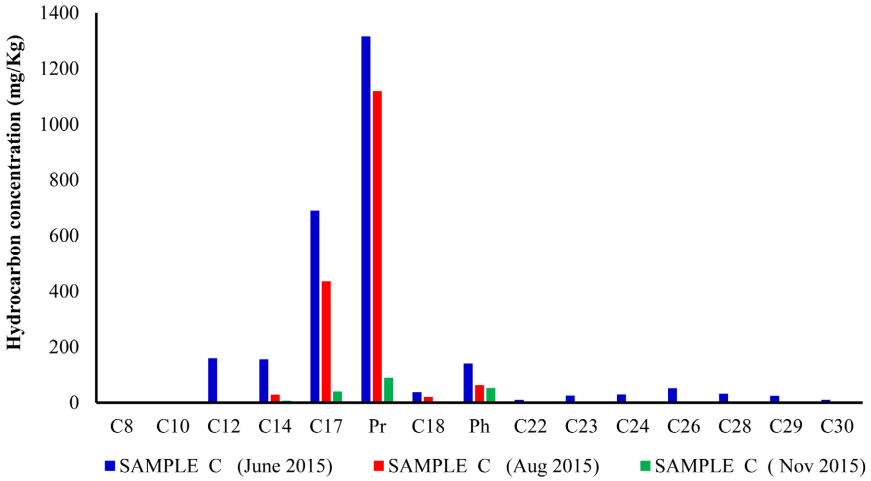

Figure 6. Plot representing hydrocarbon distribution profile in sample sets C.

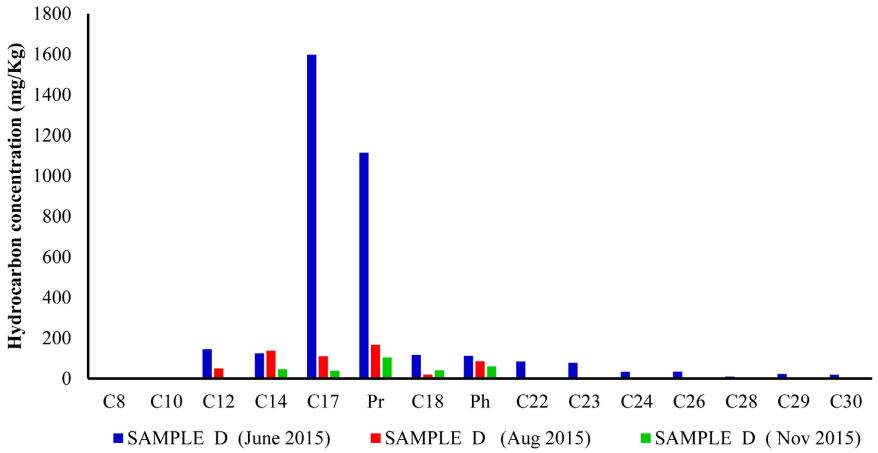

Figure 7. Plot representing hydrocarbon distribution profile in sample set D.

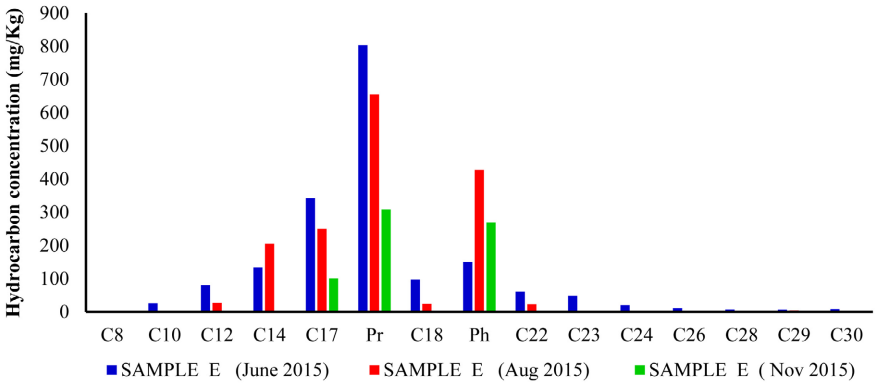

Figure 8. Plot representing hydrocarbon distribution profile in sample set E.

et al., 2007; Horel & Schiewer, 2009). The bioremediation method under evaluation is biostimulation, which involves the application of proper nutrients to the soil to enhance the activity of indigenous microorganisms (Odokuma & Dickson, 2003; Perfumo et al., 2007; Malina & Zawierucha, 2007). However, in this

study, the conditions considered were the composition of the substrate and the percent water saturation of the substrate.

Figure 4 and Figure 5 show that sample sets F and G had the highest amount of total petroleum hydrocarbon left in the soil after the given period for biodegradation. This observation to the composition of the substrate is suggested to be less favorable to the biodegradation of hydrocarbons. These sample sets had 30 g of NPK each, 50% and 80% water saturation respectively.

In a study, Xia et al. (2006) evaluated the efficiency of biodegradation in water systems; however, the emerging result showed that biodegradation rates were higher in water systems with sediments relative to water systems without sediment. This infers that the increased water saturation of sample sets F and G could retard the biodegradation efficiency of the substrates resulting in a high amount of total petroleum hydrocarbon left in the soil.

Sample set C is the control set, for which no nutrients were inoculated, however, the results in Figure 1 and Table 2 indicates that sample set C had a low amount of total petroleum hydrocarbon left in the soil. The irony of this observation is the fact that unstimulated substrates provide a good medium for the biodegradation of total petroleum hydrocarbon on the basis that biostimulation is geared towards enhancing the activity of microorganisms in the degradation of hydrocarbons by nutrient inoculation. This observation is attributed to the fact that the microorganism colony could have been exposed to the increasing toxicity of the substrates mainly due to overdosing of the substrates with regular nutrient inoculation against the backdrop of slow-releasing nutrients. The overdose results in the excessive presence of nitrates, which ionizes to form nitrous and nitric (nitrogen III and nitrogen V) acids in the soil (Pawar, 2015). The homogeneity of the nutrient in the soil presents a challenge to the availability of nutrients to the microorganisms in the soil.

Sample sets A, B, D, and E had total petroleum hydrocarbon compounds left in the samples at the end of the period for the bioremediation experiments, the concentrations of total petroleum hydrocarbons left in the soil were 466 mg/kg, 423 mg/kg, 955 mg/kg, and 416 mg/kg respectively. Sample sets A, B, and E, have total petroleum hydrocarbon left in the soil of about 400 mg/kg while the total petroleum hydrocarbon content for D was 955 mg/kg. The composition for sample set D was crude oil, 80 g NPK, and soil. The high total petroleum hydrocarbon content could be attributed to the high fertilizer content of the soil. Regular inoculation for nutrients and moisture may raise the pH level thereby rendering the substrate toxic to the microorganism, which retards their degradation capability, resulting in higher amounts of total petroleum hydrocarbon in the soil.

Exempting the control sample set which was sample set C, the best substrate composition for the series of tests in the study was that of sample set E constituting crude oil, 30 grams NPK, and 30% water saturation. The 30% water saturation could have provided the enabling environment for proper moisture, a surfactant for the microbe community to function properly.

The sample sets A and B, all have total petroleum hydrocarbon in the soil in the range of 466 and 423 mg/kg respectively as shown in Table 2 and Figure 1, all the sample sets have a similar composition with variability in the quantity of NPK fertilizer, set A has 30 g NPK while set B has 60 g NPK.

4. Conclusion

A successful biodegradation process may require strategies customized for site-specific environmental parameters of both contaminated soils and contaminants. The variability in the hydrocarbon degradation pattern of contaminated soil shows that biostimulated bioremediation achieved better results in soils with low moisture content than in soil environments with high water content (saturation). Also, nutrient overdose on the substrate could hamper the effectiveness of a biostimulated biodegradation process.

Conflicts of Interest

The author declares that there is no conflict of interest regarding the publication of this paper. Lastly, all other issues such as plagiarism, falsification of data, misconduct, and others were duly observed by the author.

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