

Full Length Research

Comparison of bioremediation capabilities of poultry droppings and avocado pear seed cake in petroleum polluted soil

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A crude oil polluted soil remediation was investigated using a mixture of avocado pear seed cake and poultry droppings. The seed cake was obtained through the Soxhlet method by extracting the oil from the pear seed. The experiment was carried out within a span of two months. Contamination was done by applying crude oil on 10 experimental reactors containing agricultural soil. The therapeutic treatment was then applied to the reactors, and the soil physicochemical properties were analyzed after set periods. The result shows that pH (6.89%), moisture content (18.1%), electrical conductivity (5430 $\mu\text{s}/\text{cm}$), total organic carbon (0.996%), total petroleum hydrocarbon (32.0 mg/kg) and total nitrogen (2.00%) which is an indication of distinct variation with time with the bacterial count of 9.20×10^7 cfu in all the reactors with an increased time. The control reactor does not show significant remediation throughout the study period. However, there was significant degradation of total petroleum hydrocarbon (TPH) in all the experimental reactors as follows: 76, 67, 55, 86, 78, 69, 81, 73 and 62% for $T_1, T_2, T_3, U_1, U_2, U_3, V_1, V_2$ and V_3 after 2months. The study revealed that good remediation was achieved by the application of poultry dropping only 0.0324 days^{-1} compared to the mixture of poultry dropping and avocado pear seed cake (0.0275 days^{-1}). Again, treatment with poultry droppings (PD) only removed 89.65% of TPH while combination of PD+ avocado pear seed cake (APSC) removed 85.41% of TPH after 70 days remediation period as predicted. The 200 g PD only (U_1) also increased the amount of nitrogen in the soil which is favorable for plant growth.

Key words: Remediation, petroleum polluted soil, poultry droppings, avocado pear seed cake.

INTRODUCTION

The fertility and size of agricultural soil is a great wealth to a nation because the increasing population of the

world depends on it. Developing countries like Nigeria which is known to be one of the major producers of crude

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oil experiences a steady rate of oil spillage which has led to pollution of the environment thereby affecting the human life, agricultural soil, and aquatic life (Olatunji et al., 2017). The department of petroleum resources estimated that about 1/89 million barrels of petroleum were spilled into the Niger Delta between 1976 and 1996; also, UNDP report states that, there have been a total of 6817 oils spills between 1976 and 2001 which accounted for a loss of three million barrels of oil (Nwaogu et al., 2008; Odiete, 1999). Environmental studies carried out in oil spilt areas showed different levels of pollution associated with exploration and production activities. The effects of crude oil spills limit the usage of areas polluted for agricultural activities thereby causing land shortage food production. Also, industries are restricted from the usage of such lands because it is very contaminated. For instance, in the USA the cost is expected to exceed \$1 trillion (Odiete, 1999; Ugochukwu et al., 2016). In the USA, 90% of the sites undergoing remediation are linked to petroleum hydrocarbon (Cole et al., 1994; Ugochukwu et al., 2016). Oil spillage has a significant impact on the ecosystem into which it is released and may constitute ecocide. Immense tracts of the mangrove forest which are susceptible to oil have been destroyed. Spills in populated areas often spread out over a wide area destroying crops and aquaculture through contamination of groundwater and the soil. Due to oil exploration and exploitation in the delta region, the environment is growing increasingly uninhabitable; hence, the need for remediation becomes imperative for the polluted sites. Different methods are applied primarily due to the cost and time consideration. Restoration of a crude oil polluted soil can be done by employing physical, chemical, and biological processes. The most common and widely used technique is the biological method (bioremediation) and it is regarded as the available means of managing a hydrocarbon polluted land. The term bioremediation as used in the context can be defined as microbiological decontamination of hydrocarbon contaminated soils (Ayotamuno, 2003; Sharma and Reddy, 2004). It involves the introduction of oil-degrading microorganisms or activation of indigenous ones are now considered as most economical methods for the decontamination of oil pollution (ISEST, 2013). Avocado pear (*Persea americana*) also called alligator pear is native to tropical America. This fruit has a very high economic, medical and market values, and hence very useful. The fruit is a greenish thick skinned drupe. When ripe, the flesh has the consistency of firm butter and a faint nut like flavour. It has around big seed inside. The cake produced from the avocados seed is rich and it is very useful in improving the soil condition. Bio-stimulation of indigenous micro-organism by the addition of nutrients is most widely used bio-remediation procedure since large quantities of carbon source (contaminants) tends to result in the rapid depletion of the available pool of major inorganic nutrients e.g. Nitrogen (N) and Potassium (K), this

method is more preferred when compared with other methods because the simplicity or ease with which avocado pear seed cake and poultry droppings are obtained. And its use of conventional equipment, convenient environmental nature and it helps in retaining the soil quality by improving the soil structure, compaction, reducing crusting, increase microbial activities and provides nutrient to the soil.

This study is aimed at utilising bio-stimulation option of bio-remediation technology with the focus on the investigation of the effectiveness of the mixture of avocado pear seed cake and poultry droppings in the remediation of the crude oil polluted soil by comparing the physicochemical properties of crude oil contaminated soils before and after the remediation period.

MATERIALS AND METHODS

The study was carried out in the research farm of the Rivers State University of Science and Technology, Port Harcourt, Nigeria. Rivers State is known for the increasing oil and gas activities within the Niger Delta region of Nigeria which is known to produce 98% of Nigeria's income (that is, crude oil). The region lies between latitude 4°47'55.68"N and 7°01'24.96"E (Olatunji et al., 2017). The area has a mean annual rainfall of about 2400 mm and the average temperatures recorded in the area is about 27°C from the experiment (Ayotamuno et al., 2006) (Figure 1).

The poultry droppings were obtained from the poultry department of the research farm of the Rivers State University, Port Harcourt, Nigeria. The poultry droppings were allowed to decompose properly before used in the remediation of the polluted soil. The avocado pear (*P. americana*) was obtained at the Opolo-Epie market at Yenagoa, Bayelsa State, Nigeria. The pear seed was then separated carefully from the mesocarp, washed and was sun-dried to a certain moisture content. The seeds were then grinded with an electrical grinding machine at mile 3 market at Port Harcourt, Rivers State, Nigeria. The ground seeds were taken to the chemical/petrochemical laboratory of the Rivers State University of Science and Technology, Port Harcourt for extraction of the oil to obtain the seed cake. The crude petroleum oil was obtained from the Nigerian Agip Oil Company Korokorosei Terminal in Bayelsa State. The reactors (bucket) were obtained from the mile 3 market, Port Harcourt. A 150 g of the ground seed was measured into an extraction bag, and 200 ml of hexane was poured into an extraction flask. This was set up in a sohxlets extraction apparatus containing condenser and was heated for 10 min using a heating mantle for a complete extraction. This process of removal continued until the required quantity of the seed cake was obtained. After the completion of the extraction, the cake was exposed to the air for some days to reduce the hexane content in the seed cake before it was used for the soil treatment and then analyzed. The avocado pear seed cake and the poultry droppings were weighed into different masses such as 200, 100, 50, 25 g, etc. in the civil laboratory of the Rivers State University of Science and Technology. Then shovel was used to dig the soil samples into the reactors.

Remediation procedure

A mass of soil weighing about 2500 g was excavated and measured into 10 treatment reactors respectively. And 1.5 L of crude petroleum oil was used to pollute the 9 treatment reactor while the 1 is for the control reactor in which no treatment was

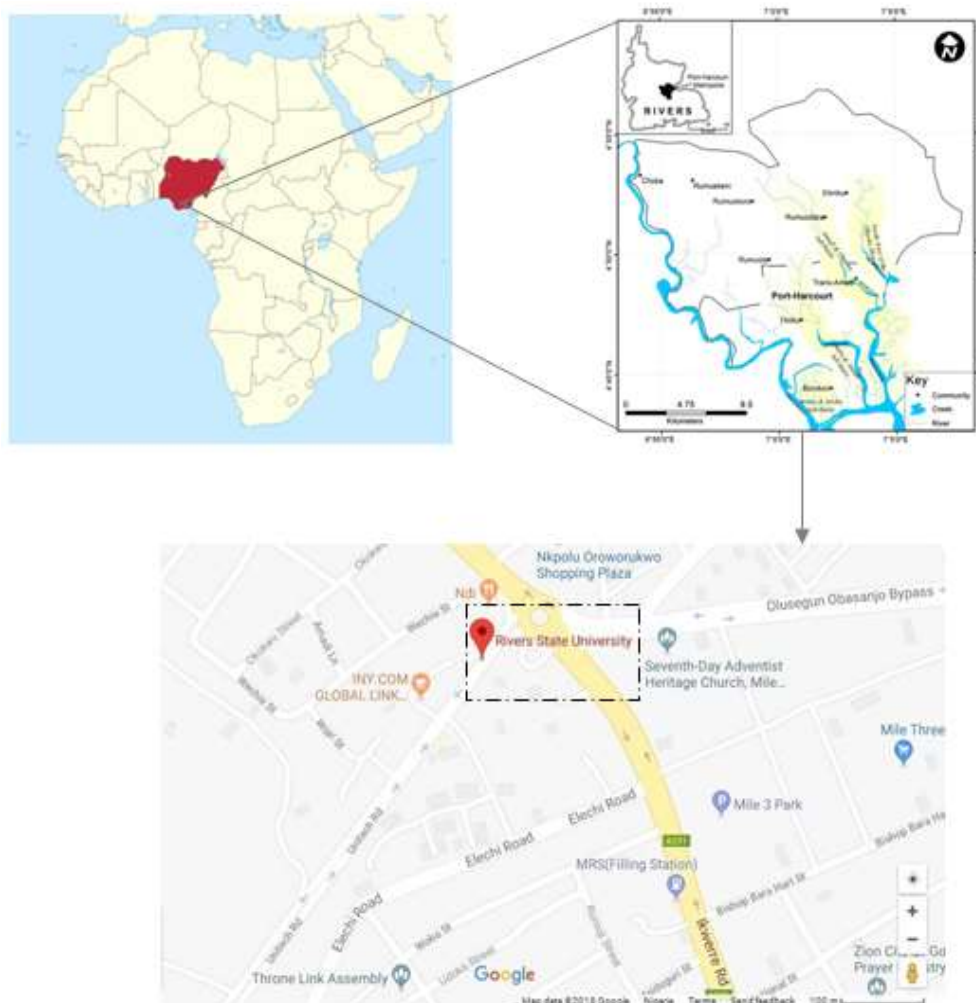


Figure 1. Map of showing the study area (2016 Google Earth-pro).

applied. After pollution, it was allowed for four days to infiltrate properly. After the four days, the soil was collected from each of the treatment reactors of composite samples with the aid of hand trowel. The unpolluted soil which is the control was also collected. The application of the agents (that is, organic manure) 200, 100, and 50 g of avocado pear seed organic manure were added to the polluted soil, respectively. Tilled soil was filled after applying the treatment to the polluted soil. The essence of this filling is to homogenize the contaminated soil nutrient and microbes for proper accretion and remediation. The tilling was done with hand trowel to break the lumps of the soil into fine particles. It is being carried out two times in a week for eight weeks. Water was applied to each treatment. The specified quantity of 0.5 g/L was added twice in a week during the eight weeks remediation period with the use of perforated cans. Tilling and watering continued for eight weeks. The fourth week, the treatment soil was collected for analysis after which tilling and watering still continued till the eight week, the treatment was also collected for the final analysis including the control sample. The soil pH, electrical conductivity (EC), total organic carbon (TOC), total nitrogen (N), total petroleum hydrocarbon (TPH), bacteria count, salinity, and moisture content were analysed using the recommended analysis procedure according to Ayotamuno et al. (2006). Table 1 shows the

description of samples used in the experiment.

Determination of total petroleum hydrocarbon

The TPH in the soil was determined according to Agamuthu et al. (2013) estimated using the standard curve derived from fresh used engine oil diluted with toluene. TPH data was fitted to first-order kinetics model Olatunji et al. (2017) using equation:

$$C_t = C_0 e^{-kt}$$

where C_t =the residual concentration (g.kg^{-1}), C_0 =the initial concentration of pollutant in the soil (g.kg^{-1}), k =the biodegradation rate constant (days^{-1}), and t =time (day).

The percentage removal of total petroleum hydrocarbon was determined using the equation:

$$\%TPH = \frac{TPH_1 - TPH_f}{TPH_1} \times 100$$

where TPH_1 =the initial amount of TPH in the soil and TPH_f =the residual amount of TPH after remediation.

Table 1. Description of treatments.

Sample label	Description
O	Control sample
T ₁	Polluted soil + 200 g of avocado pear seed cake only
T ₂	Polluted soil + 100 g of avocado pear seed cake only
T ₃	Polluted soil + 50 g of avocado pear seed cake only
U ₁	Polluted soil + 200 g of poultry dropping only
U ₂	Polluted soil + 100 g of poultry dropping only
U ₃	Polluted soil + 50 g of poultry dropping only
V ₁	Polluted soil + 200 g of avocado pear seed cake and poultry dropping
V ₂	Polluted soil + 100 g of avocado pear seed cake and poultry dropping
V ₃	Polluted soil + 50 g of avocado pear seed cake and poultry dropping

Table 2. Physico-chemical characteristics of soil sample prior to remediation.

Parameter	Uncontaminated sample	Contaminated sample
Moisture content (%)	18.1	18.7
EC ($\mu\text{s}/\text{cm}$)	5430	3002
pH	0.89	5.70
N ₂ (%)	2.00	0.090
TPH (mg/kg)	32.0	15370
TOC (%)	0.996	6.87
Salinity (mg/kg)	2468	2211
Bacterial count (cfu)	9.2×10^7	2.64×10^6

RESULTS AND DISCUSSION

Analysis of results

The soil before contamination shows a moisture content of 18.1%, electrical conductivity of 5430 $\mu\text{s}/\text{cm}$, pH value of 6.89, total nitrogen of 2.00%, total petroleum hydrocarbon of 32.0 mg/kg, total organic carbon of 0.996%, salinity of 2468 mg/kg, and bacterial count of 9.20×10^7 cfu (Table 2). After three days of contamination the moisture content of 18.7%, electrical conductivity of 3.002 $\mu\text{s}/\text{cm}$, pH value of 5.70, total nitrogen of 0.090%, total petroleum hydrocarbon of 15,370 mg/kg, total organic carbon of 6.87%, salinity of 221 mg/kg, and bacterial count of 2.64×10^6 cfu was observed (Table 2). The experimental results were analysed in Excel using graphical method which shows the standard deviation (Figure 2A-G).

It was observed that, there was significant reduction in the soil moisture content prior to remediation and increased later in all the treatments. The moisture content drop at the initial stage is because, in soils that are heavily contaminated, the water droplets adhere to the hydrophobic layer formed, and it prevents wetting of the inner part of the soil aggregates. The organic carbon degradation took place after remediation and it was more

effective in option T, U and V. The addition of nutrient enhanced the degradation of organic carbon. But change in the control also led to change in organic content. Treatment U₁ reduced the TOC level significantly which indicates that 200 g of poultry dropping only is more effective in the remediation (Figure 2b). Both the soil pH and the electrical conductivity of the soil dropped prior remediation. This was greatly observed in the 4th week of remediation and later appreciated generally during the 8th week of remediation while the control was almost the same. Treatment U₁ (200 g of poultry dropping only) significantly reduced the pH value compared to other treatments (Figure 2A).

Total petroleum hydrocarbon

The reduction in TPH for the treatment option in the result obtained shows the application of nutrients (especially 200 g of poultry dropping only, that is, treatment U₁) and adequate tillage could impact positively on biodegradation of crude contaminated soils (Figure 2H); thus, the reduction in TPH shows the effective utilization of nutrients and support the claim that nitrogenous nutrient supplied provides a suitable environment for accelerated decontamination. There is no significant

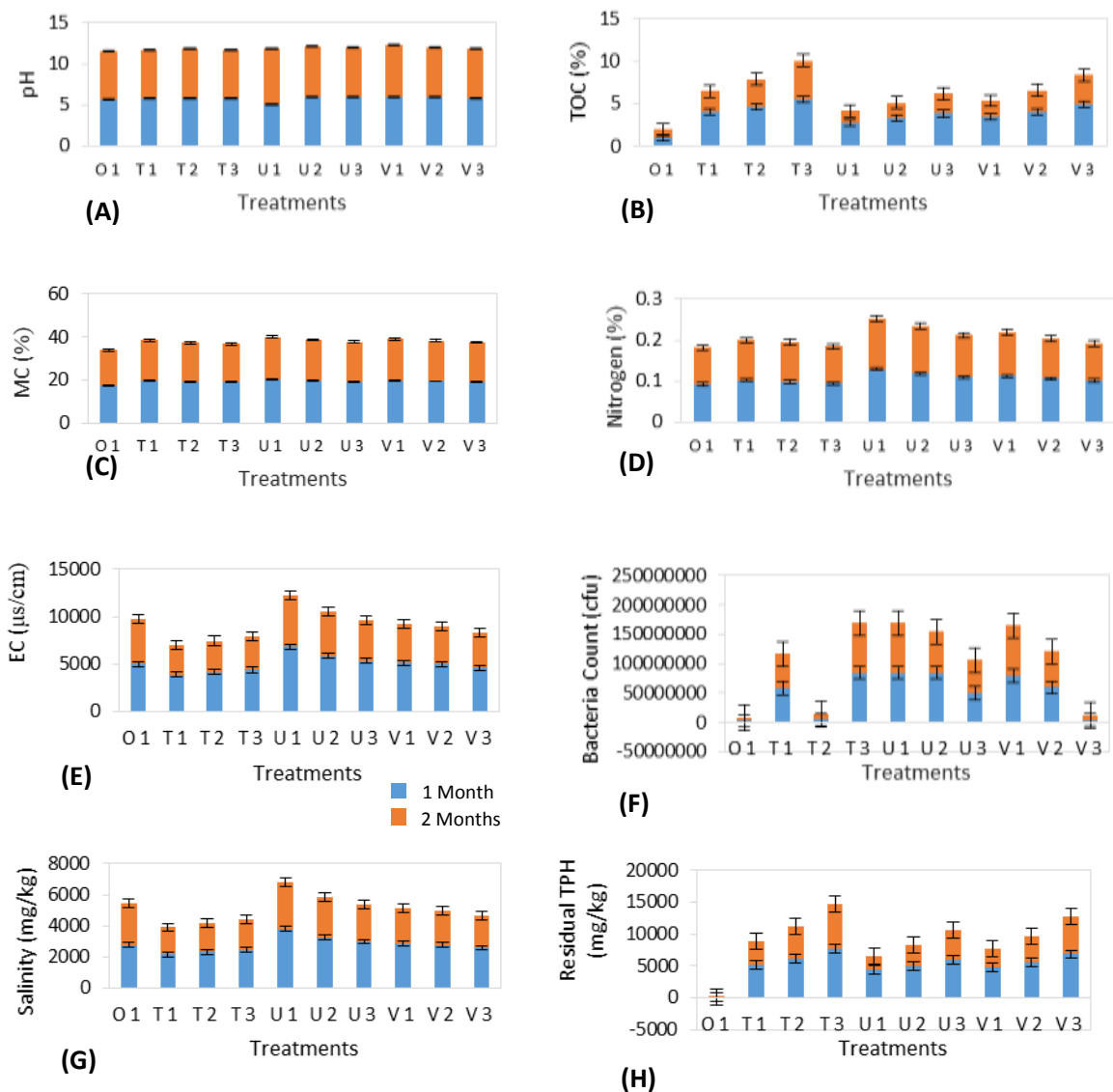


Figure 2. Variations in physico-chemical properties (A-G) and residual concentration of TPH (H).

difference between treatments in pH, moisture content, nitrogen, and bacteria count of the soil except treatment U₁ (Figure 2A, C, D, and F). However, the different treatments showed slightly significant difference on TOC, salinity, and conductivity (Figure 2B, G and E). However, the findings in this study are in agreement with Isitekhale et al. (2013) and Ogboghodo et al. (2011). Figure 2A to G shows the variations in soil physico-chemical properties after 2 weeks remediation period.

Model simulation

The mathematical model was simulated using MATLAB 2014a to predict the residual concentration of TPH after 70 days with a time interval of 14 days (Table 3).

Also, the rate of degradation was estimated using 200 g of only poultry dropping and avocado seed pear cake plus poultry droppings from a linear graph plotted in EXCEL 2016. The result showed that poultry droppings only can degrade TPH faster than a mixture of avocado seed pear cake and poultry droppings. The rates are 0.0324 and 0.0275 days⁻¹ for poultry droppings only and a mixture of avocado seed pear cake and poultry droppings (Table 4). Ogboghodo et al. (2011) showed that adding chicken manure to soil contaminated with crude oil degraded 75% of TPH in the soil within two weeks and suggested that the use of chicken manure to induce crude oil degradation in the soil could be one of the several environmentally friendly ways of minimizing TPH pollution in the ecosystem. According to Eneje et al. (2012), Kori-Siakpere (1998) and Maini et al. (2000)

Table 3. Predicted residual concentration of total petroleum hydrocarbon after 70 days of remediation.

Time (days)	TPH _{PD} (mg/kg)	TPH _{PD+APSC} (mg/kg)
0	15370	15370
14	9765	10459
28	6204	7117
42	3942	4842
56	2504	3295
70	1591	2242

Table 4. Predicted rate constants.

Amendment	Rate constant (days ⁻¹)	R ²
PD	0.0324	0.9731
PD+APSC	0.0275	0.9420

addition of organic materials such as poultry and green manure only or combination to improve the chemical properties (that is, pH, organic carbon, nitrogen, K, Mg, P, and Ca) of the oil polluted soil will enhance the solubility and removal of these contaminants, increasing the rate of degradation of TPH. Isitekhale et al. (2013) showed that application of a mixture of inorganic and organic fertilizer reduced soil TPH from 300 to 39.08 and 70.00 mg/kg, that is, net remediation of 86.97 and 76.42%, respectively. While, Ugochukwu et al. (2016) revealed that the total hydrocarbon content of the oil-polluted soil decreased from 6609.83 to 2951.37 ml/g (55.3% reduction) by applying poultry droppings. The control sample showed slight increase in microbial growth and the growth rate increased as the concentration of the poultry droppings increased.

The residual concentration of TPH was predicted using 200 g of poultry droppings only and combination of poultry droppings and avocado pear seed cake. However, treatment with poultry droppings only showed significant removal of contaminant compared to that of the combination treatment: residual TPH concentration yielded 1591 and 2242 mg/kg for poultry droppings only and combination of poultry droppings and avocado pear seed cake, respectively at 70 days remediation period (Table 3). Also, the rate of degradation is higher in treatment with PD only than PD+APSC (Table 4). Nevertheless, determination of correlation shows a strong relationship between time and residual concentration of TPH as shown in Table 4. Again, the percentage removal of TPH as shown in Table 5 indicates that treatment with PD only removed 89.65% of TPH while combination of PD+APSC removed 85.41% of TPH after 70 days remediation period.

Fundamentally, bioremediation uses microorganisms (e.g. bacteria, yeast, and fungi) to 'digest' toxic organic

contaminants (Sharma and Reddy, 2004), such as crude oil (TPH), producing water (H₂O) and carbon dioxide (CO₂) (USEPA, 2001). This can occur in the presence of oxygen or without oxygen, known as aerobic and anaerobic conditions (Figures 2 and 3). For the microorganisms to survive in the soil, a balance of a number of parameters including temperature, pH, moisture content, oxygen concentration and nutrients in the soil must be achieved (Sharma and Reddy, 2004; Agamuthu et al., 2013). However, bioremediation is most efficient according to Sharma and Reddy (2004): (1) in the temperature range of 15 to 45°C, (2) when pH is around 7 (can work with pH between 5.5 and 8.5), (3) at moisture levels of 40 to 80% of field capacity, (4) at oxygen concentrations of >2 mg/L (aerobic) and (5) <2 mg/L (anaerobic), (6) when nutrients including carbon, hydrogen, oxygen, nitrogen and phosphorous are in abundance. In this study, the poultry droppings act as nutrient (due to the presence of nitrogen, phosphorous and other elements contained in it) to the microorganisms (bio-stimulation) which makes the remediation process faster than avocado pear seed cake (Figures 3 and 4). However, Figures 3 and 4 show the summary of the comparison between treatments.

Conclusion

It was evident that 200 g of all the treatments reduced the pollutant level than the others. Nevertheless, the remediation processes can be recognized based on their order of increment as: T₁, U₁, V₁ (200 g), T₂, U₂, V₂ (100 g), T₃, U₃, V₃ (50 g). Again, it was observed that the nutrients in the poultry droppings provided the room for further degradation. Similarly, the overall performance showed that all the treatments, that is, T, U, V remediated



Figure 3. Bioremediation process using Poultry Droppings (2-step reaction).

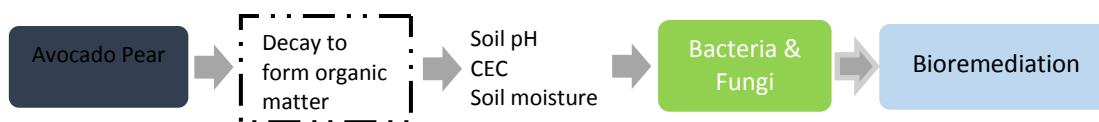


Figure 4. Bioremediation process using Avocado Pear Seed Cake (3-step reaction).

Table 5. Percentage removal of TPH after 70 days remediation period.

Time (days)	TPH _{PD} (%)	TPH _{PD+APSC} (%)
0	0.00	0.00
14	36.47	31.95
28	59.64	53.70
42	74.35	68.50
56	83.71	78.56
70	89.65	85.41

the soil at different levels. Again, the percentage removal of TPH as shown in Figure 5 indicates that treatment with PD only removed 89.65% of TPH while combination of PD+APSC removed 85.41% of TPH after 70 days remediation period. However, the comparison shows that the treatment with poultry droppings only remediated the polluted soil faster than the combination of poultry droppings and avocado pear seed cake.

RECOMMENDATIONS

This research work, advocates the utilization of the findings of the study for further remediation works on crude oil polluted soils, especially as cost effectiveness is of paramount consideration. Therefore, treatment U₁ (that is, 200 g of poultry dropping only) is recommended for the remediation of TPH, when a concentration of TPH is within the range specified in this work.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ABBREVIATIONS

EC, Electrical conductivity ($\mu\text{s}/\text{cm}$); N₂, nitrogen (%); MC,

moisture content (%); TOC, total organic carbon (%); TPH, total petroleum hydrocarbon (mg/kg); APSC, avocado pear seed cake (kg); PD, poultry droppings (kg).

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