



## Evaluation of the Potential Effects of Green-biomass for Remediation of Petroleum Polluted Soils: A case Study of Ube Leaf Mulch

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### Abstract

The effects of green-biomass applications using pulverized Ube (*Dascroides edulis*) leaf-mulch (PULM) on the water holding capacity (WHC) of petroleum product polluted soils and the immobilization of hydrocarbon within the soil matrix was investigated. Aired soil was processed and spiked with a petroleum diesel and further treated for WHC and leaching experiments according to standard procedure. The concentration of diesel range organics (DROs) in the leachate were evaluated using peak area ratio from a standard GC-MS analysis. The results revealed that composting 15% (with respect to soil mass) diesel polluted soils with 1, 2.5, 5 and 10% PULM, increased the soils' WHC by ~28.85, 33.45, 36.55, and 45.25% respectively. Also, the leaching study on 20% diesel contaminated soils, showed that, 2.5, 5 and 10% PULM prevented the leaching of the diesel range organics (DROs) by 58.5, 89.25 and 100%, respectively, from the diesel spiked soils. This implies that, the biomass could reduce the mobilization of the organic contaminants within the soil matrixes thereby increasing the accessibility of soil pollutants biodegraders (whose population is usually optimal at the top soil). Also, characterization of the PULM indicated significant amount of TOC and fibre content which have the potentials for microbial energy supply and soil aeration. Favorable soil physicochemical properties and accessibility of organic pollutants to soil microbes are vital factors for optimal bioremediation of petroleum polluted soils. Therefore, this study has revealed the use of biomass such Ube leaf mulch (and by extension, similar green-biomass) would be a positive enhancement for bioremediation of hydrocarbon (and similar organic) polluted soils.

**Keywords:** Green Composts, Soil Remediation, Soil Conditioning, Microbial Pollutants Accessibility, Hydrocarbons Polluted Soils

### Introduction

Bioremediation technology makes use of the advantages of the potentials of environmental microbes, particularly soil and aquatic micro-organisms, to break down biodegradable harmful and toxic compounds in our environment (Oghoje et al., 2024; Oghoje et al., 2023; Adekunle, 2011). Since the techniques' underlying mechanisms are microbe-driven, a number of innovative strategies for enhancing and promoting microbial growth, diversity, and metabolism have been documented (Oghoje et al., 2024; Oghoje et al., 2023; Chorom et al., 2010). However, efforts to improve methods and enhance approaches have been progressive. Good soil moisture level, pH, texture, temperature, and oxidation potential are just a few of the essential physicochemical factors that must be present for bioremediation to be efficient and effective. Previous studies have suggested a soil pH range of 6.50 to 8.50 for the best bioremediation (Atias, 1981; Bossert & Bartha, 1984). Additionally, soils' temperatures between 25 and 35 °C has been accredited for efficient and effective biodegradation of organic contaminants (White, 1987; Mmon & Deekor, 2011). Therefore, availability of nutrients, favourable soil pH and temperature, oxygen concentration, and moisture are all essential factors that could influence microbial development and metabolism. On the other hand, for the best remediation of oil contaminated or polluted soils, the accessibility of contaminants by hydrocarbons degraders is crucial (Oghoje et al., 2021; Chorom et al., 2010; Atlas, 1981).

The number of soil microorganisms is known to increase at the top soil layers and decrease as one descends the soil profile (Li et al., 2025; Marschner et al., 2012). Therefore, it's critical to prevent organic contaminants from quickly washing out of the top soil and to maintain them there. As a result, the degraders would have easier access to the pollutants, and the pollutants would be less likely to quickly reach the aquifer. The improvement of biodegradation of hydrocarbons in soils through bio-stimulation, bio-augmentation, and bio-facilitation has been the subject of numerous studies (Abdulsalam & Omale, 2009; Millioli et al., 2009; Xiao-Dong et al., 2005). Literature reviews showed that, only a few reports have been published on the use of green biomass (particularly PULM) to increase WHC and reduce the leaching of hydrocarbon pollutants from top soils. Therefore, this study's objectives include to: 1) determine the effects of PULM on the WHC of soils spiked with diesel; 2) determine the effects of PULM on the leaching of DROs from soils spiked with diesel; and 3) evaluate the significant effects of PULM on the WHC and prevention of leaching of hydrocarbon compounds from oil-contaminated soils.

## Materials and Methods

### Study Area and Soil Physicochemical Characterization

This study was carried out at University of Benin; Department of Chemistry in a greenhouse. A spade was used to collect composite soil samples at the University oil palm farm, Igue, (Within Benin metropolis, Edo State, Nigeria) at a depth of 0 to 30 cm. The soil was bulked into polymer bags and brought to the greenhouse for air drying, grind and sieved using 2mm mesh. The processed soil was then kept in a well-covered plastic container till further usage. The particle sizes of the soil and its physicochemical composition were characterized according to referenced standard and procedures. The soil and diesel were characterized prior to the soil spiking, according to standard methods and procedures. The characteristics of the soil and the diesel had earlier been published (Oghoje et al., 2024)

### The Soil Spiking with diesel

The composition of the diesel used for the study, its stabilization and sequential spiking of the air dried and processed soils was adopted from (Okeiemen & Okeiemen, 2005) and as previously reported (Oghoje et al., 2024, 2023, 2021, 2020). Evaluation of the effects of PULM on WHC of the diesel-spiked soils. The WHC of the soil utilized for the study and that after being spiked with diesel were evaluated as previously documented (Oghoje et al., 2024, 2023, 2021, 2020). The same soil spike with the diesel, had been used to investigate the effects of saw dust (Oghoje et al., 2023, Broad lime leaf mulch, Barley and Rice straws (Oghoje et al., 2024). On the enhancement of its Water retention (WHC) or WHC as well as immobilization of the DRO in soils.

### Green-biomass, Ube Leave-Mulch Sampling and Processing

Figure 1 (a – c) presents the green-biomass, Ube (*Dascroides edulis*) leaf mulch used in this study and the different stages of processing. The dropped senesced leaves (Figure 1b) were collected from a single Ube plant (Figure 1a) behind Chemistry Division building, Nigeria Institute of Oil Palm Research (NIFOR), Benin City, Nigeria. The leaf mulch was thoroughly washed using tap water to remove sand particles and dried in the greenhouse. It was then pulverized using electrical blender and sieved with 2mm mesh (< 2mm Figure 1c)



**Figure 1(a): Ube (*Dascroides edulis*) tree showing fresh leaves and fruits**



**(b) Senesced and dried PULM leave**



**Figure 1c: Pulverized (<2mm) Ube leaf mulch (PULM)**

#### **Physicochemical characterization of the soil and PULM**

The particle size analysis and physicochemical of the soil were done as documented in our previous reports (Oghoje et al., 2024, 2023, 2021, 2020). The physico-chemical characteristics of the PULM were also carried out according to standard procedures (Walkley & Black, 1934; Murphy & Riley, 1962; Bremner, 1965; Day, 1953). Sodium hydroxide hydrolysis was used to determine the fiber content of the PULM (De Meijer and Van der Werf, 1994). Other physicochemical parameters of the PULM that were assayed include pH, EC, basic cations, (WHC, TOC as well as carbon nitrogen ratio, according to standard techniques and procedures (Okalebo et al., 2000).

#### **Sampling and Characterization of the Diesel used for the study**

The diesel was obtained from a commercial filling station in Benin City. The diesel was allowed to stabilize for two weeks prior to its content characterization. During the stabilization period, the diesel was stirred every other day for least five (5) minutes in a plastic container using a polymer rod (Stirrer). The chemical analysis to ascertain the basic DROs and their concentration was done according to USEPA 8015B method (USEPA, 1996) using GC-MS (Trace 1300 GC/ITQ900 Ion Trap MS). The concentration of each diesel compound was evaluated from the GC-MS Chromatogram peaks ratio presented by each DRO compound, integrated with Thermo X CaliburQual Browser (Oghoje et al., 2020).

### Determination of the effects of the PULM on WHC of the diesel spiked soils

Diesel was added to soil samples (100g each) up to 5, 10 and 15% level of contamination. The samples were subsequently composted with the PULM at 0, 1, 2.5, 5, and 10% of the soil weight for each degree of diesel contamination. And after thorough homogenization of the samples, the WHC was evaluated in accordance with prior research (Oghoje et al., 2024). The various stages involved in the soil spiking and determining the WHC had earlier been reported (Oghoje et al., 2018, 2024). Effects of PULM on the Leaching of Diesel Compounds from Contaminated Soils. One hundred gram (100g) each of soil samples was contaminated with up to 20% diesel (to mimic heavily diesel polluted field environment). To each sample, was added approximately 25ml distilled water to maintain the moisture level of the soil at about 65% of the initial WHC. After which the samples were composted with PULM at weights of 0, 2.5, 5, and 10% of soil dry weight. By applying the OECD/OCDE 312 OECD (Organization for Economic Co-operation and Development) recommendations, 150 mL of distilled water was added to the soil column (through simulated rainfall on the samples which were properly mixed and packed into individual comparable 15 by 3 cm soil columns) (OECD, 1995). After that, dichloromethane (DCM) was used to extract the DROs from each leachate. The sample extracts were then cleaned with mixture of 3g sodium sulphate and 2.5g silica gel in a silica gel column. The cleaned extracts were then subjected to the previously reported GC-MS chemical analysis (Oghoje et al., 2024). Relative Effects of PULM on the WHC and adsorption of DROs of diesel-Spiked Soils. It is important to evaluate the relative effects of green-biomass on the WHC of the diesel polluted soil and to establish if such effects are statistical significant. Percentage relative effects relates the WHC of the un-composted and the green composted diesel spiked soils, calculated as:

$$\% RIW = \frac{WHC(gc)}{WHC(uc)} \times 100 \quad (1)$$

Where %RIW = percentage relative impact on the diesel spiked soil WHC, WHC(uc) = The water holding capacity of the un-composted diesel polluted soils and WHC(gc) = the water holding capacity of the green-biomass composted diesel polluted soils. Similarly the impact of the incorporated PULM on the adsorption of DROs as compared to the un-composted fraction of the diesel spiked soils was also evaluation as:

$$\% RIA = \frac{TDA(gc)}{TDA(uc)} \times 100 \quad (2)$$

### Data processing and Statistical Analysis

All the chemical analyses were carried out in triplicates and their mean and standard deviations were evaluated. A student T-test was used to evaluate the significant differences between the control and sample groups at 95% degree of confidence ( $p \leq 0.05$ ). SigmaPlot® software and Excel version 10 were used for the entire graphical analysis and presentations

### Results

The results of this study were as stated in tables 1 – 3 and figures 2 – 4. In table 1, the physicochemical characteristics of the PULM were enlisted; table 2 presents the data gathered on the Percentage relative and significant effects of PULM composting on the WHC of diesel polluted soils. On the other hand, figures 2 and 3 demonstrated the effects PULM on WHC of diesel polluted soils and the four sets of DROs leaching studies (using different composting rates of 1, 2.5, 5 and 10%) respectively. While figure 4 showcased the Correlation between the percentage of DROs adsorption and the amount of PULM used during leaching of 20% diesel polluted soils.

**Table 1: Physicochemical properties PULM**

Parameter assayed	Unit	Values
pH	-	6.55
Electroconductivity (EC)	( $\mu$ s/cm)	1455.65
Fibre content (Fbc)	%	43.12
Total organic matter (TOM)	%	74.38
WHC	%	72.52
Sodium (Na)	mg/kg	468.13
Potassium (K))	mg/kg	655.14
Calcium (Ca)	mg/kg	2478
Magnesium (Mg)	mg/kg	488.81
Total Phosphorus (TP)	mg/kg	1619
Organic Carbon (C)	%	53.55
Nitrogen (N)	%	1.55

C:N  
Soil Textural class

34.55  
loamy Sand

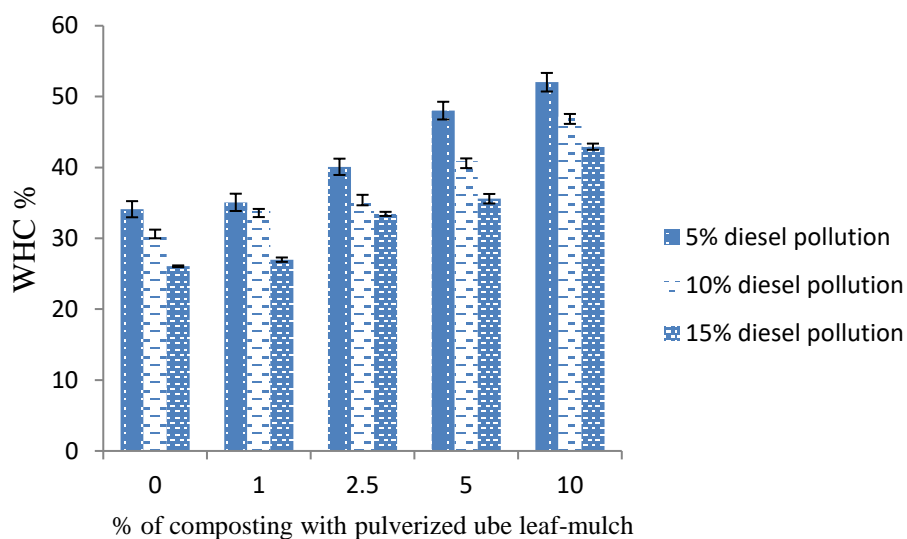
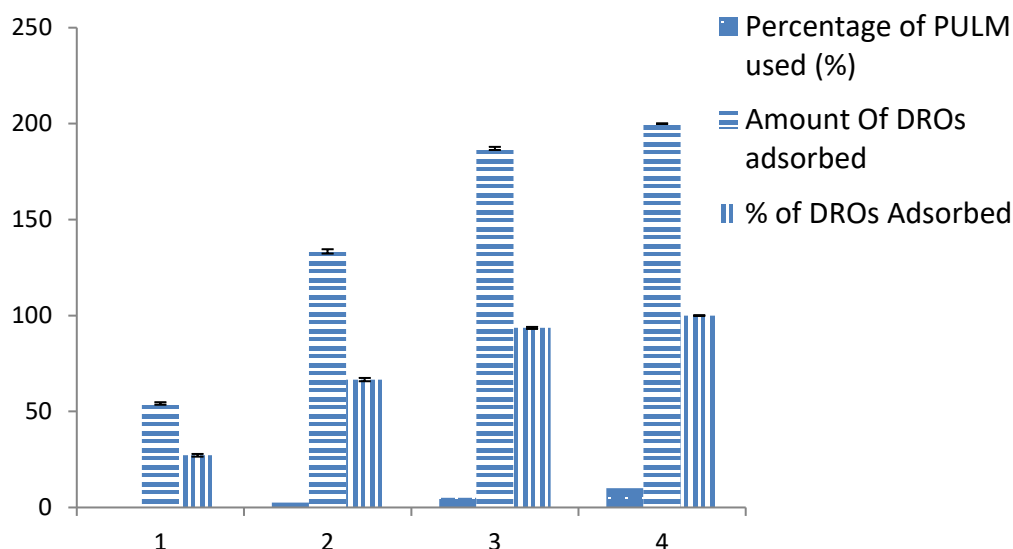


Figure 2: The effects PULM on WHC of diesel polluted soils

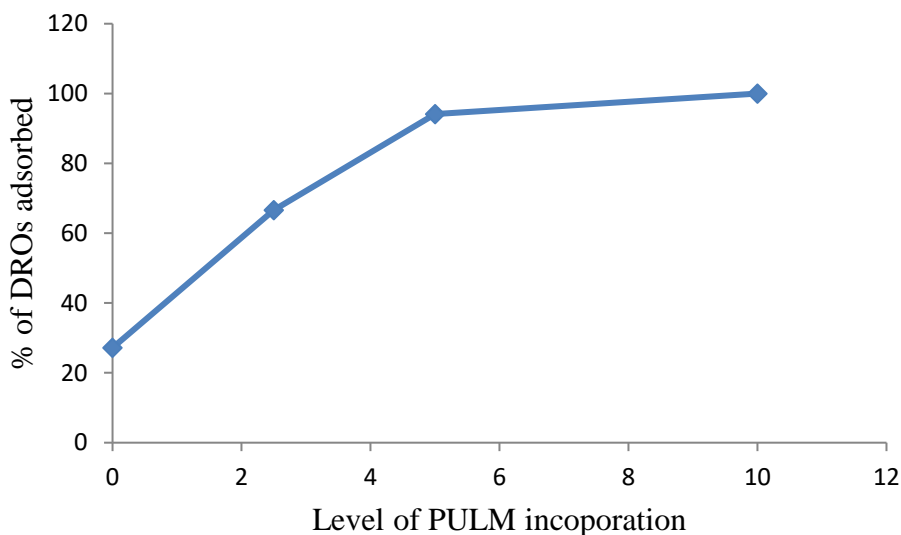
Table 2: The Percentage relative and significant effects of PULM composting on the WHC of diesel polluted soils

Level of composting	Level of Diesel Pollution (%)					
	5		10		15	
	% Rel. Effects on WHC	t-test value (p ≤ 0.05)	% Rel. Effects on WHC	t-test value (p ≤ 0.05)	% Rel. Effects on WHC	t-test value (p ≤ 0.05)
0	-	-	-	-	-	-
1	102.84	1.260E-03	109.64	3.6757E-05	136.57	8.489E-03
2.5	117.53	3.310E-06	113.06	1.060E-04	148.36	2.330E-06
5	140.78	3.2079E-08	131.93	9.820E-02	152.57	1.677E-05
10	152.54	8.928E-08	136.57	4.163E-07	164.74	3.413E-07



**Figure 3: Four sets of DROs leaching studies using different composting rates of 1, 2.5, 5 and 10% on leaching of DROs from 20% diesel polluted soils**

Level of composting (%)	% Relative effects on DROs leaching	t-test values (p ≤ 0.05 )
0	-	-
2.5	245.13	4.210E-07
5	346.39	1.198E-06
10	367.92	4.383E-05



**Figure 4: Correlation between the percentage of DROs adsorption and amount of PULM used during leaching of 20% diesel polluted soils**

## Discussion

### Physicochemical properties of Soil and PULM

The physicochemical characteristics of the soil employed in this assay have been previously reported (Oghoje et al., 2024). The PULM physicochemical characteristics are listed in Table 1. Furthermore, figure 4, collaborated the results in figure 3 indicating that as the level of composting with the PULM increased, the amount of DROs immobilized within the soil also increased. This implied that not actually up to 10% PULM was needed to curtail all the DROs in the polluted soil but just about 9.4% of the green-biomass, thereby making the process more cost effective. This kind of positive relationship between green-biomasses and immobilization of soil organic pollutants particularly petroleum products, had been documented in our previous reports using pulverized rice straws and sawdust (Oghoje et al., 2024, 2023). Specifically, Jones et al. (2011) reported a positive correlation between the amount of bio-char incorporated in organic polluted soils and the amount of the pollutant that could be leached out of the polluted soil. Soil leaching could push soil pollutants down the soil profile and consequently pollute the soil aquifer. More so, it could reduce the effectiveness of biodegradation of soil pollutants and bioremediation because the population and diversity of soil microbes as well as oxygen are usually more at the top soils' horizon (Gomoryova, 2020; Tao, 2018). The incorporation of PULM in the process of bioremediation of organic polluted soils, as revealed by this study, therefore, would increase the accessibility of the pollutants by the soils microorganism as well as facilitating a good soil conditions for optimal and cost effective bioremediation. Therefore, the use of PULM during soil remediation could be a great enhancement. Hence, PULM should be added to list of green biomasses that have been reported for remediation of petroleum polluted soils. According to one of our previous reports, the soil had a pH of 5.43 and an EC of 121.67 ( $\mu\text{s}/\text{cm}^3$ ) (Oghoje et al., 2024). This pH value was found to be consistent with earlier records regarding the sample site by other researchers (Ekebafé & Oviasogie, 2015). The physicochemical result of the PULM showed that its pH was 6.55 which was at close range to the pHs of similar green-biomass (Oghoje et al., 2024, 2023, 2018). This implied that, incorporation of green-biomasses may not significantly affect the original soil pH prior to their decomposition and humidification. The PULM (a tropical tree) fiber content was 43.12% which is relatively higher than that of Broad leave lime tree leaf mulch (*Tiliaplathyphyllos*, a temperate tree). This characteristic may be attributed to regional adaptation to a tropical climate. Lignin, hemicellulose, and cellulose, often made up the majority of the fiber matter in plants which have the ability to absorb water molecules. And as a result, could increase the WHC of soils in a similar way to paper sludge and similar biomasses that had been reported (Celik et al., 2004; Aitken et al., 1998). Furthermore, the PULM has TOM value of about 74.38%. This high value of TOM may be attributed to the easily oxidized phyto-compounds found in plant leaves, such as sugars, alkaloids, flavonoids, tanins, and fatty, some of which are hydrophilic. Therefore, the existence of these phyto-compounds may be one of the factors influencing the PULM's high WHC value of 72.51%. Consequently, the incorporation of this green-biomass in an oil polluted soil, would improve its moisture content for effective and efficient bioremediation. The soil texture, pH, EC, SOM, and other physicochemical characteristics were comparable to several soils in the Nigerian Niger Delta region (Ekebafé & Oviasogie, 2015; Puyate & Rim-Rukeh, 2008).

#### The Nutrient Values of PULM

The results (Table 1) further demonstrated that the PULM had high levels of macronutrients, with values for sodium, potassium, calcium, magnesium and total phosphorus of 468.13, 655.14, 2478, 488.81 and 1319 mg/kg, respectively. This suggests that the PULM may be a reliable source of plant macronutrients. This could be a further benefit when using the PULM for an oil polluted soil conditioning during land farming to remove oil organic pollutants. Composts' nutritive benefits for bioremediation are widely known (Radwan, 2000; Semple, 2001). On the other hand, the PULM's nitrogen and carbon nitrogen ratio values were not particularly high (Table 1), meaning that PULM would not be a significant nitrogen source for optimal bioremediation of petroleum polluted soils

#### The Effects of PULM on WHC of Diesel Polluted Soils

One of our previous reports revealed that the WHC of the diesel-spiked soils was approximately 34, 31, and 26% for 5, 10, and 15% diesel pollution accordingly without composting with green-biomass as against the initial value of 37% for the unpolluted soil (Oghoje et al., 2024, 2018). But this study showed that by incorporation of PULM, the WHC were changed to approximately 35.08, 34.00, and 26.99% (By composting at 1%), 40.09%, 35.66%, and 33.45% (By composting at 2.5%), 48.02%, 340.91%, and 35.59% (By composting at 5%), and 52.02%, 47.15%, and 42.93% (By composting at 10%), respectively, for 5%, 10%, and 15% diesel pollution accordingly. From the results (Figure 2), it was noticed that, 2.5% PULM composting was just adequate to restore the WHC of 5% diesel-polluted soil to roughly its original value in the unpolluted soil but was insufficient to normalize the WHC of soils with greater levels of diesel pollution. However, 5% PULM composting was enough to raise the WHC of up to 15% diesel-polluted soils to at least the original WHC of the unpolluted soils, 10% PULM composting significantly raised the WHC of the three levels of diesel-polluted soils above the original WHC of the unpolluted soils. Furthermore, it was observed that, the WHC of the soils decreased as the level of diesel pollution increased. And for every level of diesel contamination, the WHC increased as the level of composting

with PULM increased. These findings are consistent with previous works on the effects of green-biomasses on the WHC of oil polluted soil using pulverized rice straws (Oghoje et al., 2024), sawdust (Oghoje et al., 2023), broad leaf lime leaf mulch (Oghoje et al., 2018). Other researchers have also documented the impacts on composing on the WHC or soil moisture content (Celik et al., 2004; Aitken et al., 1988). As previously mentioned, soil moisture content is a very vital parameter for optimal bio-accessibility and remediation of various soils' pollutants. Therefore, this study has added PULM to the list of green composts that could enhance soils' moisture and consequential optimal bioremediation of petroleum oil contaminated soils.

### Relative Effects of PULM on the WHC and adsorption of DROs of diesel-Spiked Soils

The percentage relative impact of PULM on the WHC of the diesel spiked soils and their significant differences at a 95% level of confidence ( $p \leq 0.05$ ), is presented in table 2 composting at 1% and above had a substantial impact on the relative WHC of diesel-polluted soils. The data compares the extent of the impact of the green-biomass on the WHC of the diesel polluted soils with respect to the un-compost and answered the questions of where the composting is relevance and significant when compared to the un-composted samples. Four sets of DROs leaching study is presented in figure 3. The results showed that in study 1, the diesel spiked soils were not composted with PULM prior to the leaching experiment thereby serving as the control for the studies. The soil itself has the ability to adsorbed petroleum products (And Related organic compounds) due to its organic matter (including organic carbon) content. Consequently, the amount of DROs adsorbed and the percentage adsorption were  $54.19 \pm 0.66$  mg and  $27.18 \pm 0.71$  % respectively. In a similar trend, Studies 2 and 3 where the samples were composted with 2.5 and 5% PULM, and amount of DROs adsorbed were 133.43 and 187.08 respectively while the corresponding percentage adsorption were  $66.63 \pm 0.86$  and  $93.55 \pm 0.53$ % respectively. On increasing the PULM percentage to 10%, the concentration of DROs in the leachates became below detection limit (Bdl) of the GS-MS used for the analysis. This implied that the about 200 mg diesel used for the soil spiking was adsorbed in the samples representing 100 % adsorption of the DROs. These findings were in consistent with our previous reports on the enhancement or the immobility of petroleum hydrocarbon in soils using pulverized rice straws or pulverized barley straws (Oghoje et al., 2024). Similar studies by various researchers had also indicated the potentials of green biomasses and biochar in the adsorption and immobilization of soil pollutants (Particularly heavy metals and organic compounds (Banks et al., 2006; Beesley et al., 2010; Warrall et al., 2001). According to Jones et al. (2011) there was a positive correlation between the amounts of bio-char incorporated into soil and the concentration of simazine leached from simazine applied soil. Several similar documentations in this regards had been made for green-biomasses incorporated in soils (Banks et al., 2006; Beesley et al., 2010; Warrall et al., 2001). Therefore, the findings in this study as regards the leaching reduction of the DROs (And by extension organic compounds including petroleum distillates) from petroleum polluted soils has added volume to the archives. For effective and efficient bioremediation of organic pollutants in soils, the pollutants should be kept within the top soil matrix where the population of soil microbes is usually high (Gomoryova, 2020; Tao, 2018). This implies that incorporation of PULM in oil polluted would optimize the accessibility of the pollutants to soil microbes attack and the eventual degradation.

### Relative Effects of PULM on the DROs adsorption from diesel-Spiked Soils and its significant.

In every study that has control experiment, it is necessary to investigate the effects of the various treatments with regards to the control. The percentage relative effects of PULM on the DROs leaching and their significant differences ( $p \leq 0.05$ ) are shown in table 3. The results showed that the percentage relative effects (As compared to the impact of the control soils on the leaching of the DROs) were quite high ranging from about 245 to 368. And interestingly, these effects generally increased as the level of incorporation of PULM in the diesel polluted soil rose from 0 to 10%. On the evaluation of the level of their significant difference (As related to the control samples) at 95% degree of confidence ( $p \leq 0.05$ ), it was observed that the impact of the PULM composting on the leaching the DROs from the diesel polluted soils were largely significant.

### Conclusion

One of the physical constraints that restrict the usefulness and efficacy of landfarming technique for remediating petroleum-polluted soils is the quick evaporation of soil moisture brought on by tilling the soil and the leaching of oil contaminants from the top and rhizosphere soil layers during landfarming. This study has revealed that using PULM (And by extension, all other similar green biomasses) for composting diesel polluted soils improved the ability to retain water and decreased the amount of pollutants that could be leached from the top soils. Therefore, It conclude the inference that PULM could increase the accessibility of the contaminants to hydrocarbon degraders by trapping them at the top soil. In other words, the addition of green compost, such as PULM, to the bioremediation of hydrocarbon-polluted soils would improve the process. This study was conducted in the laboratory as a simulation of a real field situation, although it is possible that the results may not entirely be same for the field soils. In light of this, we advise more research into how PULM and other green composts affect the

field environment's ability during landfarming of hydrocarbon-polluted soils. As the green compost decomposes, it is also important to measure the nutrients it contributes to the whole process.

### Recommendations

1. Broad-leaf ornamental and fruit trees in the tropical region of the World including Diamond (*Pandanus candelabrum*), Cashew (*Anacardium occidentale*) and Mango (*Mangifera indica*) trees from which senesced green biomass could be generated.
2. Ube leaf-much, the potentials of these green biomass for soil physicochemical conditioning and environmental bioremediation enhancement, need to be studied in order to increase the catalogue of green composts for bioremediation of inorganic and organic substances polluted soils.

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