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Biofacilitation Potential of Sawdust on Landfarming of Petroleum Hydrocarbons Polluted Soils

S. U. Oghoje*, C. Ejeomo, U. Ugbune , J. E. Ukpebor, A. K Asiagwu and E. E. Ukpebor

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Abstract: The purpose of this study is to reveal the biofacilitation potentials of green biomass (GB) using dried and sieved sawdust (DSS) as a typical type. The effects of the DSS on water retention capacity (WRC), leaching of diesel range organics (DROs) and landfarming of 5 % diesel-spiked soils were investigated according to standard methods and procedures. Hydrocarbon analyses were carried out according to USEPA recommendations using GM-MS. The results showed that green biomass could increase the WRC of oil-contaminated soils significantly. For instance, 2.5, 5 and 10 % DSS composting of 5 % diesel-contaminated soil increased its WRC by about 35, 36 and 45 % respectively as compared to the control which was 34 % and for 10 % pollution, the effects were 31, 33, and 40 % respectively as against the control soil whose WRC was about 30 % which reveals that the higher the degree of pollution, the more the relative effects of GBM on the WRC of the polluted soils. Also, the different levels of composting reduced the leaching of the DROs by about 43, 51, and 74 % respectively. Furthermore, the weather-moist DSS was found to contain 3.5 x 10^4 and 4.0 x10^4 cfu of hydrocarbon utilizing bacteria and fungi respectively and 5 and 10 % of the GBM promoted the total petroleum hydrocarbons (TPH) removal by 47 and 52 % respectively in 56 days. The various results have revealed that DSS (And by extension all GBM) could ease the accessibility of soil pollutants by soil biodegrades and hence, optimise the bioremediation of oils polluted soils.

***Keywords**: Sawdust, Green biomass, bio-facilitation, diesel-polluted soil, landfarming*

S. U. Oghoje*
Department of Chemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria
Email: uoghoje@delsu.edu.ng

C. Ejeomo
Department of Chemistry, Faculty of Science, Michael and Cecilia Ibru University, Agbarha-Otor, Delta State, Nigeria.

U. Ugbune ,
Department of Chemistry, Faculty of Science, Delta State University of Science and Technology, Ozoro, Delta State, Nigeria

J. E. Ukpebor
Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City, Edo State, Nigeria

A. K Asiagwu
Department of Chemistry, Faculty of Science, Delta State University, Abraka, Delta State, Nigeria

E. E. Ukpebor[4]
Department of Chemistry, Faculty of Physical Science, University of Benin, Benin City, Edo State, Nigeria

1.0 Introduction

There are three Bioremediation technology has evolved in recent decades (Ang, Zhao et al. 2005; Ramfrez-García, Gohil et al. 2019). Various applications of the technology for the remediation of petroleum hydrocarbons polluted soils and water have been reported (Adoki and Orugbani 2007; Abhanzioya 2016;

Abatenh, Gizaw et al. 2017). The technology is organic-driven which involves the use of microbes, fauna and flora organisms (Dawson, Iroegbu et al. 2007; Akoachere, Akenji et al. 2008; Tanee and Akonye 2009; Agamuthu, Abioye et al. 2010; Adekunle 2011; Abatenh, Gizaw et al. 2017). The various bioremediation techniques are considered to be cost-effective, environment compactable and could be localized (Jorgensen, Puustinen et al. 2000; Mmom and Deekor 2010; Iren, John et al. 2014; Oghoje and Ukpebor 2020; Oghoje, Ukpebor et al. 2021)main domains of the bioremediation technology namely; biostimulation, bioaugmentation and biofacilitation (Leahy and Colwell 1990).

Naturally, microorganisms exist unambiguously in the soil and aquatic (Particularly surface water) environments (Anoliefo 2016). There are several types and diversity of microbial populations in soils and water bodies which include heterotrophic bacteria (HB) and Heterotrophic fungi (HF). The HB and HF are capable of degrading, decomposing or oxidizing petroleum hydrocarbons, particularly in the absence of easily oxidized carbon sources in soils and waters. But, some species of bacteria and fungi are petroleum hydrocarbons loving and are referred to as hydrocarbon-utilizing bacteria (HUB) and hydrocarbon-utilizing fungi (HUF) respectively (Akoachere, Akenji et al. 2008; Okoro 2010; Anoliefo 2016). These classes of microbes are usually attracted to petroleum hydrocarbons contaminated or polluted sites (Anoliefo 2016) especially when the level of pollution is low (Mmom and Deekor 2010; Okoro 2010). Naturally, all biodegradable pollutants in soils and water would eventually be degraded or broken down by these microbes or assimilated and immobilized by fauna or/ and flora organisms present in the polluted environment. Such process is known as natural attenuation which is usually very slow particularly when the intensity of pollution is high (Ouvrard, Chenot et al. 2013; Silva-Castro, Rodelas et al. 2013; Simpanen, Dahl *et al*. 2016).

Since bioremediation is organically driven, the need to enhance the technology by providing favour environment and nutrients or directly increasing the population and diversity of the pollutants' bio-degraders has led to intense research in recent times (Ouvrard, Chenot et al. 2013; Ramfrez-García, Gohil et al. 2019). Biostimulation aims to supply adequate nutrients particularly nitrogen, phosphorus and water to the pollutants' bio-degraders to increase the growth and biodegrading activities of the indigenous organisms (Llado, Covino et al. 2013; Koshlaf and Ball 2017). On the other, bioaugmentation involves the intentional introduction or inoculation of the polluted sites with exogenous organisms, particularly microbes to increase the population and diversity of the biodegraders with the intention that the higher the population and diversity of the biodegraders the more efficient and effective the bioremediation process would be (Leahy and Colwell 1990; Ifijen 2018; Kour, Tanvir Kaur et al. 2021). Similarly, I biofacilitation aims to provide a physicochemical environment that could enhance oxygen supplies and circulation, required temperature and increase the accessibility of biodegrades to the pollutants(Lee, Oh et al. 2008; Mmom and Deekor 2010; Iren, John et al. 2014; Koshlaf and Ball 2017; Oghoje, Ukpebor et al. 2021). Research have shown that an increase in soil water, and oxygen, modification of pH to 6.50 – 8.50, temperature range of 25 – 35^{o} C, addition of surfactant (To increase pollutants emulsification and solubility), soil moisture content of about 25 – 35 % and well-drained soil are some of the facilitation conditions for efficient and effective bioremediation of petroleum hydrocarbons polluted soils (Atlas 1981; Waiworth and Reynolds 1995; Sims, Sims et al. 2009; Angin and Yaganoglu 2011; Yousefi, Mohebbi et al. 2021)

Landfarming is a biofacilitation technique aimed at increasing the volatilization of oil pollutants and oxidative potentials of the polluted soils(USEPA 1994; Radwan, Sorkhoh et al. 1995; Jorgensen, Puustinen et al. 2000; Radwan, AL-Mailem et al. 2000; Mmom and Deekor 2010; Oghoje, Ukpebor et al. 2021). It involves tilling the soil and spreading the tillage. However, besides the increase in the volatilization of hydrocarbons and the soil oxidative potentials, tilling breaks the soil fabrics thereby increasing soil-water evaporation and leaching of pollutants particularly during rains. Also, oil reduces soil moisture content. Adequate soil moisture is a necessity for solubilization and accessibility of oil pollutants to biodegrade (Li, Feng et al. 1997). Furthermore, the population and diversity of soil microbes (Particularly aerobic microorganisms) are usually more at the topsoil and decrease down the soil profile (Bhattarai, Bhattarai et al. 2015). Hence, there is a need for pollutants to remain on the top soils for wease of bioremediation process. Some methods of achieving these conditions during landfarming of petroleum-polluted soils have been investigated including the inclusion of organic manures (Ayotamuno, Kogbara et al. 2006; Banks, Schwab et al. 2006; Ayotamuno, Okparanma et al. 2009; Beesley, Moreno-Jiménez et al. 2010). But the use of green compost such as sawdust, in particular, to increase the moisture content of oil polluted soil and enhance the biodegrades accessibility to the oil pollutants through the reduction in their leaching has not been sufficiently studied to the best of our knowledge. Therefore, this study aimed at finding a better approach to the landfarming of petroleum hydrocarbon-polluted soils. The specific objectives of the study were to 1) evaluate the effects of sawdust on the water retention capacity (WRC) of petroleum hydrocarbons polluted soils, 2) assessment of the reduction or preventive ability of sawdust on the leaching of petroleum hydrocarbons. 3) determine the best rate of sawdust application for optimal landfarming of diesel-polluted soils.

2.0 Materials and Method

2.1 The study environment, soil sampling and processing

This study was carried out in a greenhouse {Figure 1) at the Department of Chemistry, Faculty of Science, University of Benin, Benin City between March and April 2021. Soil (0 – 30 cm) was collected at the University oil palm farm, Igue, Benin City, Edo State using a spade. The soil was air dried in the greenhouse, grind and passed through 2 mm mesh. The sieved soil was kept in a plastic container for further usage.

2.2 The analytical reagents used

All chemicals used for the study were of analytical grade and were purchased from Sigma Aldrich, UK vide Pyrex International laboratory, limited, Benin City, Edo State, Nigeria. The major organic chemical include petroleum spirit (for soil spiking), dichloromethane (for extraction of TPH) and trichlorobenzene (As internal and reference standards)

2.3 Soil Physicochemical Analysis

The physicochemical analyses were carried out according to standard methods. Particle size analysis was done using the hydrometer technique as described by Day (1953). The pH and electro-conductivity (EC) were measured after appropriate treatment of the soil and calibration of the pH (A Thermo Fisher Orion 4 pH meter (model 420A, ThermoFisher, UK) meter was made using buffer solutions, 4, 7 and 10 while the EC (A Hanna conductivity meter, Model HI 99300, Hanna UK) meter was calibrated using KCl solution. The pH and EC measurements were carried out using a soil: water ratio of 1: 2.5. Total nitrogen (TN) was measured by the Kjeldahl method as described by Okalebo et al (2002). Total Organic Carbon

(TOC) was assessed according to the method of Walkley and Black (1934); Total phosphorus (TP) was done as described by Murphy and Riley (1962). A flame photometer (model 410 Sherwood, UK) was used to quantify sodium, (Na) and Potassium, (K) while Magnesium (Mg) and Calcium (Ca) were measured by atomic absorption spectrophotometer (AAS), GBC Model AA 404 Australia.

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Fig. 1: The greenhouse used for soil drying and the remediation study

2.4 Diesel Stabilization, Soil Spiking and treatment for Remediation

The diesel fuel used for the study was obtained from a commercial fuel station in Benin City. 5 litre of the diesel was poured into a plastic container and stirred for about 5 minutes daily for three weeks for stabilization (Okieimen and Okieimen, 2005). 50 g of the diesel (Density = 0.82 g/cm^3) was dissolved in 1 dm^3 of petroleum spirit and 100 cm^3 of the solution was quickly transferred into plastic containers containing 1 kg of soil each, representing 5 % diesel pollution in the soil samples. The samples (n = 3) were labelled and arranged in randomized complete block design within the greenhouse and the petroleum spirit was allowed to evaporate for 12 hours leaving the diesel in the soil. To each of the diesel spiked soils was added 100 cm^3 of water (To keep the soils moisture content to about 65 % field capacity), thoroughly mixed and allowed to stabilise and weathered for 3 weeks (Okieimen and Okieimen 2005), Before biostimulation and composting with the DSS. A solution of NPK 20:20:20:2Mg was prepared by dissolving 20 g of the fertilizer in 1 dm^3 of distilled water. To each of the samples, 100 cm^3 of the solution was added representing 2 % nutrient stimulation in all the samples. Finally for each set of triplicates, composing with the DSS was done at the rate of 0, 2.5, 5 and 10 % whereas the 0 treatment serves as the control. Then, 100 g of each sample was then collected (According to standard sampling methods for TPH samples) on day 1 (Before fertilizer addition) and 56 for total petroleum hydrocarbons (TPH) analyses at Earth Quest International laboratory, Warri, Delta State, Nigeria. During the remediation period, the samples were thoroughly mixed once a week (To mimic landfarming). Also, certain about of water were added to the samples (According to the measurement of the lysimeter set up with the samples) to maintain their moisture content throughout the remediation period.

2.4.1 Physicochemical analysis of the sawdust used for the study

Sawdust was collected from a commercial sawmill factory at Sapele, Delta State. The samples were air-dried and passed through 2 mm mesh (Figs. 2a and 2b). The various physicochemical parameters of the DSS assayed were done in similar approaches for the soil physicochemical analysis, except for the amount of DSS used. For the acid digestion and spectrometric analyses, 0.5 g was used while for the pH and EC determination, the ratio was 1: 5 (DSS: water) ((Okalebo, Gathua *et al*. 2002).

Fig. 2: (a) Unprocessed Sawdust and (b) Dried and Sieved (< 2mm) Sawdust (DSS)

The fibre content of the DSS was determined by hydrolysis method using sodium hydroxide solution as described by De Meijer and Van der Werf (1994). Five grams (5 g) of the DSS was weighed into a 200 ml beaker and 100 ml of 20 % NaOH was added to it. Then, the beaker was covered with a washed glass and heated over a magnetic stirring hot plate in a fume cupboard. The temperature of the hot plate was adjusted to 200 C but reduced to 150^o C when boiling started. The heating was done for 2 hours with magnetic stirring adjusted at 250 revolutions per minute (rpm). More NaOH solution was added to the beakers when significant volume reduction was observed. At the end of the period, the mixture was cooled and diluted with distilled water. They were then filtered under suction using Buchner funnels. The residue was washed several times with distilled water after which the residue was collected in pre-weighed clean crucibles and dried in the oven at 105^o C for 12 hours. The experiment was replicated for average values for the DSS fibre content. After the drying, the crucibles were reweighed and the percentage fibre content was calculated as:

$$\text{Sample fibre content (\%)} = (W_2 - W_1)/ / 5 \times 100 \qquad (1)$$

where W_2 and W_1 were the weights of crucible + wet residue and weight of crucible + dry residue respectively

2.4.2 The effects of Sawdust on WRC of diesel-contaminated soils

All oils including petroleum hydrocarbons and landfarming have been reported to reduce the WRC of soils. Hence, there is a need to improve the water content and WRC of oil-polluted soil before landfarming. In this study, the effect of composting diesel-spiked soil using DSS on its WRC was investigated. 100 g of soil was spiked with 0, 5, 10, and 15 % diesel (w/w, n =3). The samples at each level of diesel contamination were then composted with the DSS at 0, 1, 2.5, 5 and 10 % of the soil weight. Then, the samples were thoroughly mixed and their WRC was determined using the modified Aitken method as previously described (Oghoje et al.(2018)

2.4.4 Evaluation of the effects of Sawdust on the leaching of hydrocarbons from diesel-contaminated soils

To evaluate the potentials of DSS on the reduction or prevention of leaching of hydrocarbons pollutants from diesel-contaminated soil, 100 g soil (< 2 mm) was spiked with 20 g, 20 %, w/w) diesel (To mimic heavy hydrocarbons polluted land). Then, 25 ml of distilled water was, added to maintain the moisture content at about 65 % of the field WRC. The samples were composted with the DSS at 0, 2.5, 5, and 10 % (Concerning the soil weight). They were thoroughly mixed and packed into a soil column of 15 cm long with 3 cm internal diameter; Then, 150 ml of distilled water was added to the sample using a rainfall-simulating device following the OECD/OCDE 312 guidelines (OECD, 1995) to mimic rainfall during landfarming at field scale. The experiment was replicated for average result values. The leachates collected from the soils were solvent extracted using dichloromethane (DCM) containing 0.25 g/dm^3 of trichlorobenzene (TCB) as an internal standard. Then, the solvent extracts were cleaned in a silica gel column containing a layer of 3 g sodium sulphate. The cleaned samples were analyzed on the GC-MS a Trace 1300GC/ITQ900 Ion Trap Mass Spectrometer fitted with DB 5 column of size 30m x 0.25mm with 0.25µm inner diameter; model Restek RTS1 PONA. The carrier gas was helium set at a flow rate of 1.5 mL/min.; inlet temperature was 250^{o} C; injection mode was split; transfer line temperature was 300^{o}C; Ion source temperature was 200^{o} C and the final temperature was 320^{o} C.

2.4.5 Microbial count

The microbial population and diversity of the DSS were quantified according to standard methods for microbial analyses. The DSS was kept moist in an uncovered plastic container and weathered for 7 days after which it was thoroughly mixed. Then, the total heterotrophic microbes content was evaluated using bacteriological agar and Rose Bengal agar for bacteria and fungi populations respectively (Molina-Barahona, Rodríguez-Vázquez et al. 2004). The total hydrocarbons utilizing bacteria and fungi (THUB and THUF) were done by documented standard methods and procedures (Margesin, Zimmerbauer et al. 2000). A suspension was made by mixing 0.50g of the weathered DSS in 10 cm^3 of distilled water and then a 10-fold serial dilution was carried out to enumerate the number of colonies forming units (CFU). The Samples suspensions were prepared (n = 3) and were cultured for 8 days at a temperature of 27 ^{o}C. Then, the number of cfu was counted in each sample (Colores, Macur *et al*., 2000).

2.4.6 Data Processing and statistical analysis

The experiments were carried out in triplicates and averaging of results and standard deviations were calculated using Excel software version 2010. In the leaching experiments, the GC-MS chromatograms (Fingerprints) from the analysis of the leachates were compared and the percentage reduction in leaching of the DROs was graphically correlated to the sum of the peak area ratios of the DROs. The percentage effects of the DSS on the WRC of the diesel-polluted soils and the remediation of TPH from the soils were done according to standard procedures Determination of the significant difference ($P \leq 0,05$) of the various DSS treatments was carried out using Student's unpaired t-test with the control samples. Graphs were created using Excel software or Sigmaplot^R.

4.0 Results and Discussion

4.1 Physicochemical Properties of the Soil Used in the Study

The physicochemical characteristics of the soil used for the study had earlier been reported (Oghoje, Ukpebor *et al*., 2021). The report showed that the soil is acidic, a characteristic of typical agricultural soils in the Niger Delta region, in Nigeria. It was loamy sand by textural classification and all the other physicochemical properties were within the ranges reported for agricultural soils in the area (Udo and Dambo 1979; Ekebafe and Oviasogie, 2015; Oshomoh and Ikhajiagbe, 2015), except its relatively high TOC. The high value of TOC of the sampling site has been attributed to the organic fertilization practice which is routinely done on the oil palms (Oghoje, Ukpebor *et al*. 2018)

4.2 Physicochemical Properties of the Sawdust used in the Study

The result as presented in Table 1 showed the soil has a pH of about 6.70 which is within the pH recommended for microbial development and bioremediation. This implies that sawdust may not have negative effects on soil pH during bioremediation. Similar pH for green biomass was reported in our previous works (Oghoje, Ejeomo et al. 2017; Oghoje, Ukpebor et al. 2018)/ Another good quality of the sawdust is the significant values of macro minerals such as K, Ca, and Mg which could enhance phytoremediation

Table 1: Physicochemical properties of the sawdust used for the study

pH	**6.70 ± 0.03**
EC (µS/cm)	234.67 ± 21.10
FC (%)	65.67 ± 2.90
TOM (%)	85.83 ± 0.61
WRC (%)	66.00 ± 2.65
K mg/kg	1482.67 ± 152
Na mg/kg	380.24 ± 23.18
Ca mg/kg	3430.14 ±347
Mg mg/kg	652.93 ± 34.67
TP mg/kg	123.45 ± 8.92
TOC (%)	43.67 ± 2.11
N (%)	0.24 ± 0.01
C:N	0.01

***FC = Fibre content, TOM = Total organic matter; TP = Total phosphorus**

However, the total phosphorus and contents and carbon to nitrogen ratio were relatively low. Furthermore, the observed fibre content is relatively high which implies that the application of this substance to oil-polluted soils could improve its water retention capacity during landfarming (Oghoje *et al*. 2017; Oghoje , Ukpebor et al. 20 18). However, the fibre content of sawdust would depend on the wood material from where it is produced. This finding collaborated with previous works where the water-holding capacity of the soil was improved by the application of green compost (Oghoje, Ejeomo *et al*., 2017; Oghoje, Ukpebor *et al.*, 2018)

4.3 The effects of Sawdust on WRC of diesel-contaminated soils

Green compost refers to plant biomass that has not decomposed before application to the soil which includes straws, wood barks, leaf mulch and waste paper pulps etc. Reports have shown that plant biomass could increase the WRC when incorporated in soils (Aitken, *et al*., 1998; Oghoje *et al*., 2017; Oghoje *et al*., 2018). Green compost such as leaves, straws, bark and Sawdust is mainly made up of organic compounds of which hemicellulose, cellulose and lignin are the major components. These substances are polymeric in nature and not soluble in water or polar solvent and they form the fibre content of plants. The fibre content of a biomass plays major role in its water-holding and pollutants adsorption capacities (Aitken *et al*., 1998). Hence, they could also increase the WRC of oil-polluted soils. Fig. 2 presents the effects of DSS on the WRC of different levels of diesel-polluted soils. For the 5 % diesel pollution, 1 % composting with DSS did not produce significant effects on the WRC of the polluted soils but as the amount of DSS increased to 2.5, 5 and 10 %, the corresponding WRC of the soils were about 35, 36 and 45% respectively as compared to the control which was 34 %. The relative impact of the DSS on the soil WRC was more at high levels of diesel pollution. For instance, at 10 % of diesel pollution, the WRC of the soils were about 29, 31, 33, and 40 % respectively as against the zero composted soil whose WRC was about 30 %. It was observed that for this level of diesel pollution, the use of 1 % composting with DSS produced a negative impact on the WRC of the soil. Soil WRC depends to a larger extent, on the porosity and the water absorption potentials of the soils. In as much as plant biomass could increase water absorption potentials in soil, coarseness of the material such as the DSS, could equally increase the soil porosity and air circulations in the soil. When the effect of soil porosity is more than its water absorption capacity, the WRC would drop. This could be the reason for the drop in the 1 % DSS composted 10 % diesel-polluted soils. However, for the 15 % diesel-polluted soils, the WRC of the 1, 2.5, 5, and 10 % DSS composting were about 27, 29, 30 and 37 % respectively as compared to that of the control which was 26 %.

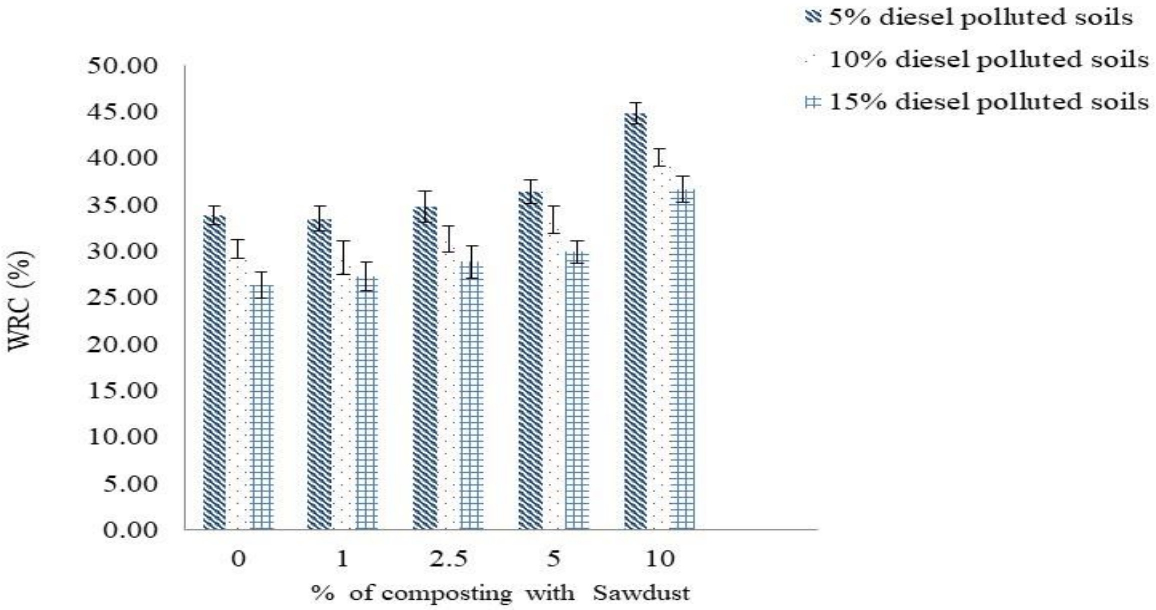

Fig. 1 1: Effects of DSS on WRC of diesel-polluted soils

Generally, it was observed that the relative effects of DSS on the WRC decreases as the levels of diesel pollution increase (Fig. 2).

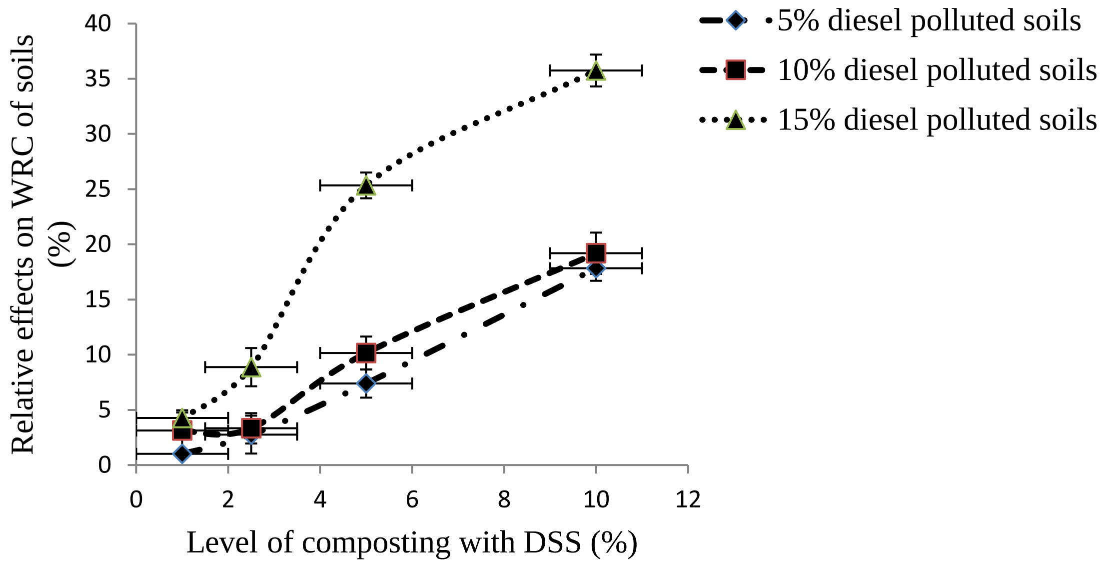

Fig. 2: Relative effects of DSS composting on WRC of diesel-polluted soils

However, the relative percentage increase in the WRC of the diesel-polluted soils was not significant ($P \leq 0.05$) if 1 or 2.5 % of DSS was used at certain levels of diesel pollution (Table 2). For instance, the use of 1 or 2.5 % of DSS did not produce significant effects on the WRC of soils polluted with diesel up to 15 % (BY this study) except for 2.5 % DSS on 5 % diesel-polluted soils. This observation supports our previous reports on the effects of barley straws and broad leave lime leaf mulch on the WRC of diesel-polluted soils (Oghoje, Ejeomo et al. 2017; Oghoje, Ukpebor et al. 2018). These results imply that green biomass can enhance the WRC of diesel and by extension, oil polluted soils but the amount of such composting would depend on the type of oil and the level of pollution. The effects of similar organic matters particularly organic wastes on the WRC of soils, agriculture and bioremediation have also been reported by researchers as mentioned earlier (Vengadaramana and Jashothan 2012; Głąba, Żabińskia et al. 2020). Insufficient soil water or moisture content could affect the solubility of oil pollutants. Water itself is a nutrient to soil organisms and metabolically, it could hinder the activities of soil organisms during bioremediation or landfarming. Beside the regulation of soil temperature, soil water enhances the movement of other nutrient and microbial organisms within the soil environment (Cho, Rhee et al. 2000; Teng, Luo *et al.* 2010). Therefore, adequate soil water could direct or indirectly enhance pollutants accessibility by soil organisms.

The leaching of petroleum contaminants during landfarming is a major constraint of this bioremediation technique. For the technique to be effective and efficient in remediating petroleum-polluted soils, the pollutants need to be held within the top soil layers. This is because the population of hydrocarbon-utilizing microbes is optimal at the top soil layers and decreases down the soil horizons (Anoliefo, 2016). SOM is the major absorber of soil pollutants (including hydrocarbon pollutants) in soils (Kisić, *et al.*, 2022). To enhance the soil absorption and adsorption for environmental contaminants, the incorporation of green composts has been reported (Jones *et al.*, 2011; Oghoje *et al.*, 2017, 2018 *et al.*, 2018).

Table 2: The effects of DSS on WRC of diesel polluted soils

Level of composting	Level of Diesel Pollution (%)					
	5		10		15	
	% Rel. Effects on WRC	t-test value	% Rel. Effects on WRC	t-test value	% Rel. Effects on WRC	t-test value
0	-	-	-	-	-	-
1	-1.01 ±0.33	1.46 x10^{-1}	3.13 ±1.83	4.75 x10^{-1}	3.41 ±0.50	2.68 10^{-1}
2.5	2.76 ±1.72	8.73 x10^{-2}	3.33 ±1.37	1.74 x10^{-1}	9.40 ±1.73	1.51 x10^{-1}
5	7.39 ±1.28	1.10 x10^{-4}	10.15 ±1.50	4.12 x10^{-2}	13.39 ±1.17	3.46 x10^{-3}
10	32.60 ±1.14	7.80 x10^{-4}	34.41 ±1.87	1.59 x10^{-3}	38.98 ±1.44	1.38 x10^{-3}

Effects of Sawdust on the leaching of hydrocarbons from diesel contaminated soils

In this study, the effects of composting 20 % diesel-contaminated soils concerning the leaching of the DROs from the top soils were investigated. Figure 3 (a-d) showed the GC-MS chromatograms (Fingerprints) of the individual DROs from the leachates of 20 % diesel-contaminated soil composted with different amounts of DSS. The results showed significant and arithmetic decreases in the sizes of the fingerprints as the levels of composting with the DSS increased. Fingerprints are functions of the concentration of the analytes in spectrometric analyses.

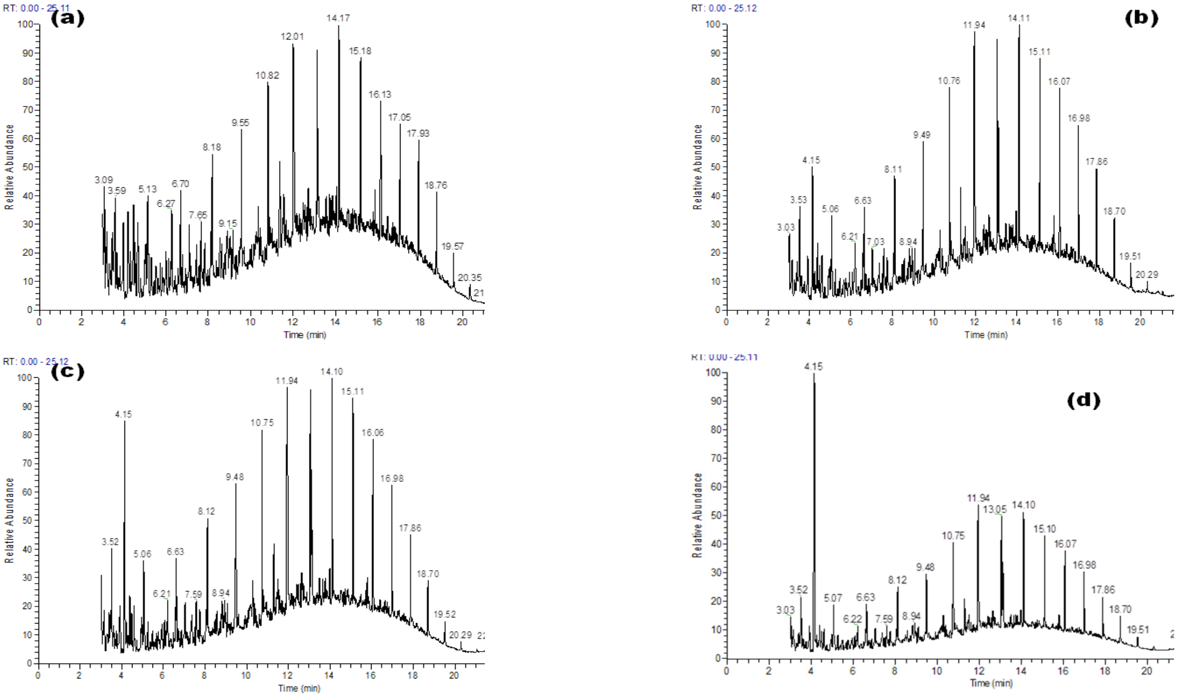

Fig. 3 (a-d): GC-MS Chromatograms of the leachate from (a) 0 %, (b) 2.5 %, (c) 5 % and (d) 10 % DSS composted 20 % diesel contaminated soils

Eighteen (18) DROs were identified and quantified in the diesel used for the soil spiking. The summation of the peak area ratio (PAR) of the 18 DROs showed a drastic reduction in the amount of DROs in the leachates which implied that much amount of DROs were held within the soils (Relative to the control) during the leaching experiments. , For instance, the ∑PAR of the DROs were 9.18, 7.83 and 4.21 for the 2.5, 5 and 10 % DSS composted diesel polluted soils as against 15.95 for the control. It is expedient to note that PAR is proportional to the concentration of the individual DROs and is a good parameter to explain the reduction of the leaching of the DROs in this study. 1t was obvious from the results that the amount of DROs leached decreased as the values of DSS incorporated into the diesel-contaminated soils increased. In other words, the peak area ratios or the sum of the DROs detected decreased as the levels of composting with the DSS increased (Table 3).

Table 3: Effects of DSS composting on the leaching of DROs from 20 % diesel-contaminated soils

DROs	**Standard (20 % diesel solution)**	**Peak Area ratios (PAR)**			
		Level of Composting with DSS (%)			
		0	2.5	5	10
C11	1.48 ±0.20	0.80 ±0.11	0.69 ±0.21	0.43 ±0.05	0.26 ±0.01
C12	2.05 ±0.09	1.00 ±0.14	0.68 ±0.00	0.57 ±0.02	0.53 ±0.42
C13	1.84 ±0.11	0.96 ±0.19	0.58 ±0.05	0.54 ±0.02	0.20 ±0.14
C14	1.60 ±0.20	0.87 ±0.12	0.58 ±0.01	0.49 ±0.02	0.25 ±0.03
C15	1.54 ±0.15	0.94 ±0.22	0.57 ±0.02	0.49 ±0.02	0.25 ±0.02
C16	1.35 ±0.15	1.05 ±0.14	0.59 ±0.01	0.52 ±0.01	0.32 ±0.03
C17	2.37 ±0.34	2.30 ±0.40	1.21 ±0.03	1.07 ±0.02	0.54 ±0.04
C18	2.06 ±0.23	1.97 ±0.55	1.12 ±0.03	0.98 ±0.02	0.50 ±0.04
C19	1.69 ±0.19	1.61 ±0.29	0.82 ±0.05	0.67 ±0.01	0.34 ±0.02
C20	1.20 ±0.14	1.13 ±0.32	0.69 ±0.02	0.60 ±0.02	0.30 ±0.05
C21	0.95 ±0.08	0.87 ±0.17	0.46 ±0.03	0.43±0.04	0.22 ±0.04
C22	0.92 ±0.07	0.82 ±0.14	0.42 ±0.02	0.38 ±0.03	0.20 ±0.02
C23	0.91 ±0.17	0.75 ±0.14	0.32 ±0.05	0.31 ±0.01	0.15 ±0.01
C24	0.63 ±0.16	0.53 ±0.12	0.25 ±0.01	0.22 ±0.01	0.10 ±0.00
C25	0.33 ±0.14	0.23 ±0.06	0.11 ±0.02	0.09 ±0.01	0.04 ±0.00
C26	0.09 ±0.03	0.09 ±0.02	0.04 ±0.01	0.03 ±0.00	0.01 ±0.00
C27	0.03 ±0.01	0.03 ±0.01	0.05 ±0.07	0.01 ±0.00	BDL
∑DROs	21.02 ±1.77	15.95 ±2.97	9.18 ±0.41	7.83 ±0.41	4.21 ±0.55
Relative reduction (%)	-	24.19 ±2.15	42.45 ±3.16	50.91. ±2.99	73.61 ±4.29

Consequently, the oil pollutants could be held in the topsoil, increasing accessibility by oil degraders and bio accumulators whose populations are usually more in the top soils (Anoliefo 2016) This finding is buttressed by our previous works where barley straws and broad leaves lime leaf mulch were used for composting diesel contaminated soils (Atlas and Bartha, 1998; Oghoje *et al*. 2017; Oghoje *et al*., 2018). Similar works on the ability of green composts, SOM and related substances to reduce or prevent the leaching of environmental pollutants have been reported (Bossert and Bartha, 1984; Radwan, Sorkhoh *et al*., 1995; Cox *et al*., 1997; Chow *et al*., 2002;;

Banks *et al*., 2006; Jose' Valarini *et al*., 2009; Beesley *et al*., *2010*, Moreno-Jiménez *et al*., 2010; Onwudike, Asawalam *et al*., 2015; Onwudide *et al*., 2016) The use of bulk agents (Bulk organic substances) on oil-polluted soil is intended to improve the oxidative potential of the soils but it could also, enhance the accessibility of the pollutants for biodegradation by reducing their fast movement down the soil profile.

4.4 Microbial potential of weathered moist sawdust

The moist weathered sawdust was found to contain a significant amount of microbes, particularly, heterotrophic fungi (Table 4). The total heterotrophic bacteria (THB) and total heterotrophic fungi were 4.8 x 10^4 and 7.2 x 10^4 (cfu) respectively. While total hydrocarbons utilizing bacteria (THUB) and total hydrocarbons utilizing fungi (THUF) were 3.5 x 104 and 4.0 x10^4 respectively.

Table 5: Microbial content of weathered moist sawdust

Parameters	Values	Parameter	Values
THB (cfu)	4.8 x 10^4	THF(cfu	7.2 x 10^4
THUB (cfu)	3.5 x 10^4	THUF (cfu)	4.0 x10^4

The population of microbes particularly, the THUB and THUF were significantly high. This implies that moist weather sawdust has microbial inoculation potential which could enhance the bioremediation of hydrocarbon-contaminated soils. The presence of a relatively high population of these microbes, particularly, the HUB and HUF in the sawdust may be due to the use of hydrocarbons as a source of fuel in the wood sawing industries. Reports have shown that hydrocarbons bio degraders are often attracted to environments contaminated with petroleum oil and its refined products (Anoliefo 2016) The spills of engine oils, diesel and lubricating oils may be the substances for the attraction of these organisms to the area before the collection of the sawdust. Besides the increase in oxygen circulation due to the incorporation of sawdust in crude oil contaminated soils, the microbial population and diversity, water retention capacity, nutrient content and ability to reduce leaching of oil pollutants from top soils of the material would be added advantages if used during bioremediation of petroleum polluted soils. This result is similar to previous reports involving the use of bulk agents during bioremediation protocols (Oghoje, Ejeomo et al. 2017; Oghoje, Ukpebor et al. 2018).

4.5 The effects of sawdust on bioremediation of diesel-contaminated soils

Table 5 presents the effects of composting 5 % diesel-contaminated soils for 56 days. The TPH concentrations at day 1 before biostimulation and composting with DSS and at day 56 (End of remediation period) showed significant removal of the diesel pollutants as the level of composting was increased. The results revealed that at day 56, 47 and 52 % of TPH were degraded in the 5 and 10 % DSS composted samples. However, the Student's unpaired t-test ($P \leq 0.05$) showed that there was no significant difference between the percentage removal in the 0 and 1 % DSS composted samples even though the 1 % DSS composted samples had greater arithmetic values of TPH removal at day 56. Similarly, there was no significant difference between the use of 5 and 10 % DSS in the TPH biodegradation even though the use of 10 % DDS composting gave a higher arithmetic percentage of TPH removal. On the other hand, the use of 5 % DSS had greater remediation efficiency than the use of 10 %

DSS. The remediation efficiency was calculated as the percentage of TPH removal (outcome) per the percentage of composting with DSS (Effort). Therefore, it would be cost-effective to use 5 % of DSS in a similar bioremediation protocol.

Table 5: The effects of sawdust on bioremediation of diesel contaminated soils

Levels of composting with DSS (%)	Parameters		
	TPH at day 1 (mg/kg)	TPH at day 56 (mg/kg)	Remediation (%)
0	27302 ±1447	22690.69	16.89
1	27302 ±1447	20902.41	23.44
2.5	28302 ±1239	19449.13	31.28
5	26202 ±1578	13826.80	47.23
10	26111 ± 1167	12410.56	52.47

However, this result is less than the values of 71 and 84 % DROs removal from diesel-contaminated soils reported by Jorgensen *et al* (2000) and Van Gestel *et al.* (2001), who used spruce bark and food waste for composting diesel-contaminated soils respectively The wide differences may be due to different environmental conditions and the type of bioremediation methods used and the duration for the remediation. Similar enhanced biofacilitation of landfarming of petroleum hydrocarbons polluted soils using similar such as plant straws, wood barks, leaf mulch and agricultural green wastes have been reported (Oghoje, Ejeomo et al. 2017; Oghoje, Ukpebor et al. 2018).

4.0 Conclusion

This study considered the facilitation potentials of sawdust incorporated in diesel-contaminated soils. The effects of the plant biomass on the water retention capacity, leaching of DROs and on biodegradation of diesel pollutants were studied. The various results obtained revealed that sawdust has the capacity of increasing the water retention capacity of diesel-contaminated soils and also, could reduce the leaching of pollutants from the topsoil down the soil profile. These properties of sawdust would make soil contaminants easily accessible to hydrocarbon biodegraders in soils. Furthermore, the weathered moist sawdust was found to contain a significant population and diversity of hydrocarbon biodegrading microbes, thereby, possessing microbial inoculant quality. The bioremediation study further revealed that the use of 5 % DSS composting was more efficient and cost-effective. The various findings obtained from this study are contributions to existing data on the use of bulk agents particularly plant biomass during bioremediation of petroleum hydrocarbons polluted soils

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