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Influence of Organic Waste on Bioremediation of Oil-Contaminated Soil

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ABSTRACT

As the transportation sector develops and urbanization increases, so does the demand for automobiles and workshop or garage services. During maintenance or workshop activities, oil may be discharged into the environment, leading to oil-contaminated soil. This study focused on optimizing the use of local organic waste to improve the bioremediation of oil-contaminated soil. The concentration of oil-contaminated soil was mixed with various organic wastes (cow manure, chicken droppings, and sewage sludge) in different ratios of organic waste (R1 100 g, R2 200 g, R3 300 g) combined with 1000 g of soil for a 56-day bioremediation trial. The results showed that the oil-contaminated soil in the case study area varied from 96.07 $mg \cdot kg^{-1}$ to 123.11 $mg \cdot kg^{-1}$. Organic wastes used contained higher levels of organic carbon, nitrogen, and phosphorus compared to the oil-contaminated soil. After 56 days, the treated soil exhibited a reduction of oil contamination by 66.9% with cow manure (CM), 61.6% with sewer sludge (SS), and 79.2% with chicken droppings (CD). The soil mixed with CD had the highest bacterial count ($35 \cdot 10^6$ $CFU \cdot g^{-1}$), while the soil mixed with SS had the lowest bacterial count ($22 \cdot 10^6$ $CFU \cdot g^{-1}$). Optimal bacterial counts were observed over the 35-day experiment, followed by a decrease in bacterial counts in all reactors. This study demonstrated that the promising technology of utilizing local organic waste has the potential to enhance bioremediation in oil-contaminated soil.

Keywords: oil contaminated soil, organic wastes, bioremediation, biodegradation.

INTRODUCTION

The development of the transport sector and the growth of urbanization resulting in the increase in the number of various transport and support facilities, such as automobile workshops for servicing and maintenances (Landquist et al., 2013). Nduka et al. (2019) pointed out that automobile could be in a two-wheeled, like a motorcycle, or three or four-wheeled, like a car or a truck. Therefore, the increase in the use of car engines and machines has also accelerated the use of oil and grease in workplaces, especially in repair, maintenance and service activities in general, which can cause an increase in soil pollution and create an alarm about soil pollution (Abioye et al., 2012). The chemicals in oil are toxic and can cause potential health risks, such as cancer development to the human body (Udeani et al., 2009). In these cases, the release of waste oil from car workshops into the environment can cause serious environmental pollution in both terrestrial and aquatic life as well as direct health threats to plants, animals and microorganisms (Haythan et al., 2016). Ogunjob and Ekanem (2017) detailed the impact of waste oil on the environment, especially in developing countries. Removal or control of hydrocarbon materials, such as oil-contaminated soil can be addressed by microbial bioremediation (Obi et al., 2022). Bioremediation exploits the biodegradation potential of microorganisms or their properties, it is simple technique and is also useful in remediation of oil-contaminated sites (Caplan 1993). In addition, this technique is relatively cost-effective (April et al., 2000). Microorganisms have found great use in the bioremediation of oil-contaminated soil. However, the biodegradation of hydrocarbons in

soil can be limited by several factors such as the type of microorganism, nutrients, moisture, and pollutant concentration (Rahman et al., 2009).

A study by Cooney (1984) pointed out that nutrients, especially nitrogen and phosphorus, are critical components in the successful biodegradation of hydrocarbon pollutants. When oil or hydrocarbon material seep out, the carbon supply increases significantly, so nitrogen and phosphorus are the limiting factors for oil degradation (Subhash 2013). Other researchers (Okolo et al., 2005) noted that addition of organic nitrogen-rich nutrients is an effective method for bioremediation. The use of organic waste as organic-rich nutrients has great potential for bioremediation of oil-contaminated soil by improving bacterial growth (Hoang et al., 2021). In addition, organic waste increases the growth and reproduction of microbial population in contaminated soil (Wu et al., 2017). Organic waste improves microbial compatibility in overcoming bioremediation limitations and promotes oil spill degradation in bioremediation (Bodor et al., 2020). Various organic wastes, such as plant residues, animal manure, green manure, composted organic materials and biosolids, have been reported in the soil contaminated with spent oil (Hoang et al., 2022). In addition, different types of organic waste have different results in decomposition of oil-contaminated soil (Jafari et al., 2023). Other researchers (Gielnite et al., 2021) reported that fermented sewage sludge has a higher potential for degradation of aliphatic hydrocarbons than others.

Therefore, the population and urbanization of the Dar es Salaam city in Tanzania has increased the need for transportation for daily economic activities which include more importation of vehicles as well as other machinery and motorcycles. In this case, more car repair shops are located within the city, and at the same time the amount of oil contaminated soil increases. Thus, based on the increase of oil-contaminated soil and the limitations of remediation of oil-contaminated soil, as well as the possibility of implementing a circular bio economy with oil-contaminated soil for maintaining a home garden and others, the aim of this study was to investigate the influence of organic waste on the bioremediation of oil-contaminated soil, with a focus on the potential of each type of organic waste and its effects on performance at different weight ratios.

MATERIALS AND METHODS

Description of study area

Tabata Dampo is a densely populated urban area in Dar es Salaam, Tanzania (Figure 1). This study area is located at -6.82306, 39.23464. There

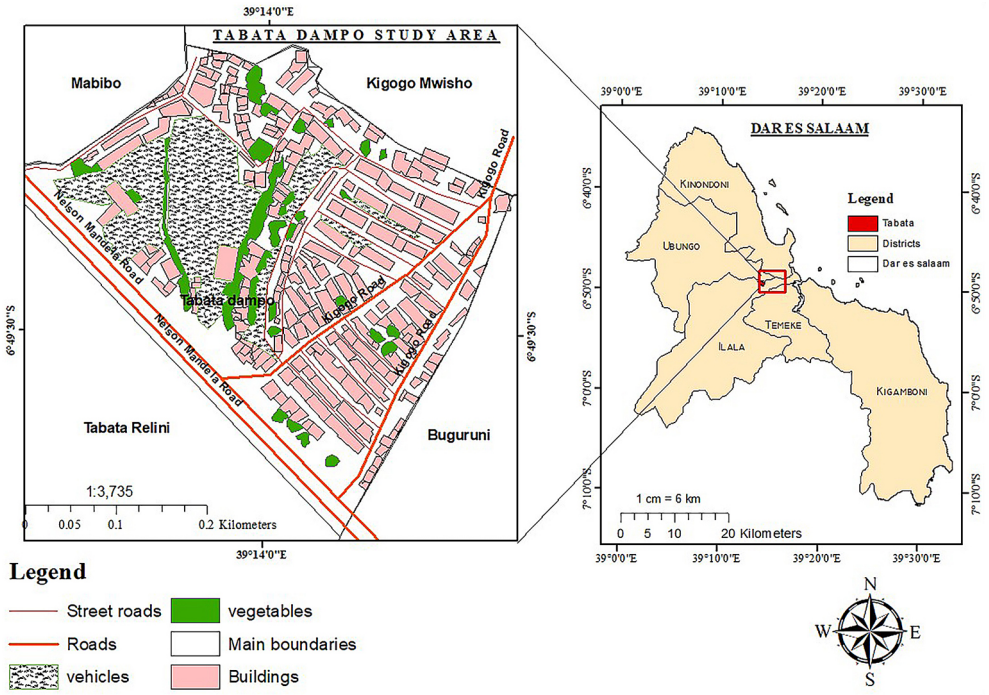

Figure 1. Location map of study area

are many garages and large shops in the area selling machinery and other related products, such as oil and grease.

Collection of oil contaminated soil sample

Soil samples were collected from fourteen (14) different locations in Tabata Dampo auto repair shops for analysis of oil concentrations, microbial counts and physicochemical analysis of oil-contaminated soil. The oil-contaminated soil samples were collected as described by Prasad et al. (2017), and the standard procedure used for this soil sample collection and handling was adopted from the ASTM standard D1586 (2018). The soil samples were collected and placed in polyethylene bags to prevent contamination and preserve their natural state. In addition, polyethylene bags are used for transporting and storing soil samples because they are durable, moisture-resistant, and have low gas permeability, which helps maintain the integrity of the sample (ASTM D1586, 2018). The soil sample collected was transported to the Environmental Engineering Laboratory at Ardhi University. The soil samples were crushed and then passed through a 2 mm sieve. A 100-gram sub-sample (this is a part of the soil sample that was prepared) was taken from the sieved soil and placed in transparent polyethylene bags for physico-chemical analysis.

Collection of organic wastes

The organic wastes used in this study were cow manure, chicken droppings and sewage sludge. Cow manure and chicken droppings were collected from the domestic farm, while sewage sludge was collected from the septic tanks at the Ardhi University. The collection procedure was taken from Muhammad et al. (2022).

Determination of oil content in soil sample (method: hexane extractable gravimetric methods)

The oil and grease content in soil samples was analyzed using the hexane extractable gravimetric method. This method involves extracting the soil sample with a nonpolar solvent, typically hexane, to dissolve and remove the oil and grease components. The extracted solution is then evaporated, leaving behind the oil and grease residues, which are subsequently weighed to determine their concentration (EPA, 2010)

Determination of nitrogen content in soil sample (method: calcium sulphate extraction)

The nitrogen content of the soil samples was estimated using the calcium sulfate extraction method. This method involves the extraction of nitrogen compounds from the soil using calcium sulfate as a reagent. The sample was ground to a fine powder using a mortar, pestle or mechanical grinder. An aliquot of soil, usually 5–10 grams, was weighed into a 50 ml centrifuge tube, 10 ml of 2 M KCl solution was added to the tube. The test tube or flask was centrifuged at 3000 rpm for 10 minutes to separate the precipitated calcium sulfate from the solution. The supernatant was transferred to a clean tube and analyzed for nitrate-nitrogen by using colorimetric or spectroscopic methods. This standard method is well described by Eaton et al. (2005).

Determine the microbial count in contaminated soil amended with selected organic waste

The standard plate count method was used technique for quantifying viable microbial cells in a given sample, as discussed in the standard methods (Eatn et al., 2005). The soil samples air-dried, homogenized, and sieved to remove any debris. A series of dilutions prepared from the soil amended with organic waste and unamended (control) to ensure that the resulting microbial count falls within the quantifiable range. A series of sterile test tubes were labeled with the desired dilution factors of 10-fold dilutions. The tubes were labeled as 10^2, 10^4 and 10^6. 1 ml of the soil sample solution, added to the first dilution tube and then 9.9 ml of distilled water was added to bring the total volume to a specific value (10 ml) in tube label 10^2. This was replicated to label tube 10^4 and 10^6 in series, consecutively. The prepared dilutions were inoculated onto the MacConkey agar. The MacConkey agar was chosen as the media due to its selectivity for gram-negative bacteria, which are commonly involved in hydrocarbon degradation. The plates incubated at the appropriate temperature for the target microorganisms, typically between 30–37°C, for 24 to 48 hours. After incubation, the colonies on the MacConkey agar plates were counted manually using visual

inspection. The microbial count was expressed as colony-forming units ($x \cdot 10^6$ CFU $s \cdot g^{-1}$)

Experimental setup and design

The aim of this part of the study was to evaluate the performance of three organic wastes in the bio remediation of oil-contaminated soil from automobile workshops. The collected soil samples (oil-contaminated soil) were divided into four tests (in reactors) (R1, R2, R3 and R4). R1 contains cow manure (CM), R2 contains chicken droppings (CD), R3 contains sewage sludge (SS), and R4 was a control without organic waste. Reactors (R1–R4) were filled with 1000 g of oil contaminated soil. In the next step, 100 g, 200 g, and 300 g of organic waste [CM, CD, and SS] were fed into the reactors according to Table 1. The mixed contents of the reactors were torn and rotated regularly to improve aeration and maintain daytime moisture in the oil-contaminated soil with distilled water. The tests were classified in categories R1 [1000 g soil + 100 g CM], R2 [1000 g soil + 100 g CD], R3 [1000 g soil + 100 g SS] and R4 [1000 g soil], this was applied similarly to 200 g and 300 g of organic waste. Each treatment unit was replicated three times.

Statistical analysis

Data analysis was statistically analyzed using the appropriate analysis of variance (ANOVA) method to assess the significance of differences between different ratios and type of organic wastes and control.

RESULTS AND DISCUSSION

Characterization of oil contaminated soil in the case study area

The results of oil contaminated soil in the case study area (Figure 1) are shown in Table 1. The minimum concentration of oil and grease contaminated soil was observed at site S6 (96.07 $mg \cdot kg^{-1}$), the maximum concentration of oil contaminated soil was at site S8 (123.11 $mg \cdot kg^{-1}$) and the average oil-contaminated soil concentration was 112.4 $mg \cdot kg^{-1}$.

The high level of oil pollution in the case study area (Figure 3) was likely caused by the large number of vehicles needing frequent maintenance and services. In the study by Ikhajiagbe and Anoliefo (2011), it was pointed out that oil released into the environment in large quantities due to manual workshop operations can inadvertently come into contact with water bodies and pollute the soil. Most of the areas have been found to have soil contaminated with high levels of oil exceeding the WHO standard, as described by Sanscartier et al. (2010). Overall, the soil contaminated with oil in the case study area (Figure 3) provided important insights into the extent and distribution of oil contamination. This information can guide the selection and design of suitable remedial measures.

Physicochemical characteristics of oil contaminated soil and organic wastes used

The physicochemical characteristics of soil and organic wastes used for this study is presented in Table 2. The pH of the oil contaminated soil was below neutral (6.12). The pH of cow manure, chicken droppings and sewage sludge were also below the neutral, reaching 6.28, 5.58 and 5.42 respectively. The values of nitrogen, phosphorous and organic carbon in oil-contaminated soil before treatment were 0.6 $mg \cdot kg^{-1}$, 0.8 $mg \cdot kg^{-1}$ and 9.5% respectively. The oil-contaminated soil had a moisture content of 17.6%, which was lower than CM (75.4%), CD (38.5%), and SS (68.42%). The organic waste used for bioremediation contained more organic carbon, nitrogen and phosphorus than the oil-contaminated soil. This study also showed that the number of bacteria in oil-contaminated soil was 3.10^6 CFU $\cdot g^{-1}$. In Table 2,

Table 1. Characteristics of oil contaminated soil at case study area

Soil sample location	Oil contaminated soil (mg/kg)
S1	117.52
S2	106.21
S3	103.48
S4	119.08
S5	104.13
S6	96.07
S7	116.74
S8	123.11
S9	110.11
S10	117.52
S11	115.31
S12	119.6
S13	107.33
S14	117.26

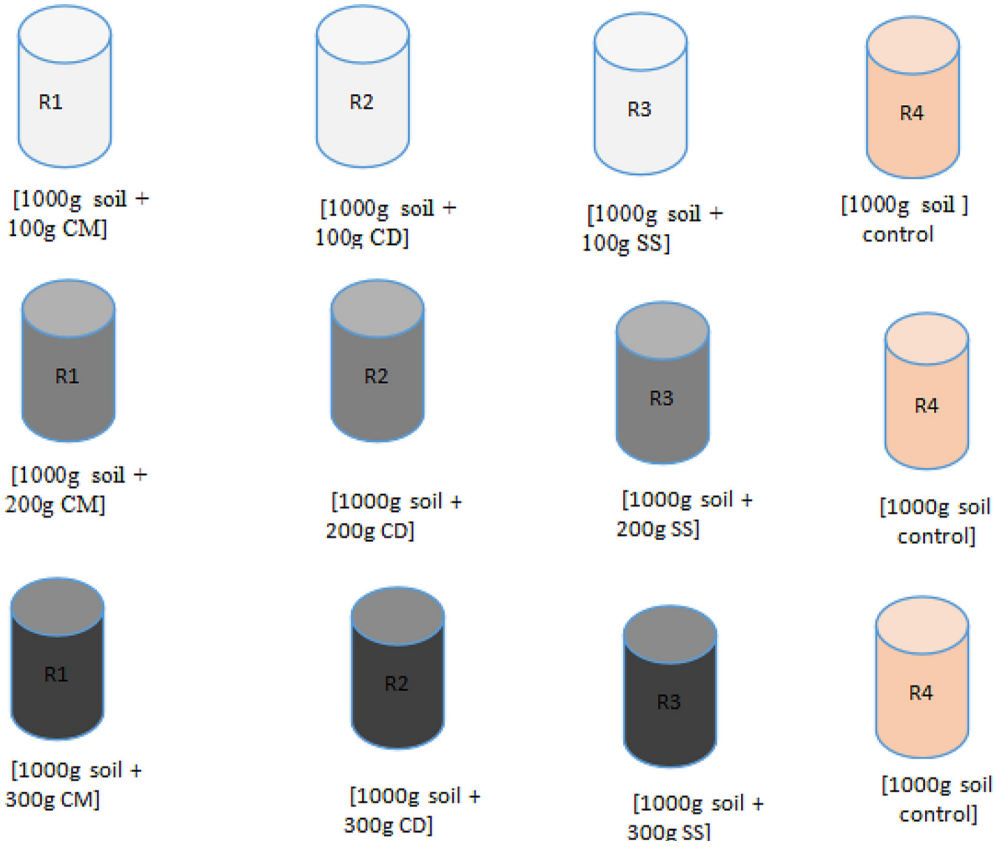

Figure 2. Experimental setup diagram

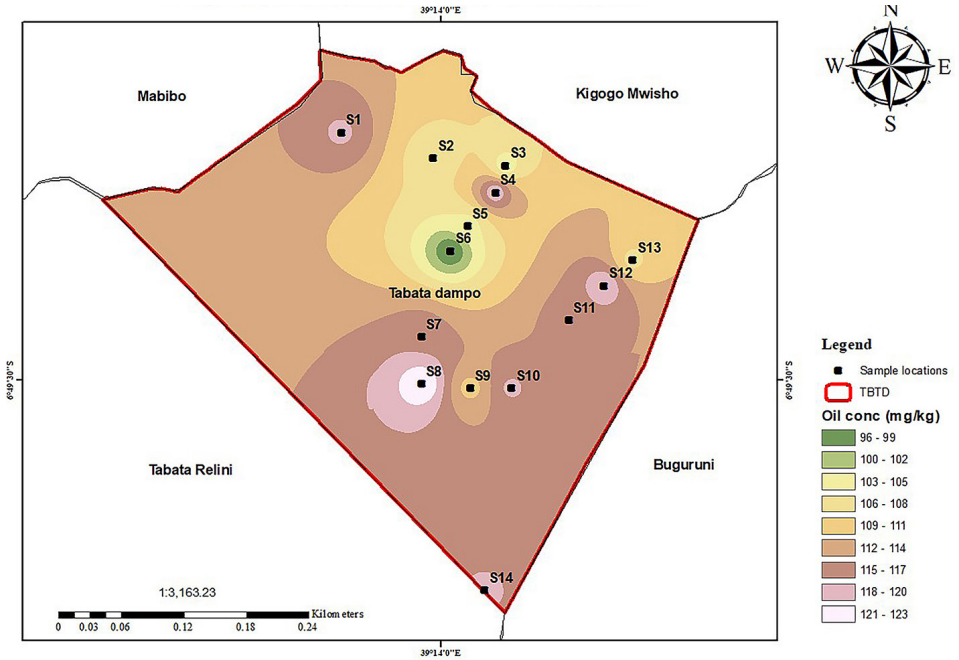

Figure 3. Distribution of oil and grease concentration at case study area

the moisture content of cow manure and sewage sludge were higher than the chicken droppings. This study is consistent with Agamuthu (2009) who pointed that the moisture content of the one of organic waste was also higher and suggested that the moisture content may favor the availability of some important microbes and promote the biodegradation of oil-contaminated soil.

Table 2. Physicochemical characteristics of organic wastes and oil-contaminated soil used

Parameters	Oil contaminated soil	Cow manure	Chicken dropping	Sewage sludge
pH	6.12	6.28	5.58	5.42
Nitrate (mg/kg)	0.6	12.7	20.2	10.6
Phosphate (mg/kg)	0.8	17.3	25.4	14.13
Moisture content (%)	17.6	75.4	38.5	68.4
Organic carbon (%)	9.5	10.5	11.4	10.1
Bacterial count (CFU/g)	3×10^6			

Note: n.d – not determined.

Nitrogen and phosphorous content in treated oil contaminated soil

Phosphate and nitrogen concentrations after bioremediation of oil-contaminated soil are shown in Figure 3. This study showed that the nitrate and phosphate levels in oil-contaminated soil treated with organic wastes (CM, CD, and SS) increased in all reactors (R1 100 g, R2 200 g and R3 300 g) compared to the initial levels of nitrate and phosphate before treatment (Table 2). However, significant increases in nitrate and phosphate were observed in chicken droppings, especially at the high dose of R3 300 g (Figure 4a and Figure 4b). Similarly, nitrate and phosphate levels were lower in sewage sludge at all organic waste dosage ratios. On the basis of this study, it can be seen that when the amount of organic waste increased (Figure 1), the nitrogen content also increased. According to a study by Okoh (2006), nitrogen is required as a nutrient to enhance the bioremediation process.

Measurements of residual oil contaminated soil after 56 days of biodegradation

The concentration of oil-contaminated soil after 56 days of biodegradation in soil treated with CM, CD, and SS is shown in Figure 5. The concentration of oil-contaminated soil in the initial stage before mixing with CM, CD, and SS was 117.7 $mg \cdot kg^{-1}$. In addition, this study found that the soil treated with different ratios of organic waste reduced the amount of soil contaminated with oil over time. However, this study showed that the CD-treated soil was the best in residual oil-contaminated soil at 24.48 $mg \cdot kg^{-1}$, and SS-treated soil was the least residual at 45.19 $mg \cdot kg^{-1}$ after 56 days of bioremediation. This study showed a statistical difference at $P<0.05$ between the soil treated with organic wastes (CM, CD and SS) and control.

The percentage of soil contaminated with oil in the treated soil after 56 days of bioremediation showed that CD had the highest reduction (79.2%), while CM and SS had 66.9% and 61.6%, respectively. The results showed a statistically significant difference at $P<0.05$ between the soil mixed with organic waste and the control.

The oil-contaminated soil treated with CD was reduced to almost 79.2% of the original oil-contaminated soil. This can be related to the high amount of organic carbon (Table 2) in the chicken droppings, which helped more microorganisms to facilitate oil degradation in the soil. The presence of organic waste in the treated soil can provide

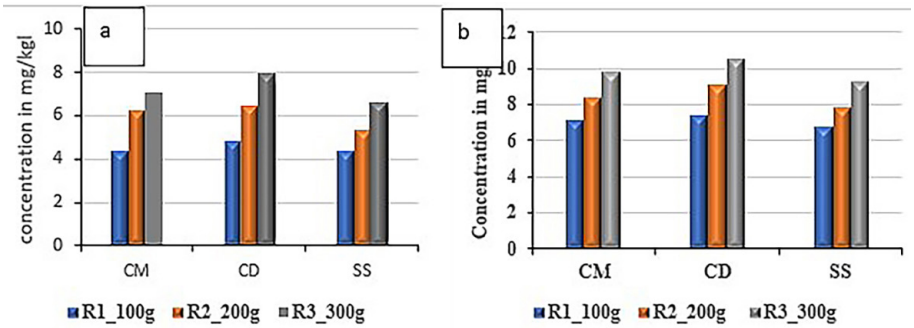

Figure 4. Average concentration in soil after 56 days of experimental run in different organic wastes of (a) nitrate, (b) phosphorus

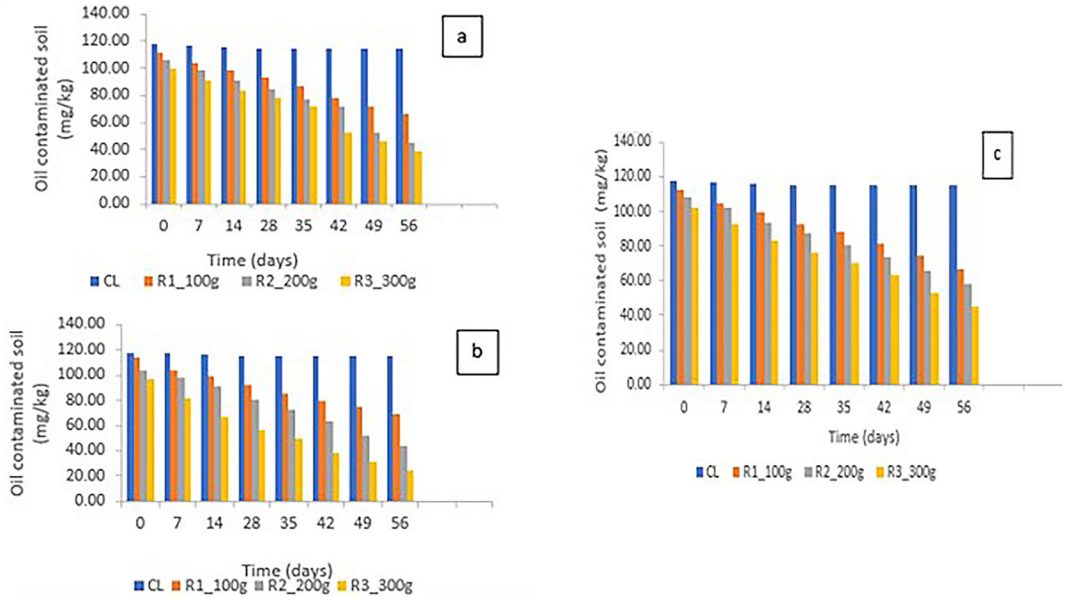

Figure 5. Residual oil-contaminated soil after 56 days of experiment mixed with (a) CM, (b) CD c – SS

more nutrients to the microbial population and thus reduce the concentration of oil in the contaminated soil (Abioye 2009). Similarly, Rahman et al. (2002) reported that adding organic carbon also affects the reduction of oil-contaminated soil. On the other hand, this study showed that in the initial phase of the bioremediation process, from day 7 to 14, no significant reduction in oil content was observed in the soil, which could also be due to the high available oil content, which makes a difficult environment for microbes activities. This trend was also reported by other researchers (Ijah and Antai 2003), who reported that high oil content can inhibit microbial availability due to the high oil content degraded in the soil. In addition, Abioye et al. (2003) reported that the low percentage of soil contaminated with oil at 28 days could be due to the toxicity of the oil to microbes, which resulted in a high concentration of oil preventing the microbial activities.

Microbial count in oil-contaminated soil after 49 days of biodegradation with different ratios organic wastes

Microbial counts of the contaminated soil amended with organic wastes (CM, CD and SS) are shown in Figure 6. During the 35 days of the experiment, the bacterial count in R3 300 g CM was $46 \cdot 10^6$ CFU·g^{-1}, while the bacterial count in R3 300 g mixed with CD and SS was $49 \cdot 10^6$ CFU·g^{-1} and $29 \cdot 10^6$ CFU·g^{-1} respectively. In this study, it was observed after 35 days that the number of bacteria began to decrease towards the end of the experiment in all treatment reactors (Figure 3). On day 49, the soil mixed with CM showed a bacterial count of 33.106 CFU·g^{-1}, CD and SS treated with R3 300 g showed bacterial counts of 35.10^6 CFU·g^{-1} and 22.10^6 CFU·g^{-1}, respectively. The soil treated with organic wastes (CM, CD and SS) and the control has a statistical difference $P<0.05$.

This study showed that the initial microbial count in oil-contaminated soil was 3.10^6 CFU·g^{-1}, but the microbial population increased as the proportion of organic waste increased (Figure 5). In the study of Abioye et al. (2009), it was because soil mixed with organic waste showed higher microbial counts than unmixed soil. In addition, higher microbial numbers were observed due to the higher nitrogen and phosphorus content of organic waste, which supplemented the nutrients required for microbial biodegradation in the soil (Joo et al., 2007). Other authors (Adenipekun and Fasidi 2005) reported that the bioremediation of oil-contaminated soil was associated with microbial mineralization. In summary, Ouedraogo et al. (2020) reported that providing oil-contaminated soil with nutrients would improve the habitat of microorganisms and ultimately affect the biodegradation of contaminated soil.

In Figure 3, on day 35 all reactors with different ratios of organic wastes indicated to have a maximum bacterial count, but the trend dropped from day 42 of the experimental run. Anyasi and Atagana (2011) reported the same observation that degradation of oil-contaminated soil was

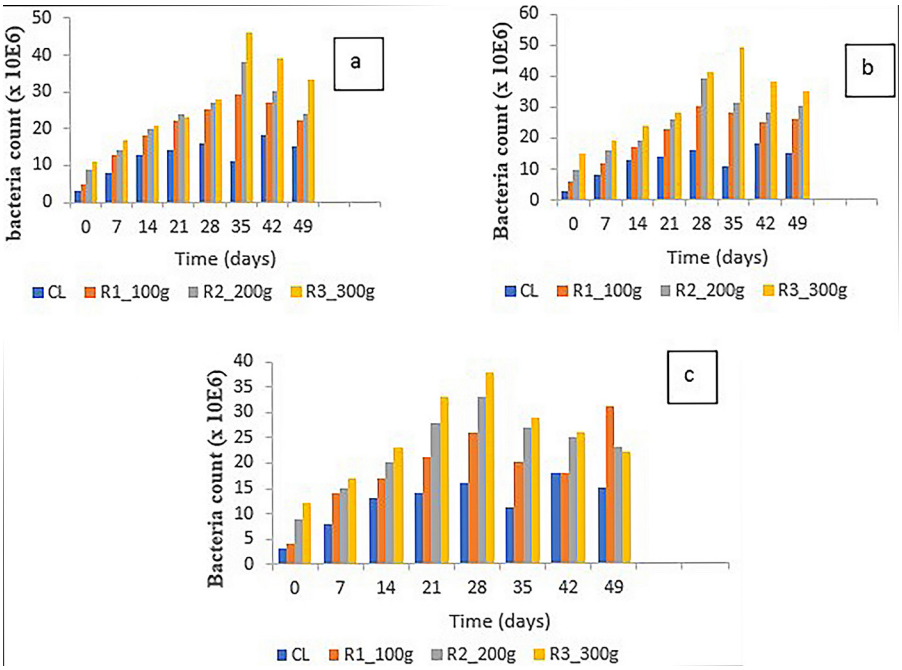

Figure 6. Microbial count in soil sample after 49 days of experiment with (a) CM (b) CD (c) SS

higher in the day 14 of the experimental run after then the degradation was slowed down. This situation might be caused by the depletion of available nutrients in the reactors to accelerate decreases of microbial population. The bioactivities within the oil contaminated soil proceeded when it is added with organic wastes as bio stimulant (Abdulyekeen et al., 2016). The higher removal of oil contaminated soil by microbes is attained due to optimized organic wastes introduced in the contaminated soil (Ogbeh et al., 2018). Chorom et al. (2010) reported that the rate of biodegradation of oil-contaminated soil depended on the presence of soil microorganisms.

CONCLUSIONS

The aim of this study was to determine the influence of organic waste on the bioremediation of oil-contaminated soil from car repair workshops and garages. This study showed that organic waste can be used to effectively reduce oil contamination in soil. The most significant reduction in performance was observed in the following order: chicken droppings, cow manure and sewage sludge. This study demonstrated that the soil mixed with 300 g of organic waste produced the best results compared to other ratios. This study showed that the number of bacteria similarly increased along with organic waste, contributing to a higher reduction of oil-contaminated soil. Therefore, the mixing ratio of 1000 g of oil-contaminated soil to 300 g of organic waste showed better reduction of oil contamination in the soil. The influence of organic wastes (CM, CD and SS) in the bioremediation of oil-contaminated soil showed better results due to the presence of essential nutrients.

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