USE OF ORGANIC WASTES FOR BIOREMEDIATION OF SPENT LUBRICATING OIL POLLUTED SOIL IN MINNA, NIGERIA

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ABSTRACT

Spent lubricating oil polluted soil was remediated with organic wastes; cow dung (CowD) and chicken droppings (ChiD) (animal wastes), corn cob (CorC) rice husk (RicH) and sorghum husk (SorH) (plant wastes). The nitrogen contents of the organic wastes were determined to be 11.45, 10.92, 9.80, 9.66 and 12.04g/kg respectively. The organic wastes were screened for ability to remediates spent lubricating oil polluted soil. The potential of the organic wastes to remediate spent lubricating oil polluted soil was monitored for 12 weeks by measuring the pH, moisture contents, microbial counts nitrogen contents and total petroleum hydrocarbon (TPH). The pH of unremediated soil ranged from 6.14– 6.31 while that of soil remediated with CowD, CorC, ChiD, RicH and SorH ranged from 6.18 – 7.25, 6.12 – 6.42, 6.38 – 7.51, 6.20 – 6.52 and 6.22 – 6.45 respectively after 12weeks. The results showed that reduction in pH of the treatments were higher compared to the control. Also there was an increase in microbial count of the treatments while there was reduction in that of the control. The nitrogen content in spent lubricating oil polluted soil (control) was lower compare to all the treatments. The nitrogen content in soil treated with CowD, CorC, ChiD, RicH and SorH reduces from 12.82– 9.24 g/kg, 11.14 – 9.46 g/kg, 12.26 – 8.16 g/kg, 11.01 – 9.52 g/kg and 13.38– 9.22g/kg respectively after 12 weeks. The percentage of oil degradation in soil remediated with organic wastes (CowD, CorC, ChiD, RicH and SorH) increased from 22.22 – 68.52%, 14.19 – 61.11%, 23.46 – 74.07%, 6.0 – 57.40% and 20.37 – 66.67% respectively after 12 weeks. The percentage of oil degradation was low in the control soil compared to the treatments, meaning that the organic wastes added to the polluted soil enhanced the activities and populations of microorganisms to remediate the oil polluted soil. This revealed that organic wastes had the potential to enhance the activities and populations of microorganisms to remediate the oil polluted soil considerably.

Keywords: Bioremediation, Organic wastes, Spent lubricating oil, Soil
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INTRODUCTION

Spent engine oil is derived subsequent to being used and dispensed off from lubricating various internal combustion engines that include all categories of motor vehicles. Engine oil is described as the oil that principally functions as cleaning the motor engines, lubricating the moving parts of motor engines, inhibiting corrosion of motor engines, improving sealing and cooling the engine by transporting heat away from the moving parts [1]. The present day engine oils are derivative of petroleum-based and non-petroleum produced chemical compounds. Engine oils are, therefore, mainly blended by employing base oils composed of hydrocarbons (organic compounds containing carbon and hydrogen exclusively), for instance mineral oil. Alaa El-din *et al*. [2] reported the pollution of the soil by petroleum products as a huge environmental concern worldwide.

The chemical and concentration of the pollutants, geological, physical and biological attributes of the polluted site play important role in the persistence of petroleum products in soil. Microorganisms utilizing the petroleum hydrocarbons in the soil depend hugely on the interaction of these factors as suitable conditions are provided [3]. This implies that the fate of petroleum products found in soil depends solely on the soil microbial community, as degradation cannot take place without these hydrocarbon utilizing microorganisms. It has been well established in the literature that microorganisms are entirely dependent on the availability of nutrients for survival [15]. The growth rate of oil degrading bacteria on contaminated soils is often restricted by the accessibility of nutrients like nitrogen and phosphorus [4]. These nutrients are the basic building blocks of life and create the enabling environment for necessary enzymes produced by the microorganisms to break down hydrocarbon compounds.

The problems of pollution have necessitated research to bring about the cleanup of the polluted soils. Bioremediation, which involves the use of microorganisms to remediate and detoxify polluted site, appears to be favoured. It is eco-friendly, cost-effective, and efficient for the abatement and decontamination of hydrocarbon pollution and many other pollutants, but might take more duration than the conventional technique because of its natural process [5,6, 7]. The process relies on microbial enzymatic activities to transform or degrade the contaminants from the environment [8]. Bioaugmentation and biostimulation are methods of bioremediation used in enhancing the process [7]. Bioaugmentation is the addition of external microbial populations, which could be indigenous or exogenous, to the oil polluted area. Sometimes, they can be genetically engineered [9]. Biostimulation involves the addition of appropriate nutrient such as organic wastes to the polluted environment. These may either occur *in-situ* or *ex-situ* [10].

Considering the increasing rates of oil spills in the country especially in oil producing States, many alternative cleanup methods should be encouraged. Inorganic fertilizer, chicken droppings and periwinkle shells have been employed to remediate oil polluted soil [11, 12]. Also, agricultural wastes such as groundnut shells, rice husk, and maize cob have been used to restore oil- spill soils [13, 14].

Using organic wastes; both plant and animal wastes to remediate spent lubricating oil polluted soil in Nigeria will solve environmental problem created by continuous availability of organic wastes in the environment. Besides, it will be cheaper than commercial bioremediation agents because organic wastes are cheap to obtain. Considering these advantages, this study focused on the use of both plant and animal wastes to bio remediate spent lubricating oil polluted soil.

MATERIALS AND METHODS

Collection and processing of samples

Spent lubricating oil polluted soil (SLOPS) was collected from abandoned mechanic workshop located at Old Airport Road, Minna, Niger State, Nigeria. It was collected at a depth of 5cm [15] into clean polythene bags and transported to the laboratory for analyses. Spent lubricating oil (SLO) used for this research was collected in a sterile jerry can from mechanic workshop at Keteren-Gwari mechanic village, Minna, Niger State, Nigeria. Organic wastes used in this study were; corn cobs (CorC), sorghum husk (SorH), rice husk (RicH), cow dung (CowD) and chicken droppings (ChiD). Corn cobs, rice husk and sorghum husk were collected into clean polythene bags from a farm in Wushishi, Niger State, Nigeria. Cow dung was collected into clean polythene bags from a private cow ranch at Barkin Sale, Minna, Niger State, Nigeria while chicken droppings were collected into clean polythene bags from a poultry farm in Nyikangbe, Minna, Niger State, Nigeria and transported to the laboratory. The organic wastes were air dried outside the laboratory and ground into fine powder using grinding mill and stored until required.

Determination of physical, chemical and microbial properties of spent lubricating oil polluted soil and organic wastes

The pH of spent lubricating oil polluted soil and organic wastes were determined using pH meter (Testronic digital pH meter, Model 3505, Jenway, United Kingdom) [16, 17]. The moisture content was determined using the dry weight method [18]. Macro-Kjeldahl method described by [19] and [20] was used to determine total nitrogen. The Bray No. 1 method of [21] was used for the determination of available phosphorus. Organic matter, Sodium and potassium were determined according to the method of [22, 20]. The method described by [23] was used for the determination of heavy metals.

Microbiological analysis of the spent lubricating oil polluted soil and organic wastes

One gramme (1g) of spent lubricating oil polluted soil and each of the organic waste were added to 10 mL of sterile deionized water respectively and shaken properly to give the first (10^{-1}) dilution. Tenfold serial dilution was made by adding 1 mL of the 10^{-1} suspension to 9 mL of sterile deionized water to make the subsequent dilutions up to 10^{-10} dilution. The serially diluted samples were inoculated on nutrient agar (NA), sabouraud dextrose agar (SDA) and oil agar (OA) for the enumeration of aerobic heterotrophic bacteria, fungi, spent lubricating oil utilizing bacteria (OA-B) and spent lubricating oil utilizing fungi (OA-F) respectively [11, 24]. The NA plates were incubated at 30ºC for 48 hours, the SDA plates were incubated at room temperature (28ºC ± 2ºC) for 5 days, while oil agar (OA; B) plates were incubated at 30ºC for 5 days (for oil degrading bacteria) and oil agar (OA; F) plates at room temperature (28ºC ± 2ºC) for 5 days (for oil degrading fungi). Nystatin (0.6 g/L) was added to OA used for isolation of SLO utilizing bacteria to suppress the growth of fungi while chloramphenicol (0.6 g/L) was added to OA used for isolation of SLO utilizing fungi to suppress the growth of bacteria [14, 24]. After incubation, distinct colonies were counted with the aid of colony counter (Mac-Anderson Instruments, UK). The counts were expressed as colony forming units per gram (cfu/g) of sample. The isolates were obtained in pure cultures by repeated sub culturing on media used for the primary isolation. The pure isolates were maintained on agar slants at 4ºC for further characterization and identification. All experiments were carried out in duplicates.

Screening of organic wastes for potential to remediate spent lubricating oil polluted soil

Completely randomized block design (CRBD) was used in this study. The bioremediation study was conducted according to the method of [25, 24]. Two kilograms (2 kg) of spent lubricating oil polluted soil moistened with distilled water was weighed into bowls labeled A-E. Zero point two kilogram (0.2 kg) each of the organic wastes cow dung (CowD), corn cob (CorC), chicken droppings (ChiD), rice husk (RicH) and sorghum husk (SorH) was mixed thoroughly with the polluted soil in the bowls and were exposed in the laboratory. Control experiment was set up in a bowl containing only spent lubricating oil polluted soil. All experiments were in duplicates and sampling was done every two weeks for a total duration of 12 weeks. pH, moisture content, microbial counts and total petroleum hydrocarbon (TPH) were monitored.

Determination of Total Petroleum Hydrocarbon (TPH)

The total petroleum hydrocarbon (TPH) in the amended soil was determined gravimetrically by diethyl ether cold extraction. Ten grammes (10 g) of soil sample was weighed into 250 mL capacity conical flask and 50 mL of diethyl ether was added, and shaken for 30 minutes in an orbital shaker, the liquid phase of the extract was measured at 420 nm wavelength using spectrophotometer. The TPH in soil was estimated with reference to the standard curve derived from fresh spent lubricating oil diluted with diethyl ether. TPH data was fitted to first- order kinetics model of Abioye *et al*. and Yeung *et al*. [14, 26].

RESULTS AND DISCUSSION

Physical, chemical and microbial properties of soil and organic wastes

Table 1 shows the physical, chemical and microbial properties of soil and organic wastes used for bioremediation studies. The pH level of the soil was generally within the range for soil as established by FEPA [27]. The moisture of the soil and that of organic wastes were within the range that helps microorganisms to proliferate well. The pH of the soil, RicH and CorC were acidic while the pH of CowD, ChiD and SorH were alkaline. Abioye *et al*. and Stephen *et al.* [14, 28] had reported the alkalinity of organic wastes favors most crude oil degrading microorganisms. The moisture content of the soil is similar to that reported by Abioye *et al*., Idowu and Ijah, Ekundayo *et al*., Debojit *et al*. and Idowu and Ijah [14, 24, 29 - 31] that microbial counts of microorganisms in soil and organic wastes can be attributed to favourable physicochemical properties of the soil and the organic wastes.

pH of soil remediated with organic wastes

The pH of unremediated soil ranged from 6.14 to 6.31 while that of remediated soil ranged from 6.12 to 7.51 after 12 weeks (Figure 1). The pH of the treatments was higher compared to that of control experiments, this may be as a result of the addition of organic wastes which was capable of increasing the pH of the polluted soil [32]. There were no significant differences (P > 0.05) among the five biostimulation treatments. The reduction in pH of the treatments and control might be as a result of acidic metabolites produced by the microorganisms during the oil biodegradation process [33]. The pH of treatments was within the ranges that support the growth of microorganisms [6]. The pH of unremediated soil were lower than that of the treatment, this might be as a result of the organic wastes added for remediation which have the ability to increase the pH of the treatments [4].

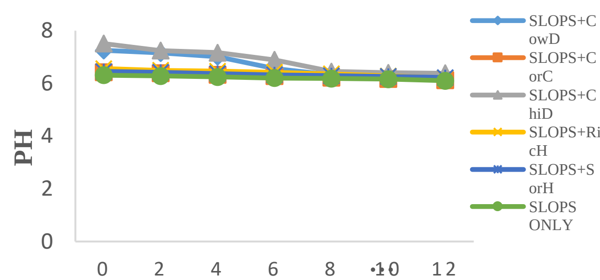

Figure. 1: pH of spent lubricating oil polluted soil remediated with organic wastes

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Table 1: Physical, chemical and microbial properties of spent lubricating oil polluted soil and organic wastes

Parameter	SOIL	CowD	ChiD	RicH	SorH	CorC
Textural class	Sandy loam	—	—	—	—	—
Sand (g/kg)	714	—	—	—	—	—
Silt (g/kg)	102	—	—	—	—	—
Clay (g/kg)	184	—	—	—	—	—
pH	6.1	8.4	9.3	6	6.8	5.8
Moisture content (%)	5.9	8.15	7.35	6.25	6.1	7.26
Organic Carbon (g/kg)	10.24	—	—	—	—	—
Organic Matter (g/kg)	—	347	462	129	55	2
Total Nitrogen (g/kg)	1.68	11.48	10.92	9.66	12.04	9.8
Available Phosphorus (g/kg)	15	0.64	11.49	1.19	1.02	0.46
Sodium (cmol/kg)	0.33	2.72	4.54	0.57	0.43	0.33
Potassium (cmol/kg)	0.11	11.7	22.43	3.96	19.7	3.43
Calcium (cmol/kg)	5.6	18.4	68.8	4.4	3.2	0.8
Magnesium (cmol/kg)	1.92	10.2	15.6	0.7	1.2	1.9
AHBC (cfu/g)	4.5×10^5	3.2×10^5	3.0×10^5	4.2×10^5	3.5×10^5	3.6×10^5
SLOUBC (cfu/g)	2.6×10^5	2.2×10^5	2.4×10^5	3.5×10^5	3.0×10^5	3.2×10^5
TFC (cfu/g)	5.2×10^2	2.5×10^2	2.6×10^2	3.8×10^2	3.4×10^2	4.0×10^2
SLOUFC (cfu/g)	3.5×10^2	2.3×10^2	2.2×10^2	3.5×10^2	3.0×10^2	3.8×10^2
Lead (mg/l)	9.08	0.24	0.33	0.06	0	0.03
Manganase (mg/l)	1.8	4.63	2.6	0.96	0.67	0.22
Copper (mg/l)	0.17	0.21	0.23	0.26	0.24	0.17

KEY: SL = Sand loamy, g/kg = gramme per kilogramme, cmol/kg = centimoles of charge per kilogramme, % = percentage, cfu/g = colony forming units per gramme, mg/l = milligramme per l

Moisture content of soil remediated with organic wastes

The moisture content of control ranged from 8.58% – 16.34% while that of the treatments ranged from 10.19% –15.95% after 12 weeks (Figure 2). The moisture content of treatments at 0 week was higher compared to the control probably due to the presence of organic wastes. There was no significant difference ($P > 0.05$) among the moisture content of both the treatments and control from 2 weeks to 12 weeks; this may be as a result of daily wetting of the experiments with water and commencement of raining season later. The moisture contents were within the range that supports the growth of microorganisms [24].

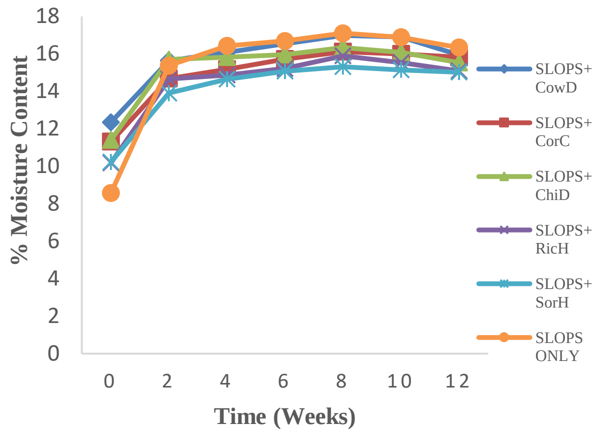

Figure 2: Moisture content of spent lubricating oil polluted soil remediated with organic wastes

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Microbial counts in soil remediated with organic wastes: Aerobic heterotrophic bacterial (AHB) counts

The AHB counts in spent lubricating oil polluted soil (control) decreased from 5.4 × 10^5cfu/g to 3.5 × 10^5cfu/g while the counts in spent lubricating oil polluted soil remediated with organic wastes (CowD, CorC, ChiD, RicH and SorH) increased from 6.9 × 10^5 cfu/g to 7.2 × 10^5cfu/g, 7.2 × 10^5 - 7.5 × 10^5cfu/g, 6.6 × 10^5cfu/g - 8.1 × 10^5cfu/g, 6.1 × 10^5cfu/g - 6.2 × 10^5cfu/g and 6.4 × 10^5cfu/g - 6.8 × 10^5cfu/g respectively (Figure 3). The reduction in the counts of AHB in spent lubricating oil polluted soil (control), could be as result of insufficient nitrogen and potassium in the polluted soil which could have served as nutrient for the proliferation of the organisms [34]. However, there were significant increases in the counts of AHB in the polluted soil treated with CowD, ChiD and SorH compared to the CorC and RicH treatment. This could be as a result of high content of nitrogen, potassium and calcium in CowD, ChiD and SorH, which has been reported by [14, 32] to enhance the growth of microorganisms and increase the pH of the treatments. Low level of nitrogen and potassium in CorC and RicH may be responsible for the slight increase in the count of AHB in the amended soils. Also, the calcium content in CorC and RicH were low, this resulted in the acidic pH which might be responsible for low count of AHB in the treatments

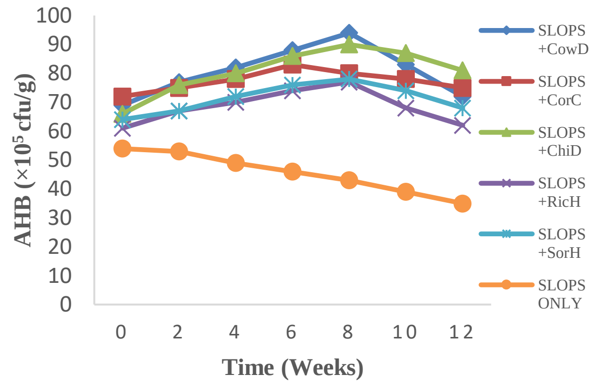

Figure 3: Aerobic heterotrophic bacterial (AHB) counts of spent lubricating oil polluted soil remediated with organic wastes:

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Spent lubricating oil utilizing bacterial (SLOUB) counts

The SLOUB counts in spent lubricating oil polluted soil (control) ranged from 4.4 × 10^5 cfu/g to 2.5 × 10^5cfu/g while the counts in spent lubricating oil polluted soil remediated with organic wastes (CowD, CorC, ChiD, RicH and SorH) ranged from 4.3 × 10^5cfu/g to 5.2 × 10^5cfu/g, 3.7 × 10^5 cfu/g - 4.8 × 10^5cfu/g, 4.7 × 10^5cfu/g - 5.7 × 10^5cfu/g, 3.8 × 10^5cfu/g to 4.7 × 10^5cfu/g and 4.4 × 10^5cfu/g - 5.3 × 10^5cfu/g respectively (Figure 4). These results indicated decrease in the counts of SLOUB in the control soil compared to the treatments which showed increase in the counts of SLOUB. This may be as a result of inadequate nutrient such as nitrogen and potassium which can stimulate the growth of microorganisms in the polluted soil [32]. The increase in the counts of SLOUB in CowD, ChiD and SorH were higher compared to CorC and RicH treatments. This may be as a result of high content of nutrients in CowD, ChiD and SorH compared to CorC and RicH [34]. CowD, ChiD and SorH have high content of nitrogen and potassium which are essential nutrients for microbial growth. Also, CowD and ChiD calcium contents were high and this may be responsible for the high pH which support microbial growth [15].

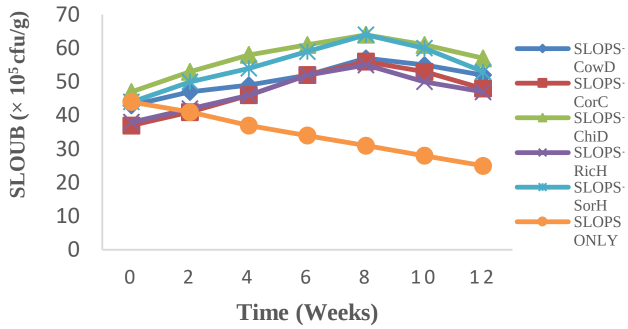

Figure 4: Spent lubricating oil utilizing bacteria (SLOUB) counts of spent lubricating oil polluted soil remediated with organic wastes:

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Total fungal (TF) counts

The TF counts in the control soil showed a decrease from 5.3 × 10^2cfu/g to 3.5 × 10^2cfu/g while TF counts of polluted soil treated with CowD, CorC, ChiD, RicH and SorH increased from 6.7 × 10^2cfu/g to 7.8 × 10^2cfu/g, 5.8 × 10^2cfu/g - 6.7 × 10^2cfu/g, 6.5 × 10^2cfu/g - 8.5 × 10^2cfu/g, 6.2 × 10^2cfu/g - 7.1 × 10^2cfu/g and 5.6 × 10^2cfu/g - 6.6 × 10^2cfu/g respectively (Figure 5). The decrease in TF counts in the control experiment might be as a result of insufficient nutrients, such as nitrogen and phosphorous which can stimulate the growth of the fungi while the increase in the TF counts in the treatments may be attributed to the organic wastes which served as sources of nutrients that stimulated the growth of the fungi [2, 35].

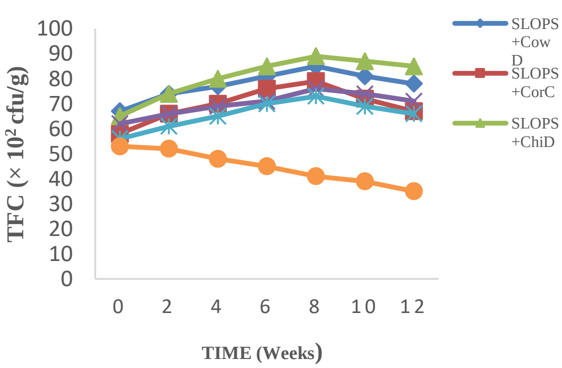

Figure 5: Total fungi counts (TFC) of spent lubricating oil polluted soil remediated with organic wastes:

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Spent lubricating oil utilizing fungal (SLOUF) counts

The SLOUF counts of control soil decreased from 3.9 × 10^2 cfu/g to 2.7 × 10^2 cfu/g while that of the treatments ranged from 4.9 × 10^2 cfu/g to 6.2 × 10^2 cfu/g, 4.4 × 10^2 cfu/g - 5.3 × 10^2cfu/g, 5.8 × 10^2 cfu/g - 8.3 × 10^2 cfu/g, 5.4 × 10^2 cfu/g - 6.5 × 10^2cfu/g and 4.9 × 10 cfu/g - 5.9 × 10 cfu/g respectively (Figure 6). The decrease in SLOUF counts in the control soil may be as result of limited ability of the microbes to utilize the carbon and energy source (SLO) present in the polluted soil, due to the competition for inadequate nutrient, while the significant increase in CowD, ChiD and SorH compared to CorC and RicH may be attributed to adequate nutrient which amount to high level of nitrogen and potassium in them compared to CorC and RicH [35].

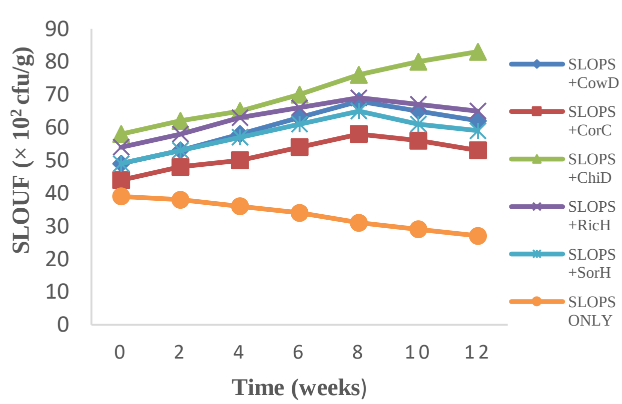

Figure 6: Spent lubricating oil utilizing fungi counts (SLOUF) of spent lubricating oil polluted soil remediated with organic wastes:

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Nitrogen content in soil remediated with organic wastes

Figure 7 shows the nitrogen content in soil remediated with organic wastes. The nitrogen content in spent lubricating oil polluted soil (control) was lower compare to all the treatments. The nitrogen content in soil treated with CowD, CorC, ChiD, RicH and SorH reduces from 12.82 to 9.24 g/kg, 11.14 – 9.46 g/kg, 12.26 – 8.16 g/kg, 11.01 – 9.52 g/kg and 13.38 - 9.22 g/kg respectively after 12 weeks. The high content of nitrogen in the treatments serves as nutrient for microbes to grow, also the reduction in nitrogen content shows the rate at which the microorganisms utilize the nitrogen to proliferate [2, 4].

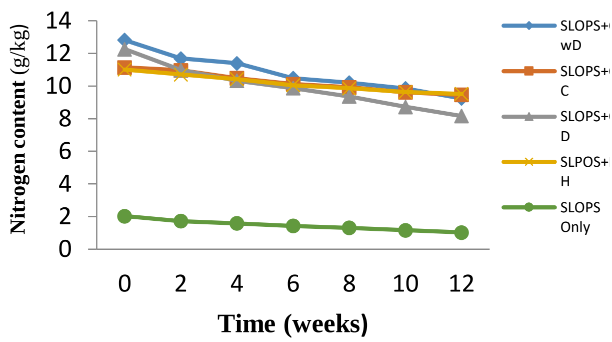

Figure 7: Nitrogen content in soil remediated with organic wastes

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

Total Petroleum Hydrocarbon (TPH) in soil remediated with organic wastes

The percentage degradation of oil in soil remediated with organic wastes (CowD, CorC, ChiD, RicH and SorH) increased from 22.22 – 68.52%, 14.19 – 61.11%, 23.46 – 74.07%, 6.04 – 57.40% and 20.37 – 66.67% respectively after 12 weeks while that of control increased from 1.23 – 11.73% (Figure 8). The percentage degradation of oil was high in the treated soil compared to the control, which indicates that the organic wastes added to the polluted soil enhanced the remediation of the oil polluted soil. The percentage degradation of oil in polluted soil, remediated with CowD, ChiD and SorH were higher than that of CorC and RicH, indicating that CowD, ChiD and SorH enhanced the spent lubricating oil degradation better than CorC and RicH. This may be due to high contents of nitrogen and potassium in CowD, ChiD and SorH compared to CorC and RicH. Alaa El-din *et al.*, Abioye *et al.* and Atiku *et al.* [2, 14, 32] recorded similar results.

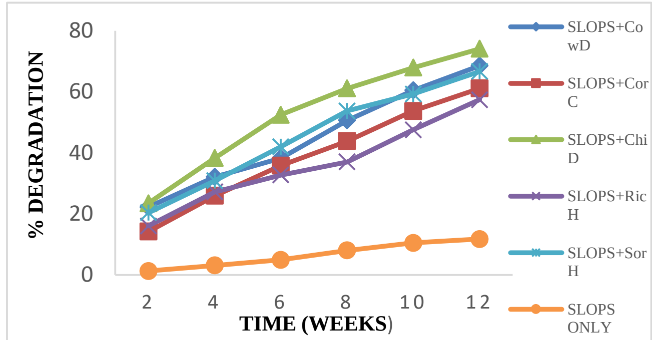

Figure 8: Percentage degradation of oil in soil remediated with organic wastes**:**

KEY: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

CONCLUSION

This study investigates the potential of both plant and animal wastes for remediation of spent lubricating oil polluted soil. The results revealed high reduction in the pH of unremediated soil compared to the treatments. Also there was an increase in microbial counts of the treatments while the microbial counts of control decreased. The nitrogen content in spent lubricating oil polluted soil (control) was lower compare to all the treatments, this shows that the organic wastes used have high nitrogen contents which serve as nutrients for microorganisms to proliferate well. Also the oil percentage degradation was low in the control soil compared to all the treatments, which implies that the organic wastes added to the polluted soil enhanced the remediation of the spent lubricating oil polluted soil considerably.

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