SCREENING OF ORGANIC WASTES AND MICROORGANISMS FOR BIOREMEDIATION OF SPENT LUBRICATING OIL POLLUTED SOIL

[1]IDOWU, F. C., [1]IJAH, U. J. J., [1]BALA, J. D.[2]., & TSADO, P. A.
[1]Department of Microbiology, School of Life Sciences,
Federal University of Technology, Minna, Niger State, Nigeria
[2]Department of Soil Science,
School of Agriculture and Agricultural Technology,
Federal University of Technology, Minna, Niger State, Nigeria.
E-mail: arabambicaroline@gmail.com
Phone No: +234-703-706-3073

Abstract
Microbes were isolated from spent lubricating oil polluted soil and organic wastes; cow dung (CowD), corn cob (CorC), chicken droppings (ChiD), rice husk (RicH) and sorghum husk (SorH) and were identified as species of Aspergillus, Penicillium, Mucor, Trichophyton, and Fusarium (fungi) and Pseudomonas, Bacillus, Salmonella, Staphylococcus and Escherichia (bacteria). The organisms and the organic wastes were screened for ability to utilize spent lubricating oil as a source of carbon and energy. The isolates utilized the oil at varying rates. However, five isolates (22.8%) utilized the oil maximally while eight isolates (31.8%) utilized it moderately and nine isolates (40.9%) utilized the spent lubricating oil minimally. The potential of the organic wastes to remediate spent lubricating oil polluted soil was monitored for 12 weeks by measuring the pH, microbial counts and total petroleum hydrocarbon (TPH). The pH of unremediated soil ranged from 6.14 - 6.31 while that of remediated soil ranged from 6.18 - 7.25, 6.22 - 6.42, 6.38 - 7.51, 6.20 - 6.56 and 6.22 - 6.45 respectively after 12weeks. The results showed that reduction in pH of the treatments were higher compared to the control and there were no significant differences (P > 0.05) in the pH of the five biostimulation treatments. Also there was an increase in microbial count of the treatments while there was reduction in that of the control. The percentage degradation of oil in spent lubricating oil polluted soil remediated with organic wastes; CowD, CorC, ChiD, RicH and SorH were 68.52%, 61.11%, 74.07%, 57.40% and 66.67% respectively while that of the control was 11.73%. This revealed that organic wastes had the potential to remediate spent lubricating oil polluted soil considerably.

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

Introduction
The contamination of land and water by improper disposal of spent lubricating oil (SLO) is on the increase, leading to ecological imbalance and soil infertility. Spent lubricating oil can also be released into the environment via engine leaks, automobiles and application into rural roads for dust control (Agency for Toxic Substances and Disease Registry, ATSDR, 1997; (Stephen, Obaka, & Ekeji, 2016). The presence of different types of automobile and machinery has also resulted in increase in the use of lubricating oil. Onuoha, Olugbe, Uraka, and Uchendu (2011) reported that oil spills from industries, fuel serving stations, activities in petroleum depots during loading, transportation and auto-mechanic workshops, all combine to contribute to soil contamination. Spent lubricating oil contains heavy metals and polycyclic aromatic hydrocarbons (PAHs) that could be mutagenic and carcinogenic in nature (Abioye, Agamathu, & AbdulAzez, 2012). No matter how small, pollution influences the natural microbial community and affects both physical and chemical properties of the polluted environment (Ijah & Antai, 2003; Idowu & Ijah, 2018).

Spent engine oil is the brown-to-black, oily liquid removed from the engine of a motor vehicle, it is typically referred to as used motor oil that has been collected from motor vehicle servicing workshops, garages, and industry sources such as hydraulics oil, turbine oils, process oil and metal working fluids (Hamawand, Yusaf, & Ibrahim, 2013). Used engine oil is similar to unused oil except that it contains additional chemicals that are produced or that build up in the oil when it is used as an engine lubricant (Aucelio, de Souza, de Campos, Miekeley, & Da Silva, 2007). Used engine oil has many of the characteristics of unused oil. It smells like unused oil and contains the chemicals found in unused oil. It contains a mixture of different chemicals including low to high molecular weight (C15-C21) compounds, lubricants, additives, decomposition products and heavy metals which have been found to be harmful to the soil and human health (Duffus, 2002). These include straight chain (aliphatic) hydrocarbons and aromatic or polycyclic aromatic hydrocarbons (PAHs), which are distilled from crude oil, and various additives that improve the performance of the oil in the engine (Aucelio *et al*., 2007; Hamawand *et al*., 2013). Aliphatic hydrocarbons are molecules with carbon atoms in simple or branched chains while aromatic hydrocarbons are compounds with unsaturated carbons in six-membered rings and with properties similar to benzene. PAHs are complex organic compounds containing three or more aromatic rings (Aucelio *et al*., 2007; Hamawand *et al*., 2013).

In addition to the chemicals found in unused oil, used oil contains chemicals that are formed when the oil is exposed to the high temperature and pressure inside an engine as it runs. It also contains metals such as aluminum, chromium, copper, iron, lead, manganese, nickel, silicon, and tin that comes from engine parts as they wear down. Also, used oil contains small amounts of water, gasoline, antifreeze, and chemicals that come from gasoline when it burns inside the engine (Singhabhandhu & Tezuka, 2010; Udonne, 2011). The chemicals found in used oil vary depending on the brand(s) and type of engine oil used, whether gasoline or diesel fuel was used, the mechanical condition of the engine that the oil came from, the various sources of used oil (e.g., automobiles, airplanes, trains, ships, tractors, lawn mowers), and the number of miles driven between oil changes (Singhabhandhu & Tezuka, 2010; Udonne, 2011). Due to its chemical composition, worldwide dispersion and effects on the environment, used motor oil is considered a serious environmental problem (Aucelio *et al*., 2007; Abioye *et al*., 2012; Abdulyekeen *et al*., 2016).

Most current motor oil lubricants contain petroleum base stocks, which are toxic to the environment and difficult to dispose after use (Marai, 2015). Over 40% of the pollution in America's waterways is from used motor oil (Fox, 2007). Used oil is considered the largest source of oil pollution in the United States of America. By far, the greatest cause of motor oil pollution in Nigeria comes from drains and urban street runoff, much of which is from improper disposal of engine oil (Aucelio *et al*., 2007).

The problems of pollution have led to the search for many remedial methods 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 (Adekunle 2011; Ijah *et al*., 2013; Effenyi *et al*., 2017). The process relies on microbial enzymatic activities to transform or degrade the contaminants from the environment (Philip & Atlas, 2005). Biostimulation involves the addition of substrates, vitamins, oxygen and other compounds that can stimulate microorganism activity and increase their population so that they can degrade petroleum hydrocarbon faster. Stimulation of microorganisms by the addition of nutrients brings large quantities of carbon sources which tend to result in a rapid depletion of the available pools of major inorganic

nutrients such as Nitrogen and Phosphorous (Kumar *et al*., 2011). When fertilizer (nutrient) is added to the oil polluted soil, the organisms can rapidly degrade the oil, utilizing it as the carbon source and the fertilizer as the nitrogen and phosphorous source (Onuoha, 2011). Combinations of inorganic nutrients often are more effective than single nutrients where a low level of macronutrients and a high level of micronutrients are required to stimulate the activities of indigenous microbes (Onuoha, 2011). Addition of nutrients also promotes the growth of heterotrophic microorganisms which are not innate degraders of total petroleum hydrocarbon thereby creating a competition between the resident micro flora (Adams *et al*., 2014).

Biostimulation which involves the addition of organic and inorganic nitrogen rich nutrients is an effective approach to enhance the bioremediation process. Addition of oil-degrading microbial consortia to supplement the indigenous populations (bioaugmentation) is another strategy for the bioremediation of oil contaminated environments (Rahman *et al*., 2002; Idowu & Ijah, 2017).

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 (Stephen *et al*., 2016) 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, 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, Nigeia. 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 Nykamgbe, 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 and Chemical Properties of Organic Wastes

The pH of organic wastes was determined using pH meter (Testronic digital pH meter, Model 3505, Jenway, United Kingdom) (Obire, 2012; Olalemi & Arotupin, 2012). The moisture content was determined using the dry weight method (Li & Mira, 2010). Macro-Kjeldahl method described by Black (1965) and Agbenin (1995) was used to determine total nitrogen. The Bray No. 1 method of Bray and Kurtz (1945) was used for the determination of available phosphorus. Organic matter, Sodium and potassium were determined according to the method of IITA (1979) and Agbenin (1995). The method described by Obi *et al*., (2016) was used for the determination of heavy metals.

Microbiological Analysis of Organic Wastes

One gramme (1g) 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 organic waste 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 (Ijah & Antai, 2003; Idowu & Ijah, 2017). The NA plates were incubated at 30^{o}C for 48 hours, the SDA plates

were incubated at room temperature (28^{o}C ± 2^{o}C) for 5 days, while oil agar (OA; B) plates were incubated at 30^{o}C for 5 days (for oil degrading bacteria) and oil agar (OA; F) plates at room temperature (28^{o}C ± 2^{o}C) 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 (Abioye *et al.*, 2010; Idowu & Ijah, 2017). 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 subculturing on media used for the primary isolation. The pure isolates were maintained on agar slants at 4^{o}C for further characterization and identification. All experiments were carried out in duplicates.

Utilization of Spent Lubricating Oil by Microbial Isolates

Bacterial isolates

Five millilitres (5 mL) of mineral salt medium was dispensed into bottles containing 0.05 mL of spent lubricating oil (Ijah *et al.*, 2013). The bottles were sterilized at 121°C for 15 minutes, the medium was allowed to cool and 0.1 mL of nutrient broth grown culture (1.2 × 10^3spores) of spent lubricating oil degrading bacterial isolates were inoculated and incubated at room temperature (28 ± 2°C) for 14 days. The turbidity, which developed as a result of bacterial growth was monitored visually and spectrophotometrically at the end of the incubation period and assigned +, ++ to +++, and determined the optical density (OD) respectively depending on the degree of turbidity (Abioye *et al.*, 2012). All experiments were carried out in duplicates.

Fungal isolates

The ability of the fungal isolates to grow on, and utilize spent lubricating oil, as a source of carbon and energy was determined using the dry weight method of Li and Mira (2010). This was done by inoculating 0.1 mL of fungal spores (1.2 × 10^3spores) into 10 ml of Bushnell and Haas (1941) medium (BHM) and 1% (v/v) of spent lubricating oil contained in test tubes. Control experiments were set up in test tubes containing 10ml of BHM plus 1% (v/v) of spent lubricating oil, but without added fungi. The tubes were incubated at room temperature (28± 2°C) for 21 days under a stationary condition. At the end of the incubation period, the growth of the inocula was determined by removing the growth, which was then rinsed with diethylether to remove the residual oil. It was put in crucible that has been weighed and then weighed again to note the weight of the crucible and the organism before drying. It was dried in an oven at 150°C for 1hour after which the weight of the dried cells and the crucible was determined. The weight of the cells was obtained by subtracting the weight of the crucible from the weight of the crucible plus dried cells. The extent of degradation of the incorporated spent lubricating oil by the fungal isolates was determined by the gravimetric analysis method of Odu (1972), Ijah and Antai (2003) and Idowu and Ijah (2017).

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 Baldrian *et al.* (2000) and Idowu and Ijah (2017). 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, microbial counts and total petroleum hydrocarbon (TPH) were monitored.

Determination of pH of remediated soil

The pH of soil samples was determined using pH meter (Testronic digital pH meter, Model 3505, Jenway, United Kingdom). Five grammes (5g) of the soil sample was suspended in 25ml of distilled water and mixed well. The pH meter was standardized at pH 7.0 and pH 4.0 using phosphate buffer solution after which the pH of the samples was determined in duplicates by inserting the electrode into the soil suspension and noting the reading on the screen (Obire, 2012; Olalemi & Arotupin, 2012).

Microbiological analysis of remediated soil

Serially diluted soil samples were inoculated on Nutrient agar (NA), Sabouraud dextrose agar (SDA) and Oil agar (OA) for the enumeration of aerobic heterotrophic bacteria, fungi, SLO utilizing bacteria (OA-B) and SLO utilizing fungi (OA-F) respectively (Ijah & Antai, 2003; Idowu & Ijah, 2017). The NA plates were incubated at 30^{o}C for 24 - 48 hours, SDA plates were incubated at room temperature (28^{o}C ± 2^{o}C) for 3-5 days, while oil agar (OA; B) plates were incubated at 30^{o}C for 3-5 days (for oil degrading bacteria) & oil agar (OA; F) plates at room temperature (28^{o}C ± 2^{o}C) for 5-7 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 (Abioye *et al.*, 2010; Idowu & Ijah, 2017). After incubation, district 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 subculturing on media used for the primary isolation. The pure isolates were maintained on agar slants at 4^{o}C for further characterization and identification.

Characterization and Identification of Isolates

Bacterial isolates

Bacterial isolates were characterized based on their cultural and morphological characteristics, Gram staining as well as biochemical tests such as, production of catalase, indole, oxidase, urease, citrate test and methyl red-Voges Proskauer test (Fawole & Oso, 1988). The identities of the isolates were known by comparing their characteristics with those of known taxa using Bergey's Manual of Determinative Bacteriology (George *et al.*, 2009).

Fungal isolates

Two drops of seventy per cent (70%) alcohol was placed on a clean microscope slide, cultures of filamentous fungi isolates were transferred into the drops of alcohol using sterilized inoculating needle, two drops of lactophenol cotton blue dye was added and covered with a coverslip. It was initially examined using a low power objective lens (x10) and then a higher power objective lens (x40) for more detailed examination of spores and other structures. Results obtained were characterized based on the colour of aerial and substrate mycelium, nature of hyphae, shape and kind of asexual spore, appearance and characteristics of spore head while the identification was carried out using the scheme of Domsch and Gams (1970) and Nagamani *et al.* (2006).

Determination of Total Petroleum Hydrocarbon (TPH) in remediated soil

Total petroleum hydrocarbon content of the remediated soil was determined gravimetrically by diethyl ether cold extraction. Ten grammes (10g) of soil sample were 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 optical density 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 (Yeung *et al*., 1997; Abioye *et al*., 2010).

Data Analysis

Data generated from this study were analyzed using the computer package SPSS (Version 20), (Pallant, 2005). One way analysis of Variance (ANOVA) was used to establish significant differences at $p<0.05$ in the treatments and oil biodegradation by formulated bioremediation agents.

Results and Discussion

Microbiological and physicochemical properties of organic wastes

The physical, chemical and microbial properties of organic wastes used for bioremediation studies were presented in Table 1. The pHs of organic wastes (CowD, ChiD, RicH, SorH and CorC) were 8.40, 9.30, 6.00, 6.80 and 5.80 respectively. The moisture content were 8.15%, 7.35%, 6.25%, 6.10% and 7.26% respectively, organic matter were 347.00 g/kg, 462.00 g/kg, 129.00 g/kg, 55.00 g/kg and 2.00 g/kg respectively, nitrogen were 11.48 g/kg, 10.92 g/kg, 9.66 g/kg, 12.04 g/kg and 9.80 g/kg respectively, phosphorus were 0.64 g/kg, 11.49 g/kg, 1.19 g/kg, 1.02 g/kg and 0.46 g/kg respectively, sodium were 2.72 g/kg, 4.54 g/kg, 0.57 g/kg, 0.43 g/kg and 0.33 g/kg respectively, potassium were 11.70 g/kg, 22.43 g/kg, 3.96 g/kg, 19.70 g/kg and 3.43 g/kg respectively, calcium were 18.40 g/kg, 68.80 g/kg, 4.40 g/kg, 3.20 g/kg and 0.80 g/kg respectively and magnesium were 10.20 g/kg, 15.60 g/kg, 0.70 g/kg, 1.20 g/kg and 1.90 g/kg respectively. The heavy metals in the organic wastes were, Lead; 9.08 mg/L, 0.24 mg/L, 0.33 mg/L, 0.06 mg/L, 0.00 mg/L, and 0.03 mg/L respectively, Manganese; 1.80 mg/L, 4.63 mg/L, 2.60 mg/L, 0.96 mg/L, 0.67 mg/L and 0.22 mg/L respectively, Copper; 0.17 mg/L, 0.21 mg/L, 0.23 mg/L, 0.26 mg/L, 0.24 mg/L and 0.17 mg/L respectively. The pH level of the organic wastes is generally within the range as established by Federal Environmental Protection Agency, (1991).The moisture content of organic wastes were within the range that helps microorganisms to proliferate well. The acidic pH of the organic wastes (RicH, SorH and CorC) had also been reported by Abioye *et al* (2010), and Stephen and Abioye (2013), to favour most crude oil degrading microorganisms. The counts of aerobic heterotrophic bacteria and diesel oil utilizing bacteria of the organic wastes were 4.5×10^5 cfu/g, 3.2×10^5 cfu/g, 3.0×10^5 cfu/g, 4.2×10^5 cfu/g, 3.5×10^5 cfu/g, 3.6×10^5 cfu/g, and 2.6×10^5 cfu/g, 2.2×10^5 cfu/g, 2.4×10^5 cfu/g, 3.5×10^5 cfu/g, 3.0×10^5 cfu/g, 3.2×10^5 cfu/g respectively while those of the fungi and diesel oil utilizing fungi were 5.2×10^2 cfu/g, 2.5×10^2 cfu/g, 2.6×10^2 cfu/g, 3.8×10^2 cfu/g, 3.4×10^2 cfu/g, 4.0×10^2 cfu/g and 3.5×10^2 cfu/g, 2.3×10^2 cfu/g, 2.2×10^2 cfu/g, 3.5×10^2 cfu/g , 3.0×10^2 cfu/g, 3.8×10^2 cfu/g respectively. Ekundayo and Obuekwe, (2000); Abioye *et al., (*2010); and Debojit *et al*., (2010) have recorded similar microbial counts in organic wastes and attributed it to favourable physicochemical properties of the organic wastes.

Table 1: Physical, Chemical and Microbial Properties of Organic Wastes

Parameter	CowD	ChiD	RicH	SorH	CorC
pH	8.40	9.30	6.00	6.80	5.80
Moisture content (%)	8.15	7.35	6.25	6.10	7.26
Organic Matter (g/kg)	347.00	462.00	129.00	55.00	2.00
Total Nitrogen (g/kg)	11.48	10.92	9.66	12.04	9.80
Available Phosphorus (g/kg)	0.64	11.49	1.19	1.02	0.46
Sodium (g/kg)	2.72	4.54	0.57	0.43	0.33
Potassium (g/kg)	11.70	22.43	3.96	19.70	3.43
Calcium (g/kg)	18.40	68.80	4.40	3.20	0.80
Magnesium (g/kg)	10.20	15.60	0.70	1.20	1.90
THBC (cfu/g)	3.2×10^5	3.0×10^5	4.2×10^5	3.5×10^5	3.6×10^4
SLOUBC (cfu/g)	2.2×10^5	2.4×10^5	3.5×10^5	3.0×10^5	3.2×10^4
TFC (cfu/g)	2.5×10^2	2.6×10^2	3.8×10^2	3.4×10^2	4.0×10^2
SLOUFC (cfu/g)	2.3×10^2	2.2×10^2	3.5×10^2	3.0×10^2	3.8×10^2
Lead (mg/L)	0.24	0.33	0.06	0.00	0.03
Manganase (mg/L)	4.63	2.60	0.96	0.67	0.22
Copper (mg/L)	0.21	0.23	0.26	0.24	0.17

Key: g/kg = gramme per kilogramme, % = percentage, cfu/g = colony forming units per gramme, mg/L = milligramme per litre.

Isolates from Organic Wastes

Identification of bacteria: Species of the following bacteria genera were identified in the organic wastes: *Pseudomonas, Bacillus, Salmonella, Staphylococcus* and *Escherichia*. The bacteria were mainly members of *Bacillus* (31.58%), followed by *Escherichia* (26.32%), *Salmonella* (21.05%), *Pseudomonas* (10.53%), and *Staphylococcus* (10.53%). Species of *Bacillus* were more abundant than other species; this may be due to its ability to compete well with the other organisms present in the wastes. This agrees with the findings of Ekundayo *et al*., (2012). *Pseudomonas, Salmonella, Staphylococcus* and *Escherichia* were also found in organic wastes due to their ability to utilize the wastes as source of energy (Obire, 2012; Alaa Eldin *et al*., 2017).

Identification of fungi: Species of the following fungal genera were identified in the organic wastes: *Aspergillus*, *Penicillium*, *Mucor*, *Trichophyton*, and *Fusarium.* The fungi were mainly members of *Aspergillus* (36.4%), followed by Mucor (22.7%), Penicillium (13.6%), *Fusarium* (13.6%) and Trichophyton (9.09%), in that order. Species of *Aspergillus* were more abundant than other species because they were able to grow well in organic wastes. This agrees with the findings of Nadia (2011), Alaa Eldin *et al*., 2017. Ekundayo, Olukunle and Ekundayo (2012) also reported that species of *Aspergillus* are ubiquitous in nature. *Penicillium*, *Mucor*, *Trichophyton*, and *Fusarium* were also found in the organic wastes, this may be as a result of their ability to utilize the organic wastes as source of nitrogen and phosphorous to proliferate (Obire, 2012; Idowu & Ijah, 2017).

Growth of microbial isolates on spent lubricating oil

Bacterial isolates: The utilization of spent lubricating oil (SLO) as source of carbon and energy by bacterial isolates is presented in Table 2. The nineteen microbes isolated utilized oil at varying rates. It was observed that six isolates (31.6%) utilized the SLO maximally while thirteen isolates (68.4%) utilized it moderately (Table 2). Some members of the genera *Staphylococcus*, *Bacillus, Escherichia, Pseudomonas and Salmonella* utilized the SLO at maximum rate, while other strains of the organisms utilized the SLO at moderate rate as source of carbon and energy (Table 2). This result revealed that these bacteria have the ability to utilize SLO as source of carbon and energy to grow well. Kristin *et al*. (2003); Mai,Schormann *et al*. (2004) and Al-Nasrawi, (2012), have also reported the potential of microorganisms in utilization of hydrocarbons.

Table 2: Utilization of spent lubricating oil by bacterial isolates

Coded bacterial isolates	Extent of growth in SLO medium after 14 days	Optical density (OD at 420nm)
Staphylococcus aureus SA1	++	1.025
Staphylococcus aureus SA2	+++	1.350
Bacillus subtilis BS1	+++	1.044
Bacillus subtilis BS2	++	0.470
Bacillus subtilis BS3	++	0.362
Bacillus subtilis BS4	++	0.296
Bacillus subtilis BS5	++	0.251
Bacillus subtilis BS6	++	0.196
Escherichia coli EC1	++	0.419
Escherichia coli EC2	+++	1.250
Escherichia coli EC3	++	0.312
Escherichia coli EC4	++	0.268
Escherichia coli EC5	++	0.224
Pseudomonas aeroginosa PA1	+++	1.448
Pseudomonas aeroginosa PA2	+++	1.321
*Salmonella spp.*SS1	+++	1.270
*Salmonella spp.*SS2	++	0.986
*Salmonella spp.*SS3	++	0.724
*Salmonella spp.*SS4	++	0.526

Key: ++ = Moderate growth, +++ =Maximum growth,

Fungal isolates: The utilization of SLO as carbon source by fungal isolates is presented in Table 3. Twenty-two microbes isolated utilized the oil at varying rates. It was observed that five isolates (22.8%) utilized the SLO maximally, eight isolates (31.8%) utilized it moderately while nine isolates (40.9%) utilized the SLO minimally as a source of carbon and energy. Members of the genera *Aspergillus niger*, *Aspergillus flavus, Penicillium chrysogenum* and *Fusarium venenatum* utilized the SLO at maximum rate, while *Mucor mucedo* and *Trtchophyton venenatum* utilized the SLO at moderate rate (Table 3). This result support the findings of Idowu and Ijah, 2017, which reported that *Aspergillus spp*. and *Penicillium spp*. are able to utilized diesel oil as source of carbon and energy maximally. Al-Nasrawi, (2012) and Idowu and Ijah (2018), have also reported that species of *Aspergillus* and *Penicillium* were abundant in hydrocarbon polluted soil.

Table 3: Utilization of spent lubricating oil by fungal isolates

Coded fungal isolates	Extent of growth in SLO medium after 21 days
Aspergillus niger A01	+++
Aspergillus niger A02	+++
Aspergillus niger A03	++
Aspergillus niger A04	++
Aspergillus niger A05	+
*Aspergillus flavus*B06	++
Aspergillus flavus B07	+++
Aspergillus flavus B08	+
Penicillium verrucosum C09	+++
Penicillium verrucosum C10	++
Penicillium verrucosum C11	+
Penicillium verrucosum C12	+
*Fusarium spp.*D13	+++
*Fusarium spp.*D14	++
*Fusarium spp.*D15	+
Mucor mucedo E16	+
Mucor mucedo E17	+
Mucor mucedo E18	++
Mucor mucedo E19	+
Mucor mucedo E20	++
*Trichophyton soudanense*F21	++
Trichophyton soudanense F22	+

Key: + = Minimal growth, ++ = Moderate growth, +++ = Maximum growth.

The pH of unremediated soil ranged from 6.14 - 6.31 while that of remediated soil ranged from 6.18 - 7.25, 6.22 - 6.42, 6.38 - 7.51, 6.20 - 6.56 and 6.22 - 6.45 respectively after 12weeks (Table 4). The pH of the treatments were higher compared to that of control experiments, this may be as a result of the addition of organic wastes which is capable of increasing the pH of the polluted soil (Atiku *et al.*, 2018). There were no significant differences ($P > 0.05$) among the five biostimulation treatments. The reduction in pH might be as a result of acidic metabolites produced by the microorganisms (Hidayat *et al.,* 2012). The pH of treatments were within the range that support the growth of microorganisms (Ijah *et al.*, 2013).

Table 4: pH of Remediated Soil

Time (Weeks)	SLOPS+CowD	SLOPS+CorC	SLOPS+ChiD	SLOPS+RicH	SLOPS+SorH	SLOPS Only
0	7.25 ± 0.40^{b}	6.42 ± 0.06^{d}	7.51 ± 0.10^{d}	6.52 ± 0.29^{a}	6.45 ± 0.03^{d}	6.31 ± 0.01^{d}
2	7.15 ± 0.40^{b}	6.38 ± 0.04^{d}	7.24 ± 0.04^{c}	6.49 ± 0.03^{a}	6.42 ± 0.02^{cd}	6.28 ± 0.03^{cd}
4	6.51 ± 0.38^{a}	6.32 ± 0.06^{cd}	7.16 ± 0.03^{c}	6.46 ± 0.04^{a}	6.37 ± 0.01^{bcd}	6.24 ± 0.03^{bcd}
6	6.56 ± 0.13^{a}	6.28 ± 0.04^{bc}	6.89 ± 0.04^{b}	6.42 ± 0.08^{a}	6.32 ± 0.06^{abcd}	6.20 ± 0.02^{abc}
8	6.32 ± 0.40^{a}	6.22 ± 0.04^{ab}	6.45 ± 0.03^{a}	6.36 ± 0.09^{a}	6.29 ± 0.03^{abc}	6.18 ± 0.03^{ab}
10	6.28 ± 0.03^{a}	6.17 ± 0.01^{ab}	6.40 ± 0.04^{a}	6.28 ± 0.04^{a}	6.26 ± 0.00^{ab}	6.16 ± 0.02^{ab}
12	6.18 ± 0.03^{a}	6.12 ± 0.02^{a}	6.38 ± 0.03^{a}	6.20 ± 0.05^{a}	6.22 ± 0.06^{a}	6.14 ± 0.02^{a}

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 Remediated Soil

Aerobic heterotrophic bacterial (AHB) counts: The AHB counts in spent lubricating oil polluted soil (control) decreased from 5.4 × 10^5 cfu/g - 3.5 × 10^5 cfu/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 - 7.2 × 10^5 cfu/g, 7.2 × 10^5 - 7.5 × 10^5 cfu/g, 6.6 × 10^5 cfu/g - 8.1 × 10^5 cfu/g, 6.1 × 10^5 cfu/g - 6.2 × 10^5 cfu/g and 6.4 × 10^5 cfu/g - 6.8 × 10^5 cfu/g respectively (Fig. 1). 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 to grow well (Agbor *et al.*, 2012). 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 and potassium in CowD, ChiD and SorH, which has been reported (Abioye *et al.*, 2010; Atiku *et al.*, 2018) to enhance the growth of microorganisms. 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.

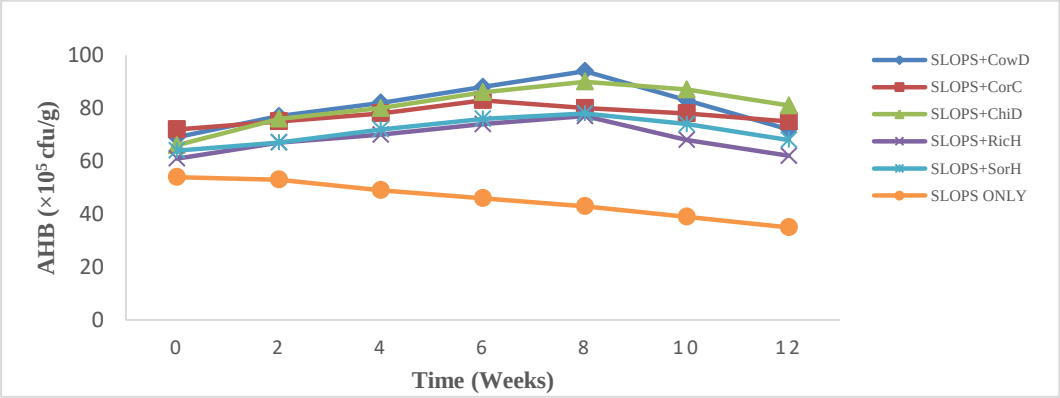

Fig. 1: Aerobic heterotrophic bacterial (AHB) counts of spent lubricating oil polluted soil remediated with organic wastes: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk.

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 - 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 - 5.2 × 10^5cfu/g, 3.7 × 10^5 cfu/g - 4.8 × 10^5 cfu/g, 4.7 × 10^5cfu/g - 5.7 × 10^5cfu/g, 3.8 × 10^5cfu/g - 4.7 × 10^5cfu/g and 4.4 × 10^5cfu/g - 5.3 × 10^5cfu/g respectively (Fig. 2). 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 microorganism in the polluted soil (Atiku *et al.*, 2018). 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 nitrogen and potassium in CowD, ChiD and SorH compared to CorC and RicH (Agbor *et al.*, 2012).

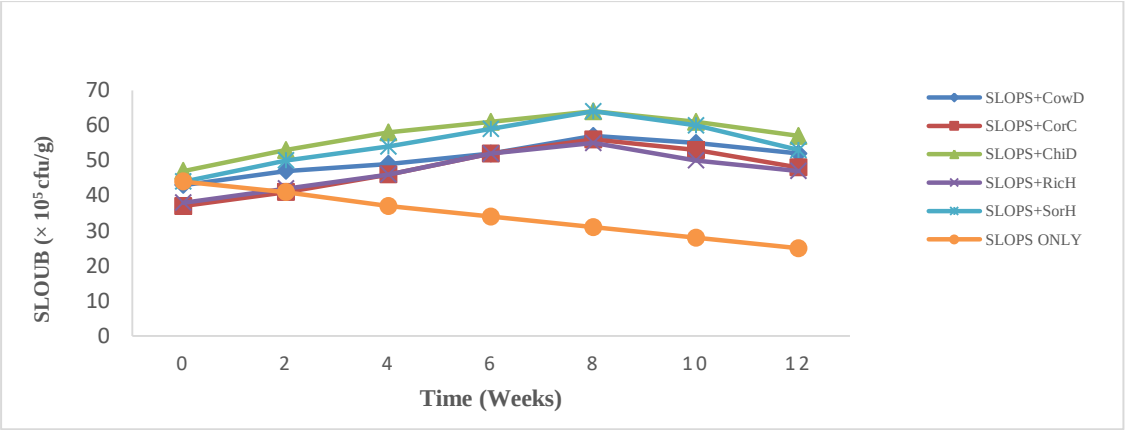

Fig. 2: Spent lubricating oil utilizing bacteria (SLOUB) counts of spent lubricating oil polluted soil remediated with organic wastes: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk

Total fungal (TF) counts

The TF counts in the control soil showed a decrease from 5.3 × 10^2cfu/g - 3.5 × 10^2cfu/g while that 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 to 6.7 × 10^2cfu/g, 6.5 × 10^2cfu/g to 8.5 × 10^2cfu/g, 6.2× 10^2cfu/g to 7.1 × 10^2cfu/g and 5.6 × 10^2cfu/g to 6.6 × 10^2cfu/g respectively (Fig. 3). The decrease in TF counts in the control experiment might be as a result of acidic nature of the polluted soil and insufficient nutrients, such as nitrogen and phosphorous which can stimulate the growth of the organisms while the increase in the TF counts in the treatments may be attributed to the presence of organic wastes which increases the pH of the soil and serves as sources of nitrogen and potassium that stimulate the growth of microbes (Agboun *et al*., 2016; Alaa El-Din *et al*., 2017).

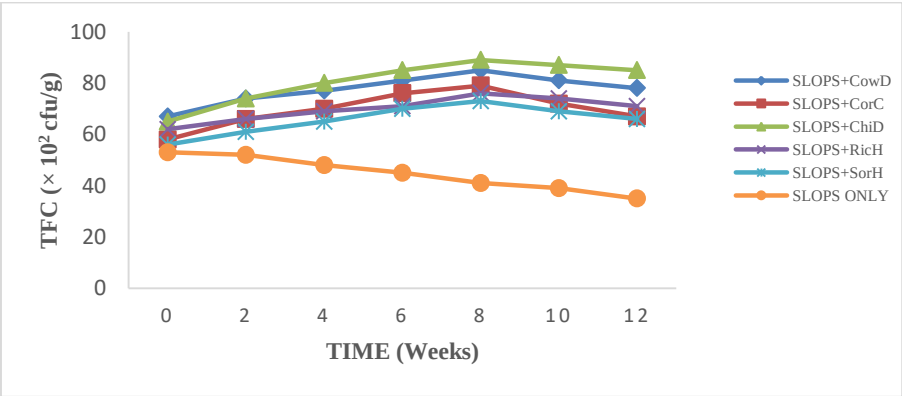

Fig. 3: Total fungi counts (TFC) of spent lubricating oil polluted soil remediated with organic wastes: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk

Spent lubricating oil utilizing fungal (SLOUF) counts

The SLOUF counts of control soil ranged from 3.9 × 10^2 cfu/g - 2.7 × 10^2 cfu/g while that of the treatments ranged from 4.9 × 10^2 cfu/g - 6.2 × 10^2 cfu/g, 4.4 × 10^2 cfu/g - 5.3 × 10^2 cfu/g, 5.8 × 10^2 cfu/g - 8.3 × 10^2 cfu/g, 5.4 × 10^2 cfu/g - 6.5 × 10^2 cfu/g and 4.9 × 10 cfu/g - 5.9 × 10 cfu/g respectively (Fig 4). 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

present in the polluted soil, due to the competition for the 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 (Agboun *et al*., 2016).

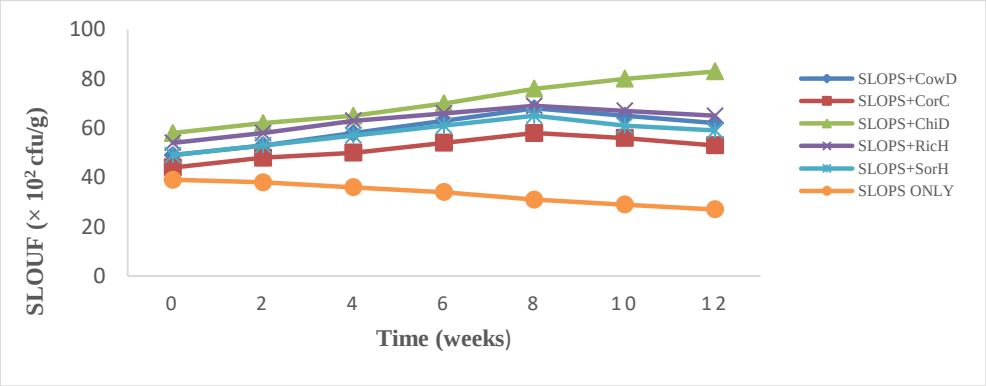

Fig. 4: Total fungi counts (TFC) of spent lubricating oil polluted soil remediated with organic wastes: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk

Identification and frequency of occurrence of microbial isolates in remediated soil Fungi

Species of the following fungal genera were identified in the soil: *Aspergillus*, *Penicillium*, *Mucor*, *Trichophyton*, and *Fusarium*. Their frequencies of occurrence were *Aspergillus* (36.4%), Mucor (22.7%), *Penicillium* (13.6%) and *Trichophyton* (9.09%), in that order (Table 5). Species of *Aspergillus* were more abundant than other species probably because of its wide spread distribution in the environment and also, for its ability to grow well in spent lubricating oil polluted soil. This agrees with the findings of Ekundayo *et al*., (2012). Similarly, Idowu and Ijah (2018) reported that species of *Aspergillus* had highest degradative capacity. *Penicillium*, *Mucor*, *Trichophyton*, and *Fusarium* were also found in spent lubricating oil polluted soil due to their ability to utilize the oil to proliferate (Obire, 2012; Idowu & Ijah, 2017).

Table 5: Frequency of occurrence of fungal isolates in bioremediated soil

Organisms	Number of Isolates	% Frequency of occurrence
Aspergillus flavus	3	13.64
Apergillus niger	5	22.73
Penicillium verrucosum	4	18.18
Trichophyton soudanense	2	9.09
Mucor mucedo	5	22.73
Fussarium venenatum	3	13.64
Total	22	100

Bacteria

Species of the following bacterial genera were identified in the soil: *Pseudomonas, Bacillus, Salmonella, Staphylococcus* and *Escherichia*. Their frequencies of occurrence (Table 6) were *Pseudomonas* (10.53%), *Bacillus* (31.58%), *Salmonella* (21.05%), *Staphylococcus* (10.53%)

and *Escherichia* (26.32%). Species of *Bacillus* were more abundant than other species; this might be as a result of its ability to form a tough protective endospore, allowing it to tolerate extreme environmental conditions. Also, because of its ability to utilized hydrocarbon as source of carbon and energy. This agrees with the findings of Ekundayo *et al*. (2012) and Atiku *et al*. (2018). *Pseudomonas, Salmonella, Staphylococcus* and *Escherichia* were also found in spent lubricating oil polluted soil due to their ability to utilize the oil to proliferate (Obire, 2012; Idowu & Ijah, 2017).

Table 6: Frequency of occurrence of bacterial isolates in bioremediated soil

Organisms	Number of Isolates	% Frequency of occurrence
Staphylococcus aureus	2	10.53
Pseudomonas aeruginosa	2	10.53
Bacillus licheniformis	6	31.58
Escherichia coli	5	26.32
Salmonella enterica	4	21.05
Total	19	100

Total Petroleum Hydrocarbon (TPH) in Remediated Soil

The residual TPH in remediated soil with organic wastes (CowD, CorC, ChiD, RicH and SorH) decreaced from 0.152g - 0.051g, 0.152g - 0.063g, 0.158g - 0.042g, 0.159g - 0.069g and 0.153g - 0.054g respectively after 12 weeks (Fig. 5). The residual TPH was high in the control soil compared to the treatments, meaning that the organic wastes added to the polluted soil enhanced the remediation soill. The residual TPH in polluted soil, remediated with CowD, ChiD and SorH was lower 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. Abioye *et al.* (2010), Alaa El-Din *et al*., (2017) and Atiku *et al*., (2018) recorded similar results.

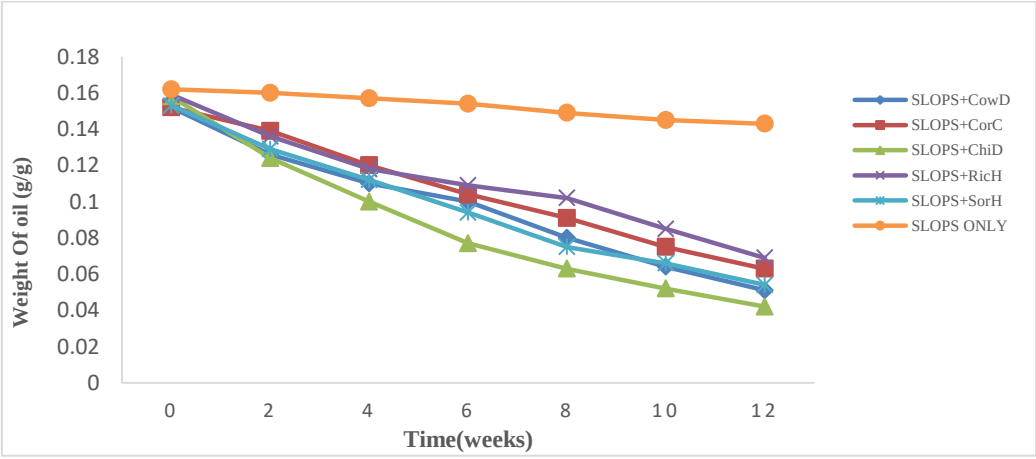

Fig. 5: Residual TPH of spent lubricating oil polluted soil remediated with organic wastes: SLOPS; spent lubricating oil polluted soil, CowD; cow dung, CorC; corn cob, ChiD; chicken droppings, RicH; rice husk, SorH; sorghum husk

Conclusion

This study screened organic wastes and microorganisms for potential to remediate spent lubricating oil polluted soil. The organic wastes harboured bacteria and fungi of various

genera, particularly *Bacillus* and *Aspergillus*. These organisms utilized spent lubricating oil at varying rates. Microbial counts in the amended oil polluted soil were higher compared to the control soil. The study also revealed that CowD, ChiD and SorH enhanced the spent lubricating oil degradation better than CorC and RicH. The residual total petroleum hydrocarbon in remediated soil showed that these organic wastes have the potential to remediate spent lubricating oil polluted soil.

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