A Comparative Study on the Bioremediation of Spent Engine Oil Contaminated Soil Using Cow Dung, Poultry Manure and Saw Dust

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ABSTRACT

The contamination of the soil with spent engine oil has become a threat on the health of the public as hazardous chemicals are released into the environment. In this study, three organic wastes (cow dung (CD), poultry droppings (PD) and saw dust (SD)) were used for bioremediation of spent engine oil contaminated soil to determine the potential of these organic wastes in enhancing biodegradation of spent engine oil in the soil. This research was undertaken in eight phases. These wastes were added to the spent engine oil contaminated soil individually and in combination (SD ONLY, CD ONLY, PD ONLY, CD+SD, PD+CD, SD+PD, CD+SD+PD) and oil contaminated soil only as control. The rates of bioremediation of the spent engine oil was studied for a period of 8 weeks under laboratory conditions. Physicochemical analysis, isolation and enumeration of bacteria and fungi were done. Characterization and identification tests of isolates revealed that a microbial consortium comprising of the following Hydrocarbon Utilizing Bacteria genera; *Bacillus subtilis*, *Pseudomonas aeriginosa*, *Proteus vulgaris*, and *Klebsiella aerogenes* Similarly, the Hydrocarbon Utilizing Fungi encountered were *Aspergillus niger*, *Aspergillus flavus* and *Mucor sp*. Hydrocarbon-utilizing bacterial and fungal counts were high in all the organic waste-amended soil when compared to unamended control soil throughout the 8 weeks of study. Spent engine oil-contaminated soil amended with a combination of cow dung and poultry manure as biostimulant showed the highest spent engine oil loss in 8 weeks compared to other treatments. These results revealed that a combination of poultry manure and cow dung was the best of the organic wastes used. The results obtained demonstrated the potential of organic wastes for oil bioremediation in the order PM+CD>PM>CD>PM+SD>SD+PM+CD >CD+SD >SD. Hence, all the biostimulants showed the ability to stimulate the growth of microorganisms for used engine oil degradation.

Keywords: Bioremediation, Spent engine oil contaminated soil, Organic wastes, Bacteria, Fungi

INTRODUCTION

Soil is the most valuable natural resource, crucial for every nation's sustainable, economic and social growth. Crude oil, a naturally occurring liquid obtained from the earth crust is one of Nigeria's abundant natural resources, and the country heavily depends on it for revenue.

The widespread usage of petroleum products and ongoing oil and gas industry activities in Nigeria have greatly harmed the environment (Aghalibe *et al*., 2017). This is as a result of leaks from damaged storage tanks, accidental spills, and inappropriate waste disposal procedures (Ofoegbu *et al.*, 2015, Allebrandt *et al.*, 2015). These oil spills leads to soil pollution (Zhang *et al.*, 2020) and it is a threat to the survival of people, animals, and also vegetation (Adeleye *et al*., 2018) as oil spill can penetrate to a depth of about 10-20cm, altering the soil physiochemical properties (Ofoegbu *et al*., 2015) and results in loss of soil fertility.

The persistence of petroleum products in the soil is dependent on a variety of factors including the chemical composition and concentration of the pollutants, the geological and physical attributes of the polluted site (environmental factors) and most importantly the presence of hydrocarbons utilizing microorganisms in the

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soil (Rahman *et al*., 2002; Amran *et al*., 2022) as it has been established that microbes completely depend on the presence of nutrients to survive.

One of these oil spills that has and continue to go unnoticed in Nigeria is that of mechanic workshop where spent engine oil (the engine oil obtained after servicing and afterwards draining from vehicles and generator engines) are released accidentally or deliberately to the environment (Stephen *et al*., 2015).

Engine oil is one of several by-products of crude oil refining (Mahmood *et al*., 2017). It is an intricate mixture of hydrocarbons, other organic compounds and some organometallic components. Engine oil lubricates automotive engine components, prevents engine corrosion, improves sealing, and cools the engine by transferring heat away from moving parts (Abioye *et al*, 2012). The most important characteristic of automotive engine oil is its viscosity. Most engine oils are made from heavier, thicker petroleum hydrocarbon base stock obtained from crude oil that have been upgraded with various additives to develop specific properties (Yerima *et al*., 2020).

Engine oil changes during use as a result of the breakdown of additives, contamination with combustion by-products, and the accumulation of metals like Lead and mercury from the engine's wear (Agarry & Ogunleye, 2012) during the course of its operation. The engine oil obtained after servicing and afterwards draining from vehicles and generator engines is termed **spent engine oil**. Spent oil is dark brown to black in colour and is harmful to the soil environment when improperly discarded. Discarded spent engine oil is a recalcitrant pollutant with accumulation of heavy metals like Lead and mercury.

The high incidence of spent engine oil spill as well as the delay in the natural recovery of spent engine oil polluted soils is a great concern to environmentalists in Nigeria and has led to an increase in research into finding ways to assist in the oil spillage bioremediation strategies as communities affected by such cases are denied access to their agricultural lands for a long time (Ebuehi *et al.*, 2005).

Bioremediation refers to the removal, destruction, or transformation of contaminants to less harmful substances in the environment or to levels below concentration limits established by regulatory authorities (Abatenh *et al*., 2017). This is a naturally occurring degradation process in which microorganisms break down complex organic molecules into harmless substances such as carbon dioxide, fatty acids and water in order to obtain energy and nutrients (Osinowo *et al*., 2020).

The main principle of bioremediation is based on biodegradation and transformation of pollutants such as hydrocarbons, heavy metal, pesticides etc. That is carried out in enzymatic way through metabolizing, (Abatenh *et al*., 2017). For successful biodegradation, microorganisms develop a catabolic activity by the following processes: new metabolic capabilities development by changes of genetic, induction of specific enzymes and eclectic enrichment of microorganisms that are capable to convert the pollutants. When conditions are favourable to the microorganisms, biodegradation of Petroleum Hydrocarbons will reach a maximum level. An important process in the absorption of Petroleum Hydrocarbons by microorganisms is the formation of an emulsion by their own production or by the help of biosurfactants (Al-Hawash *et al*., 2018).

Therefore, in order to minimize and prevent the destruction to the ecosystem, contaminated sites need to undergo a treatment process through bioremediation which can increase and maximize growth of native bacteria and biological degradation. The use of organic wastes has been reported to enhance the bioremediation of spent oil-polluted soil, hence this research is aimed at a comparative study on the bioremediation of oil contaminated soil using cow dung, poultry manure and saw dust.

MATERIALS AND METHODS

The study was carried out in Abuja. Abuja is the capital of Nigeria which lies in the central part of the

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country located between the latitude of 9.07^0N and 7.6 ^{0}E. It is surrounded by Kaduna, Kogi, Nasarawa and Niger states to the north, south, east and west respectively (Orisakwe *et al*., 2017).

The soil sample was collected from a specific location within the mechanic workshop that had heavy spillage of used engine oil after confirmation from the attendants. The sites with the oil spillage had a characteristic black colour and hardened surfaces. There were no grasses growing on the location.

Samples Collection and Preparations

The contaminated soil sample was collected from an auto mechanic workshop in Asokoro, Abuja. At this sampling point, samples were collected at a depth of 0-15 cm by digging up the soil with a hand- dug soil auger and transferred directly into clean, sterile containers which were immediately transported in cold storage containers to the laboratory for analysis. The poultry manure and Cow dung were collected from a farm in Mambilla Barracks while the Sawdust was collected from Kugbo timber market located in Abuja metropolis, FCT, Nigeria.

Large debris and unwanted particles in the collected soil sample were removed. The contaminated soil, saw dust, cow dung and poultry manure was air dried for one week in the Laboratory and ground into powdered form using mortar and pestle. The samples were passed through a 2 mm standard mesh sieve thereafter, portion of the powdered samples was analysed for the determination of its minerals content such as carbon, nitrogen, phosphorus and pH. This was carried out to ascertain the remediating properties of the organic wastes used (Ofoegbu *et al.*, 2015; Onuoha e*t al.*, 2014; Oludele *et al.*, 2019). These samples were weighed using an electronic weighing balance. Table 1 summarized the design and treatment of the contaminated soil.

Table 1: Experimental Layout for Bioremediation of Oil Contaminated Soil

Design	Treatment
A	1kg Oil Contaminated Soil (control)
B	1kg Oil Contaminated Soil + 120g Cow Dung
C	1kg Oil Contaminated Soil + 120g Saw Dust
D	1kg Oil Contaminated Soil + 120g Poultry manure
E	1kg Oil Contaminated Soil + 60g Cow Dung + 60g Saw Dust
F	1kg Oil Contaminated Soil + 60g Cow Dung+ 60g Poultry manure
G	1kg Oil Contaminated Soil + 60g Saw Dust + 60g Poultry manure
H	1kg Oil Contaminated Soil + 40g Cow Dung+ 40g Saw Dust + 40g Poultry manure

Isolation and Enumeration of Microorganisms from the Oil Contaminated Soil

The microbial counts of soil samples were determined using spread plate method on nutrient agar (NA) and Sabouraud Dextrose Agar (SDA). Total microbial (bacterial and fungal) counts was carried out in triplicates by inoculating one (1) gram of oil contaminated soil into 9ml of sterile water. The mixture was shaken thoroughly to produce a well dispersed solution and was serially diluted (ten-fold dilution) from the stock sample using sterile water. 0.1ml of each dilution was inoculated unto sterile Petri dishes with already prepared nutrient agar and Sabouraud Dextrose Agar (SDA) to culture bacteria and fungi respectively (Adams *et al.*, 2014) by means of sterile pipette (Wokem & Madufuro, 2020). All media, sterile distilled water were sterilized by autoclaving at 121^0C for 15minutes.

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The SDA plates was incubated at $28^0C \pm 2^0C$ for 72 hours while Nutrient Agar was incubated at 37^0c for 24 hours. Chloramphenicol antibiotic was added to the SDA to suppress bacterial growth on fungal plate and nystatin used on NA plates to suppress fungal growth (Ahmad *et al*., 2015; Oludele *et al*., 2019; Wokem & Madufuro, 2020). Discrete colonies that developed was counted using the formula below and expressed in colony forming unit per gram (cfu/g).

Microbial counts (cfu/g) = Number of Colonies X Dilution factor

gram of soil sample

On completion of the culture, discrete colonies on Nutrient Agar and SDA plates was purified by sub culturing several times. The pure cultures of microbial isolates were further examined (morphological and biochemical studies) and microbial strains was identified.

Isolation and Enumeration of Hydrocarbon Utilizing Microorganisms

The isolation and enumeration of hydrocarbon utilizing microbes was performed using mineral salt agar (MSA). The components of this medium are [1.8g K_2HPO_4, 4.0g NH_4Cl, 0.2g $MgSO_4 . 7H_2O$, 1.2g KH_2PO_4, 0.01g $FeSO_4 . 7H_2O$, 0.1g NaCl, 20g agar]. 1g of the samples was suspended in 9ml of sterile saline. Subsequent ten-fold dilution was made from this initial dilution. 0.1ml aliquot was inoculated on mineral salt agar plates in duplicates and using the vapour phase transfer method, the hydrocarbon was supplied by placing sterile Whatman No. 1 filter paper saturated with used engine oil. The plates were inverted over the dish covers containing 9cm Whatman No. 1 filter paper earlier impregnated with sterile used engine oil and incubated in an inverted position at 37^OC for 48 hours (Onuoha e*t al.*, 2014; Wokem & Madufuro, 2020). Nystatin was added to the bacterial plates to suppress fungal growth (Okafor *et al.*, 2016). For enumeration of Hydrocarbon Utilizing Fungi, chloramphenicol antibiotics was incorporated into the SDA medium to suppress growth of bacteria and incubated at 30^oC for 3-7 days. Discrete colonies that developed was counted and expressed in cfu/g (Adams *et al*., 2014).

Characterization and Identification of Bacteria and Fungi Isolates

The bacterial colonies from each cultured plate were characterized based on their colonial and morphological characteristics. Gram reaction and biochemical characterization (catalase, coagulase, indole, urease, MR-VP, citrate and oxidase tests) according to the methods (Cheesbrough, 2006). Fermentation of sugars was also carried out to identify pure colonies. The isolated fungal strains were identified by visual observation. The cultural as well as microscopic features according to the fungal classification based on the nature of mycelium and growth patterns using lactophenol cotton blue stain as mordant (David *et al*., 2007).

Gram staining

A clean and grease free slide was obtained and a drop of normal saline was placed at the edge of the slide. A wire loop was then flamed and allowed to cool. The 24 hour old bacteria colony was picked and mixed homogeneously with normal saline. It was allowed to air dry and then it was heat fixed using a Bunsen burner. Crystal violet (primary stain) was flooded on the slide and allowed to stain for 1 minute and then rinsed with distilled water. It was then covered with iodine for 1 minute (this act as mordant). The iodine was then decolorized with alcohol and rinsed with distilled water. The smear was then counter stain with safranin (secondary stain) for 30 seconds. Washed off with distilled water and allowed to dry and it was then observed under the microscope using x 100 oil immersion objective.

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Gram positive organism retains the dark blue/purple colour due to the iodine/crystal violet complex, while Gram negative organisms appears red maintaining the colour of the secondary dye.

Physicochemical analyses of samples

Physicochemical parameters including pH, total nitrogen, phosphorus and organic carbon contents were carried out on the soil, cow dung, saw dust and poultry manure before amendment and only on the soil sample after amendment. The soil sample analysis after amendments was carried out every two weeks throughout the study period.

Determination of pH

The pH of the oil contaminated soil and organic wastes was determined using pH meter. Ten grams of samples was weighed into a 100ml beaker and 25 ml of distilled water was added. The suspension was shaken with the aid of a mechanical shaker for 25-30 min, then allowed to stand for 50 min and stirred occasionally with a glass rod. The electrode was rinsed with water and dried by dabbing with a piece of tissue. The electrode was inserted into the partly settled suspension to be analyzed and the pH range of the solution was measured. The pH meter was standardized at pH 7.0 (Oludele *et al.*, 2019; Awari *et al.*, 2020).

Determination of Total Nitrogen

The total nitrogen for the samples was determined using an ultraviolet (UV) spectrophotometric method. Five gram (5 g) of each sample was weighed into a flask and 125 ml of distilled water was added and shaken for 10 minutes on a rotary shaker and then filtered to obtain the extract. 1 ml of the extract was transferred into 10 ml volumetric flask and 0.5ml of Brucine reagent was added. Subsequently, 2 ml of concentrated sulphuric acid was rapidly added and mixed for about 30 seconds. The flask was allowed to stand for 5 minutes. Two millilitres (2mls) of distilled water was then added and mixed for about 30 seconds. Flasks was allowed to stand in cold water for about 15 minutes. The absorbance of the samples was measured using the spectrophotometer at the wavelength of 470 nm (Awari *et al.*, 2020).

Determination of Phosphorus

The phosphate levels for the samples was determined using an ultraviolet (UV) spectrophotometer. Twenty-five millilitres (25mls) of 2.5% Acetic acid was added to 1 g of sample and shaken for 30minutes**.** The suspension was filtered through a filter paper. 5 ml of the extract was transferred into 25ml volumetric flask. The extract was diluted with distilled water until the flask was about two-thirds full. 5 ml of ammonium molybdate reagent was added and mixed with extract, 1ml of tin chloride was also added and mixed and the solution was diluted to 25 ml mark with distilled water. The flask was allowed to stand for 30minutes and the absorbance was measured at the wavelength of 690 nm (Awari *et al.*, 2020).

Determination of Organic Carbon

In a 250 ml volumetric flask, 0.5 g of sieved contaminated soil sample was added, then 10 mL of 1M $K_2Cr_2O_7$ was added and swirled gently to disperse the sample. 20 mL of concentrated H_2SO_4 was also carefully added. The solution was shaken gently and allowed to cool at room temperature. After standing for 30 minutes, 100 mL of distilled water was added, mixed and allowed to stand overnight. In addition, a blank solution was prepared but this time without soil sample to standardize the dichromate. 3 – 4 drops of indicator were added and titrated with 0.5M ferrous sulphate solution on a white background. As the endpoint is approached, the solution takes on a greenish cast and then changes to dark green. At this point, ferrous sulphate was added drop by drop until the colour changes sharply from blue to red (maroon colour) in reflected light against a white background. Samples concentrations were calculated using the following

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equation (Abdulkarim *et al*., 2019).

$$\% \text{ Organic Carbon} = \frac{(\text{Blank} - \text{Sample TV}) \times 0.003 \times f) \times 100}{\text{Weight of sample}}$$

Where: correction factor, f = 1.33

TV= Titre Value

Determination of Spent Engine Oil Loss (Biodegradation)

The amount of spent engine oil biodegradation in soil was determined by suspending 10g of oil contaminated soil in 25ml of diethyl ether in a 250ml capacity Erlenmeyer flask. The flask was shaken vigorously to extract the oil. The solvent-oil mixture was transferred slowly into a beaker of known weight. This was repeated until all the oil was extracted from the soil. The solvent – oil mixture was exposed at room temperature overnight to allow the solvent to evaporate completely. The new weight of the beaker (now containing residual oil) was recorded and the percentage of oil degraded was calculated (Ijah *et al*., 2013).

$$\text{Thus: \% biodegradation} = \frac{\text{weight of oil (control)} - \text{weight of oil (degraded)} \times 100}{\text{weight of oil (control)}}$$

RESULTS AND DISCUSSION

Results

Total Heterotrophic Bacterial and Fungal Counts in the Contaminated Soil

The total heterotrophic bacteria and fungi population counts in the amended oil contaminated soil are presented in Table 2. The combination of cow dung and poultry manure amended soil recorded the highest heterotrophic bacteria count (8.10×10^7), while the oil contaminated soil amended with saw dust only has the highest heterotrophic fungal count (6.0×10^4) compared to other treatments this could be as a result of the nutrient composition of these amendment materials.

Hydrocarbon Utilizing Bacterial and Fungal count

As shown in table 3, the highest Hydrocarbon Utilizing bacterial count was obtained from contaminated soil amended with combination of poultry manure and cow dung (3.1×10^5) while highest Hydrocarbon Utilizing fungi count was obtained from contaminated soil amended with a combination of saw dust and poultry manure (3.33×10^3).

Identification of Bacteria and Fungi Isolated from Hydrocarbon Contaminated Soil

Table 4 and 5 shows the bacteria and fungi isolated and identified from spent engine oil contaminated soil.

Physicochemical Parameters

Table 6 shows the result of the physicochemical parameters (pH, carbon, nitrogen and phosphorus) of the oil contaminated soil and the amendment materials examined during the study. In the table, all the physicochemical parameters increased on the application of the different amendment materials. There was

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significant difference in all treatment compared with the control.

The highest pH was observed in poultry manure amended soil ranged from 7.22 to 8.00 while the least pH was observed in the saw dust amended soil ranged from 6.24 to 7.02. The highest organic carbon was observed in poultry manure amended soil 18.31% while the least organic carbon was observed in the saw dust amended soil 8.81%. The organic carbon values differed from one organic manure to the other.

Nitrogen content was generally higher in all amended soil than control. The phosphorus content of saw dust amended soil lower in relative to other treatments. Cow dung amended soil has the highest in phosphorus content 38.23mg/kg.

Table 2 Total Heterotrophic Bacterial and Fungal Counts in the Amended Soil

Treatments	Time	10^7 cfu/g THBC	10^4cfu/g THFC
Oil contaminated soil (control)	Day 0	2.58 ± 0.07	2.10 ± 0.85
	Day 14	2.42 ± 0.12	1.90 ± 0.36
	Day 28	2.38 ± 0.07	1.70 ± 0.61
	Day 42	2.12 ± 0.11	1.60 ± 0.46
	Day 56	2.04 ± 0.03	1.40 ± 0.40
OCS+ cow dung	Day 0	2.10 ± 0.01	2.00 ± 0.6
	Day 14	5.46 ± 0.21	3.00 ± 0.58
	Day 28	7.64 ± 0.31	4.00 ± 0.26
	Day 42	5.14 ± 0.05	3.00 ± 0.40
	Day 56	4.08 ± 0.02	2.30 ± 0.61
OCS+ saw dust	Day 0	2.00 ± 0.02	2.67 ± 0.58
	Day 14	2.68 ± 0.11	5.00 ± 0.9
	Day 28	3.62 ± 0.08	6.00 ± 0.85
	Day 42	3.24 ± 0.01	4.67 ± 0.58
	Day 56	2.64 ± 0.04	3.33 ± 0.58
OCS+ poultry manure	Day 0	2.17 ± 0.03	2.67 ± 0.58
	Day 14	5.70 ± 0.26	4.33 ± 0.68
	Day 28	7.82 ± 0.12	5.00 ± 1.00
	Day 42	5.30 ± 0.11	4.67 ± 1.53
	Day 56	4.62 ± 0.08	2.50 ± 0.87
OCS+CD+PM	Day 0	2.22 ± 0.18	2.30 ± 0.61
	Day 14	6.40 ± 0.44	3.67 ± 0.58
	Day 28	8.10 ± 0.05	4.33 ± 0.58
	Day 42	5.60 ± 0.09	4.00 ± 1.00
	Day 56	4.76 ± 0.10	2.17 ± 0.76
OCS+CD+SD	Day 0	2.05 ± 0.03	2.00 ± 0.30
	Day 14	4.48 ± 0.09	3.00 ± 1.00
	Day 28	6.22 ± 0.06	4.00 ± 1.00
	Day 42	4.08 ± 0.02	3.00 ± 0.95

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	Day 56	3.55 ± 0.03	2.70 ± 1.47
OCS+SD+PM	Day 0	2.10 ± 0.02	2.00 ± 0.17
	Day 14	5.38 ± 0.03	3.30 ± 1.21
	Day 28	7.22 ± 0.02	4.00 ± 1.00
	Day 42	4.70 ± 0.02	3.00 ± 1.00
	Day 56	3.96 ± 0.01	2.33 ± 0.58
OCS+PM+SD+CD	Day 0	2.00 ± 0.05	2.00 ± 0.10
	Day 14	3.72 ± 0.05	3.17 ± 0.76
	Day 28	4.06 ± 0.02	4.00 ± 1.00
	Day 42	3.84 ± 0.03	2.97 ± 1.05
	Day 56	2.98 ± 0.02	2.00 ± 1.00

Values are mean of triplicate analyses ±S.D. OCS =Oil contaminated soil, PM= poultry manure, CD=Cow dung, SD=Saw dust, THBC = Total Heterotrophic Bacterial Count, THFC= Total Heterotrophic Funga l Count

Table 3 Hydrocarbon Utilizing Bacterial and Fungal Counts in the Amended Soil

Treatments		10^5 cfu/g HUBC	10^3 cfu/g HUFC
Oil contaminated soil (control)	Start 0	2.02 ± 0.06	1.00 ± 0.71
	Week 2	2.32 ± 0.03	1.10 ± 0.14
	Week 4	2.54 ± 0.03	1.20 ± 0.14
	Week 6	2.46 ± 0.07	1.10 ± 0.17
	Week 8	2.13 ± 0.17	1.00 ± 0.01
OCS+ cow dung	Start 0	2.36 ± 0.23	1.10 ± 0.07
	Week 2	2.51 ± 0.24	2.07 ± 1.28
	Week 4	2.98 ± 0.06	2.63 ± 0.78
	Week 6	2.84 ± 0.20	2.27 ± 0.71
	Week 8	2.76 ± 0.20	2.03 ± 0.64
OCS+ saw dust	Start 0	2.10 ± 0.17	1.63 ± 0.07
	Week 2	2.34 ± 0.23	2.87 ± 0.71
	Week 4	2.63 ± 0.41	3.33 ± 1.41
	Week 6	2.53 ± 0.40	2.57 ± 1.41
	Week 8	2.45 ± 0.35	2.20 ± 0.70
OCS+ poultry manure	Start 0	2.21 ± 0.08	1.50 ± 0.07
	Week 2	2.64± 0.01	2.50 ± 0.71
	Week 4	3.02 ± 0.06	2.60 ± 0.71
	Week 6	2.87 ± 0.03	2.43 ± 1.20
	Week 8	2.76 ± 0.04	2.10 ± 0.50
OCS+CD+PM	Start 0	2.30 ± 0.14	1.53 ± 0.21
	Week 2	2.86 ± 0.17	2.67 ± 0.71
	Week 4	3.10 ± 0.14	3.00 ± 0.71
	Week 6	2.88 ± 0.01	2.30 ± 0.71
	Week 8	2.80 ± 0.14	2.17 ± 0.78

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OCS+CD+SD	Start 0	2.15 ± 0.01	1.50 ± 0.07
	Week 2	2.38 ± 0.08	2.60 ± 1.56
	Week 4	2.69 ± 0.06	2.67 ± 1.49
	Week 6	2.55 ± 0.06	2.33 ± 0.71
	Week 8	2.48 ± 0.03	2.10 ± 0.99
OCS+SD+PM	Start 0	2.20 ± 0.14	1.50 ± 0.14
	Week 2	2.43 ± 0.14	2.70 ± 1.34
	Week 4	2.72 ± 0.07	3.00 ± 0.71
	Week 6	2.57 ± 0.03	2.50 ± 1.06
	Week 8	2.50 ± 0.03	2.17 ± 0.35
OCS+PM+SD+CD	Day 0	2.18 ± 0.01	1.50 ± 0.14
	Day 14	2.39 ± 0.03	2.27 ± 0.57
	Day 28	2.60 ± 0.06	2.53 ± 0.99
	Day 42	2.56 ± 0.01	2.17 ± 0.35
	Day 56	2.49 ± 0.01	2.10 ± 0.49

Values are mean of duplicate analyses ±S.D. OCS =Oil contaminated soil, PM= poultry manure, CD=Cow dung, SD=Saw dust, HUBC = Hydrocarbon Utilizing Bacterial Count, HUFC= Hydrocarbon Utilizing Fungal Count

Table 4: Cultural, Morphology and Biochemical characteristics of Bacterial Isolates

			Biochemical test								Sugar fermentation				
Cultural	Morph	G/S	Mo	In	Ca	MR	Ci	Co	Ox	UR	Gl	Su	La	Mal	Organisms
Rough, milkfish On NA	Rods	+	+	-	+	-	+	-	-	-	+	+	-	+	*Bacillus subtilis*
Green colonies on NA, brown on MAC	Rods	-	+	-	+	-	+	-	+	-	-	-	-	-	*Pseudomonas aeruginosa*
Mucoid and greyish -white on NA, pink-red on MAC	Rods	-	-	-	+	-	+	-	-	+	+	+	+	+	*Klebsiella aerogenes*
Mucoid and brownish On NA	Rods	-	+	-	+	+	-	-	-	+	+	-	-	-	*Proteus vulgaris*

Table 5: Cultural and Microscopic Identification of hydrocarbon utilizing Fungal Isolates

Cultural characteristics	Microscopy	Tentative Identification
Brownish black mycelia were growing on the plate	Smooth conidospores from long broad thick smooth wall	Aspergillus niger
Army green mycelia growth was seen in colonies on growth media	Rough conidiophores upright, bearing phialides, at the apex was observed.	Aspergillus flavus
Gray colonies seen on the surface growing at a rapid speed	Non septate broad hyphae branched sporangiophores were seen	Mucor sp

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Table 6: Physicochemical Parameters of amended soil sample.

Parameters	Oil Contaminated Soil	OCS+ Cow	OCS+ Saw Dust	OCS+ Poultry manure	OCS+ Cow Dung +Saw Dust	OCS+ Poultry manure +Saw Dust	OCS+ Cow Dung +Poultry manure	OCS+ Cow Dung +Poultry manure +Saw Dust
Changes in pH								
Start 0	6.02	7.14	6.24	7.22	6.31	6.58	7.2	6.24
Week 2	6.01	7.43	6.45	7.69	6.59	7.17	7.55	6.44
Week 4	6	7.66	7.02	8	7.1	7.22	7.93	7.04
Week 6	6	7.57	7	7.91	7.08	7.2	7.84	7.01
Week 8	5.98	7.53	6.57	7.87	7.06	7.19	7.77	6.59
Changes in Nitrogen(%)								
Day 0	0.14	0.24	0.1	0.26	0.22	0.22	0.28	0.2
Day 14	0.12	0.46	0.17	0.58	0.41	0.41	0.62	0.4
Day 28	0.11	0.55	0.15	0.7	0.55	0.57	0.78	0.56
Day 42	0.11	0.55	0.12	0.65	0.52	0.55	0.73	0.51
Day 56	0.1	0.52	0.11	0.63	0.5	0.51	0.7	0.48
Changes in phosphorus(mg/kg)								
Day 0	8	26.47	12.67	32.7	16	20.01	28.02	21.33
Day 14	7.82	37.89	12.79	34.43	16.86	20.46	28.88	21.59
Day 28	7.69	38.23	13.09	35.21	16.58	20.75	29.19	21.79
Day 42	7.57	38.15	13.01	35.19	16.43	20.69	29.09	21.72
Day 56	7.49	38.05	12.91	35	16.33	20.67	28.97	21.7
Changes in OrganicCarbon (%)								
Day 0	7.92	14.55	9.45	19.28	9.6	11.47	16.64	9.66
Day 14	7.02	14	9.07	18.76	9.15	11.13	16.05	9.19
Day 28	6.5	13.77	8.81	18.31	8.86	10.59	15.74	8.9
Day 42	6.32	13.53	8.59	18.09	8.66	10.46	15.56	8.67
Day 56	6.17	13.48	8.45	17.97	8.54	10.33	15.37	8.57

Results are presented in Mean. OCS= Oil contaminated soil, PM= poultry manure, CD=Cow dung, SD=Saw dust.

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Percentage (%) of Spent Engine Oil Loss

The percentage loss of spent engine oil in both contaminated and amended soil during the bioremediation period is shown in Figure 7. The results showed that application of amendment materials reduced the spent engine oil content of the contaminated soil. From the results, it was also revealed that percentage of spent engine oil loss was highest in soil amended with a combination of poultry manure and cow dung (85.53±0.28%) while the least was that of soil amended with sawdust (30.31±0.27%) at the end of week 8.

Table 7 Percentage (%) Spent Engine Oil Loss in Soil Amended with Organic Wastes during the Bioremediation Period

Soil Treatment	2 weeks	4 weeks	6 weeks	8 weeks
OCS+PM	41.01±0.71	55.95±0.14	64.63±0.4	69.07±0.07
OCS+SD+PM	28.19±0.13	40.47±0.34	47.23±0.34	50.18±0.03
OCS	6.13±0.10	7.75±0.28	8.11±0.14	8.97±0.07
OCS+SD	6.27±0.11	25.05±0.07	28.67±0.08	30.31±0.27
OCS+CD+PM	50.28±0.18			
OCS+CD+SD	20.71±0.41	31.05±0.25	35.67±0.14	38.31±0.49
OCS+ CD	35.88±0.17	52.21±0.21	59.65±0.04	63.41±0.24
OCS+SD+CD+PM	22.47±0.34	33.08±0.14	37.72±0.61	40.07±0.51

Values are mean of duplicate analyses ±S.D. OCS= Oil contaminated soil, PM= poultry manure, CD=Cow dung, SD=Saw dust.

DISCUSSION

From the data in table 2, the microbial population increased with time in amended samples compared to the control. This increase in the heterotrophic and hydrocarbon-utilizing microbial counts recorded may be due to the microbial utilization of the hydrocarbon in the spent engine oil as the source of carbon and energy. Similar results were reported by other researchers who observed counts of heterotrophic and hydrocarbon utilizers in oil-polluted soil to be higher than in uncontaminated soil samples (Ijah *et al.*, 2013; Abdulkarim *et al*., 2019).

The results also showed that soil amended with a combination of cow dung and poultry manure had highest total heterotrophic bacteria 8.10 ±0.05 x 10^7 cfu/g and Hydrocarbon utilizing bacteria (HUB) 3.10 ±0.14 x10 ^{4} cfu/g population while the soil amended with only Saw dust had the highest total heterotrophic fungi 6.00 ±0.85 x 10^4 cfu/g and Hydrocarbon utilizing fungi (HUF) population 3.33 ±1.41 x 10^3 cfu/g. This could be attributed to difference in nutrient composition of cow dung, poultry manure and sawdust. Cow dung is known to contain more organic nutrients as a result of the presence of degradative bacteria in rumen of cows. Poultry manure has a high nutrient content thereby having more organic nutrients supporting increase in microbial growth. The highest fungal counts observed in the saw dust could be due to high cellulose content of saw dust being product of plant (Wokem & Madufuro, 2020). The microbial populations during the bioremediation period increased from 0 to 4 weeks and decreased from 6 to 8 weeks. This may be attributed to decline in the availability of nutrients for microbial utilization as reported by Onuoha *et al*., 2014.

Table 6 showed that the physicochemical parameters (Nitrogen, pH, Phosphorus and Carbon) of the spent engine oil contaminated soil increased after the application of amendment materials (cow dung, saw dust

and poultry manure), this could be as a result of the degradation activities of the spent oil by the microorganisms (bacteria and fungi). The pH of the spent oil contaminated soil was 6.02 (acidic), this is supported by the study of Muhammad *et al.*, (2019) that the presence of TPH have the ability to release hydrogen ions. The soil pH increased in the amendment samples, this is in support of the findings of (Obasi *et al*., 2013) that addition of agricultural wastes to the engine oil polluted soils raises the soil pH. This pH value was obtained as a result of the alkaline nature of the amendment materials used. This condition of alkalinity is responsible for the high counts of total heterotrophic and hydrocarbon-utilizing bacteria, this is supported by the reports of (Williams & Ameachi, 2017) that the survival of most microbial species is dependent on a certain pH range, soil pH is essential. The nitrogen and phosphorus contents was higher in the amended soil compared to the contaminated soil. This may be due to the presence of high nitrogenous and phosphate compounds in the amendment materials. A similar result was reported by (Obasi *et al*., 2013) and (Adams *et al*., 2014) who worked on organic manure. This result also agrees with the findings of (Osazee *et al*., 2019) who reported that the addition of organic nutrient elevated the nutrient concentration in phosphorus, potassium and nitrogen meaning a positive effect on nutrient concentration. The organic carbon contents were higher in the amended soil samples than control but there was a reduction in organic carbon concentration in all soil samples. This was because carbon was utilized by the microorganisms as nutrients for their growth and also required for metabolic activities. Findings in this study agrees with Ideriah *et al*., (2018).

After characterization, a total of four bacterial genera and three fungi species were isolated from the hydrocarbon contaminated soil. The bacteria isolated and identified were *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella aerogenes* and *Proteus vulgaris*. These bacteria species have earlier been reported as hydrocarbon degraders by the authors (Abioye *et al*., 2012); (Onuoha *et al.*, 2014); (Osinowo *et al*., 2020); (Wokem & Madufuro, 2020). On the other hand, the HUF isolates obtained in this study belonged to the *genera Aspergillus niger, Aspergillus flavus,* and *Mucor sp*. These genera have also been reported by other researchers (Wokem & Madufuro, 2020).

The low percentage of engine oil loss observed in the unamended soil showed the possibility of natural degradation by indigenous Hydrocarbon Utilizing bacteria and Hydrocarbon Utilizing fungi in the soil which occurs rather slowly. The combination of poultry manure and cow dung showed the highest percentage spent engine oil loss 85.53±0.28% while the least was that of soil amended with sawdust (30.31±0.27%) at the end of week 8. The percentage of spent engine oil loss was enhanced by addition of amendment materials (cow dung, poultry manure and saw dust) as reported by other researchers (Abdulkarim *et al*., 2019; Wokem & Madufuro, 2020).

As observed in this study, the addition of organic wastes enhances the availability of nutrients, which positively impacts on the population of the indigenous microorganisms that aid in utilization of hydrocarbon in our environment.

CONCLUSION

This study showed that the application of a combination of poultry manure and cow dung as amendment materials had the highest percentage (%) bioremediation efficiency than other amendments.

It was also observed that the microbial biomass and the physiochemical parameters analysis revealed an increase as bioremediation increases with time after the application of amendment materials.

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