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Bioremediation of Spent Engine Oil Polluted Soil Enhanced with Blends of Poultry Manure and Pig Dung

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The disposal of spent oil into open vacant plots and farms, gutters and water drains is an environmental risk. This study was conducted to explore the bioremediation of spent engine oil polluted soil enhanced with blends of poultry manure and pig dung, as well as its effects on

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microbiological composition of the soil. Top soil (0-15 cm depth) samples were collected from areas with history of spent engine oil contamination within Anyigba, Kogi State, Nigeria. One kilogram of soil was measured into nine clean dry containers of three litres each. The prepared blends of poultry manure and pig dung (PMPD) was mixed with the soil samples at the rate of 0, 50 and 100 g kg^{-1} soil in triplicates. The experiment used a Completely Randomized Design. Soil samples were taken from each container at 0 and 28 days for hydrocarbon utilizing bacteria and total petroleum hydrocarbon determination using standard methods. Data obtained from the experiment were subjected to descriptive and inferential statistics. The species identified were *Enterobacter sp* and *Pseudomonas aeuginosa. Enterobacter sp* was the most predominant isolate. The total petroleum hydrocarbon ($mgkg^{-1}$) of the soil on day 0 was 59.78±1.84. After the amendments (at control, 50 and 100 g kg^{-1}), the total petroleum hydrocarbon ($mgkg^{-1}$) values were 44.92 ±2.26, 35.54 ± 2.78 and 29.52±1.28 at 28 days respectively. The blends of PMPD and the high level carbon utilizing bacteria, *Enterobacter sp,* showed promising potentials in the bioremediation of spent engine oil polluted soil by significantly enhancing the biodegradation process, as an impressive 50.62% remediation efficiency was achieved 28 days after amendment in soil treated with 100g of PMPD blend.

Keywords: Bioremediation; poultry manure; pig dung; hydrocarbon-degrading bacteria; total petroleum hydrocarbon.

1. INTRODUCTION

"Several reports on the effects of petroleum products on living organisms have focused on crude oil, diesel and gasoline" [1,2] which get to the environment through accidental spillage. However, "through the activities of automobile, generator, other machines, and servicing engineers (mechanics) spent oil is released to the environment promiscuously" [3].

"Spent engine oil refers to used motor oil collected from mechanical or automobile, workshops, garages, and industry sources like hydraulics oil, turbine oils, process oil and metal working fluids" [4]. Spent oil consists of different chemicals [5]. "Spent oil contains polycyclic aromatic hydrocarbons (PAHs) and chemical additives like lead (Pb), zinc (Zn), sulphur (S), phosphorus (P), magnesium (Mg), iron (Fe), nickel (Ni), phenols, amines and benzenes" [6]. "The concentration of PAHs in spent oil increases with time of usage" [7].

"Spent oil is usually obtained after servicing and subsequent draining from automobile and generatorengines" [8]. "Spent oil is a common and toxic environmental contaminant not naturally found in the environment" [9]. It gets to the environment due to discharge by motor and generator mechanics [10] and from the exhaust system during engine use and due to engine leaks [11,12].

"The disposal of spent oil into open vacant plots and farms, gutters and water drains is an environmental risk" [10]. "Since spent oil is liquid, it easily migrates into the environment and eventually pollutes either water or soil" [4]. Unfortunately, this contaminant becomes difficult to remediate completely from the soil. Several studies have been conducted on the use of poultry manure, as well as pig dung for the removal of oil contaminant from soils [13,14,15,16,17]. These studies reported the effectiveness of organic matters of animal origin. Therefore, objectives of this study aimed to determine the effects of blends of poultry manure and pig dung on total petroleum hydrocarbon (TPH), TPH degrading bacteria count and identification, and soil physical and chemical properties.

2. MATERIALS AND METHODS

2.1 Study Area

The research was conducted at the Department of Animal and Environmental Biology of Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria. The laboratory within the department was used to provide a suitable setting for the study under standard and controlled conditions.

2.2 Samples Collection and Preparation

Top soil (0-15 cm depth) samples were randomly collected in triplicates [18] from areas with history of spent engine oil contamination within Anyigba, Kogi State, Nigeria, using a soil auger. As described by [14,19], "the soil samples were air dried in clean, well ventilated laboratory, homogenized by crushing and sieved by passing through a 2 mm mesh sieve. One kilogram of soil

was measured into nine clean dry containers of three litres each. The poultry manure and pig dung samples were collected from Prince Abubakar Audu University Teaching and Research Farm, Anyigba, Kogi State, Nigeria. These samples were air dried, pulverized, mixed, sieved with a 2 mm sieve and stored in polythene bag for use, as described" [14,19].

The prepared blend of poultry manure and pig dung (PMPD) was added at the rate of 0 (control), 50 and 100 g kg^{-1} soil in triplicates. The PMPD was thoroughly mixed with the soil and the nine containers were arranged in a Completely Randomized Design in the laboratory. Soil samples were taken from each container at 0 (zero day refers to the day PMPD was added to the soil) and 28 days for pH, organic carbon, nitrogen, phosphorus, potassium, hydrocarbon degrading bacteria count, hydrocarbon utilizing bacteria and total petroleum hydrocarbon determination.

2.3 Laboratory Analysis

Soil Chemical Properties: The pH, organic carbon, total nitrogen, potassium and available phosphorus were determined in the soil samples using the methods described in [20].

Cultural Characterization of Bacteria: "Pure cultures of representative bacteria colonies were randomly picked from inoculated plates and were grouped on the basis of their colonial characteristics such as colony elevation, colour, size, opacity, shape, consistency, and edge" [21].

Morphological Characterization of bacteria: "Cultural grouping was followed by microscopic examination of isolates for cellular morphology. Day-old cultures of the bacteria isolates were stained with cotton blue lacto-phenol blue and observed microscopically for cell shape, size and sporulation" [21].

Biochemical Characterization of Bacteria: A modified method of [22] was used for Gram staining, catalase test, urease test, citrate utilization test, indole test, motility test, coagulase test and sugar fermentation test [23].

Determination of Total Hydrocarbon Utilizing Bacteria Count: Total hydrocarbon utilizing bacteria count was carried out on mineral salt medium (MSM) agar as described by [24]; and the isolated microorganisms were identified using Bergey's manual of systemic bacteriology [25].

Determination of Total Petroleum Hydrocarbon: "Ten grams of the petroleum products-polluted soil sample was weighed into a clean bottle and 25ml of dichloromethane was added, the mixture was allowed to stand on a mechanical shaker for a period of 3- 4 hours. The procedure was repeated twice and the aliquots were collected and mixed together in a beaker. The aliquots were concentrated on a steam bath reducing the extracts to about 5 ml. The concentrate was passed through a pipette packed with anhydrous sodium sulphate on top of a glass wool to remove moisture and other impurities. The final extract was analyzed using a Hewlett-Packard 5890 series GC system coupled to a mass spectrophotometer(VG TRIO 2000) to determine the quantity of total petroleum hydrocarbons" [22].

The concentration degraded and percentage degradation was calculated using equation 1 and 2 respectively as described by [26,27].

Concentration degraded = C1 – C2 Equation (1)

(%) Biodegradation = $\frac{C1-C2}{C1} \times 100$ - Equation (2)

2.4 Data Analysis

Data obtained were subjected to descriptive (mean and standard deviation) and inferential (ANOVA) statistics. Means were separated using Duncan Multiple Range Test (DMRT).

3. RESULTS AND DISCUSSION

Effect of Blends of Poultry Manure and Pig Dung on the Soil Chemical Properties: In this study, attempt was made to assess the efficacy of PMPD on total petroleum hydrocarbon degradation, soil chemical and microbial properties of spent engine oil contaminated soil. The effect of PMPD on the soil chemical properties is shown in Table1. A significant ($p<0.05$) increase from 6.90± 0.10 to 7.30± 0.10 and 6.90±0.10 to 7.20±0.10 in pH was observed in contaminated soil with 50g and 100g of PMPD between days 0 and 28 days respectively, as compared with the control (0g) which decreased from6.90±0.10 to 6.80±0.10. The total value of Nitrogen (gkg^{-1}) in soil containing 0g, 50g and 100g of PMPD respectively, on day 0 was 6.90±0.10. However, there was a significant decrease in total Nitrogen on day 28 in soil amended with 100g with a value of 1.21±0.01 respectively. Although no significant decrease was obtained in soils amended with 0g and 50g

of PMPD with a value of 1.13±0.02 on day 28. The total Phosphorus (Mgkg^{-1}) was observed to have decreased from 1.6×10^{2}±2.87 to 1.12×10^{2}±2.16 and 1.6×10^{2}±2.67 to 1.37×10^{2}±1.25 at 28 days in soil amended with 50g and 100g of PMPD respectively, indicating a downward trend. There was a significant decrease from 1.24±0.30 to 0.41±0.20 of total potassium (Colkg^{-1}) in soil treated with 50g of PMPD on day 28. However, level of potassium significantly increased on day 28 from 1.24±0.30 to 2.24±0.02in soil amended with control 0g of PMPD, respectively. Levels of organic carbon significantly decreased in soil treated with 0g and 50g of PMPD, from 65.48±2.90 to 48.29±2.97and 65.48±2.90 to 54.76±0.73 on days 0 and 28 respectively. But there was a non-significant increase from 65.48±2.90 to 67.37±0.94 on day 28 in soil amended with 100g of PMPD.

Effects of Blends of Poultry Manure and Pig Dung on Total Petroleum Hydrocarbon (TPH) of Spent Engine Oil Polluted Soil: The result for the effect of blends of poultry manure and pig dung (PMPD) on TPH of spent engine oil polluted soil is shown in Table 2. The PMPD treatment significantly remediated the TPH in the polluted soil. The lowest level of TPH after amendment was recorded on day 28 in soil treated with 100g of PMPD, reducing the TPH from 59.78±1.84 to 29.52±1.27 at days 28 indicating that an impressive 30.26 (50.62%) of TPH was degraded. A total of 24.24 (40.54%) of degraded TPH was recorded in soil treated with 50g of PMPD, decreasing TPH from 59.78±1.84 to 35.54±2.78 at 28 days. The least percentage of degraded TPH of about 14.86 (24.86%) was observed and recorded for the control at 28 days.

Effect of Blends of Poultry Manure and Pig Dung on Hydrocarbon Degrading Bacteria (THDB) Counts and Identification: A 100g of PMPD treatment of spent engine oil polluted soil significantly decreased THDB count from 2.13×10^{4}±3.51×10 to 1.47×10^{4}±2.51×10 on days 0 and 28 respectively (Table 3). Significantly ($p<0.05$) increased THDB count was observed in the control (0g) from 2.13×10^{4}±3.51×10 to 9.17×10^{4}±7.37×10 on days 0 and 28 respectively.. However, there was a recorded significant decrease from 2.13×10^{4}±3.51×10 to 1.28×10^{4}±2.51×10 on days 0 and 28 in THDB count for soil amended with 50g of PMPD.

The morphological characteristics of bacteria isolated from the polluted soil amended with PMPD at 28 days are presented in Table 4. The size of the bacteria ranged between 1-5mm. The bacterial isolates had irregular, round and smooth shapes, grey-white, green and yellow colours, dry and wet consistencies, rough and smooth edges, flat, raised and slightly raised elevations and were all opaque.

Table 1. Effects of Blends of Poultry Manure and Pig Dung Application on the Soil Chemical Properties

PMPD (g)	Days	pH	Nitrogen (gkg^{-1})	Phosphorus (Mgkg^{-1})	Potassium (Colkg^{-1})	Organic Carbon (gkg^{-1})	Moisture Content(gkg^{-1})
0	0	6.90±0.10^{a}	1.33±0.08^{c}	1.6×10^{2}±2.87^{b}	1.24±0.30^{b}	65.48±2.90^{c}	2.00±0.50^{a}
	28	6.80±0.10^{a}	1.13±0.02^{a}	1.25×10^{2}±2.67^{a}	2.24±0.20^{c}	48.29±2.97^{a}	1.65±0.04^{a}
50	0	6.90±0.10^{a}	1.33±0.08^{c}	1.6×10^{2}±2.87^{b}	1.24±0.30^{b}	65.48±2.90^{c}	2.00±0.50^{a}
	28	7.30±0.10^{b}	1.13±0.02^{a}	1.12×10^{2}±2.16^{a}	0.41±0.20^{a}	54.76±0.73^{b}	1.79±0.10^{a}
100	0	6.90±0.10^{a}	1.33±0.08^{c}	1.6×10^{2}±2.87^{b}	1.24±0.30^{b}	65.48±2.90^{c}	2.00±0.50^{a}
	28	7.20±0.10^{b}	1.21±0.01^{b}	1.37×10^{2}±1.27^{a}	1.31±0.10^{b}	67.37±0.94^{c}	1.90±0.05^{a}

Values are mean ± SD of three replicates. Different superscripts in the same column indicate significant difference at p< 0.05 (DMRT).
PMPD- Poultry Manure and Pig Dung

Table 2. Effects of Blends of Poultry Manure and Pig Dung on Total Petroleum Hydrocarbon (TPH) of Spent Engine Oil Polluted Soil

PMPD(g)	Days	TPH(mgkg^{-1})	TPH Degraded	Degradation %
0	0	59.78±1.84^{d}	14.86	24.86
	28	44.92±2.26^{c}		
50	0	59.78±1.84^{d}	24.24	40.54
	28	35.54±2.78^{b}		
100	0	59.78±1.84^{d}	30.26	50.62
	28	29.52±1.28^{a}		

Values are mean ± SD of three replicates. Different superscripts in the same column indicate significant difference at p<0.05 (DMRT).
PMPD- Poultry Manure and Pig Dung.

Table 3. Effects of Blends of Poultry Manure and Pig Dung on Hydrocarbon Degrading Bacteria (THDB) Counts and Identification

PMPD(g)	Days	THDB (CFU g^{-1})
0	0	$2.13\times10^{4}\pm3.51\times10^{2d}$
	28	$9.17\times10^{4}\pm7.37\times10^{2a}$
50	0	$2.13\times10^{4}\pm3.51\times10^{2d}$
	28	$1.28\times10^{4}\pm2.51\times10^{2b}$
100	0	$2.13\times10^{4}\pm3.51\times10^{2d}$
	28	$1.47\times10^{4}\pm2.51\times10^{2c}$

Values are mean ± SD of three replicates. Different superscripts in the same column indicate significant difference at p<0.05 (DMRT).
PMPD- Poultry Manure and Pig Dung.

Table 4. Morphological Characteristics of Bacteria Isolated from the Spent Engine Oil Polluted soil Amended with Blends of Poultry Manure and Pig Dung at 28 Days

Isolate Code	Size (mm)	Shape	Colour	Consistency	Edges	Elevation	Opacity
CTRL (0g)	3-5	Irregular	Grey-white	Dry	Rough	Flat	Opaque
PMPD(50g)	3-4	Round	Green	Wet	Smooth	Raised	Opaque
PMPD(100g)	1-2	Smooth	Yellow	Wet	Smooth	Slightly Raised	Opaque

CTRL – Control
PMPD – Poultry Manure and Pig Dung.

Table 5. Types and Relative Abundance of Micro-organisms in soil

Isolate Code	GR	SP	CP	CA	CO	MO	IN	OX	CI	UR	MR	VP	G	I	M	Probable Organsm
CTRL 0g	GNB	-	-	+	-	+	-	-	+	-	+	-	A	A	-	*Enterobactersp*
PMPD 50g	GNB	-	-	+	-	+	-	+	+	-	-	+	-	-	-	*Pseudomonas aeuginosa*
PMPD 100g	GNB	-	-	+	-	+	-	-	+	-	+	-	A	A	-	*Escherichiacoli*

Keys: *GR-Gram staining, SP- Spore staining, CA- Capsule staining, CT- Catalase, MO-Motility, IN- Indole, OX- Oxidase,CI-Citrate, UR- Urea, MR- Methyl-red, VP- Vogesproskeur, G- Glucose, L- lactose, S- Sucrose, M- Mannitol, A-Acid production, g = Gram, - =Absent, + = Present, A = Abundant, CTRL – Control; PMPD– Poultry Manure and Pig Dung.*

The types and relative abundance of microbial communities in microcosms due to natural attenuation and biostimulation treatment methods recorded in the contaminated soil are shown in Table 5. Two hydrocarbon utilizing bacteria were identified from the spent engine oil contaminated soil. The hydrocarbon utilizing bacteria identified belong to the genera *Enterobacter* and *Pseudomonas*. *Enterobacter* species were the most predominantly isolated bacterial species across the treatments.

Effect of Blends of Poultry Manure and Pig Dung on the Soil Chemical Properties: "The increase in pH as biodegradation progressed recorded in this study was connected to the biodegradation process which removed the contaminant and introduced some substances or ions from the naturally alkaline PMPD" [28]. "Soil pH range between 6.9 and 7.5, as recorded in this study, and an earlier study" [14,19], is good for most hydrocarbon utilizing bacteria [29,30]. Watkinson and Morgan [31] reported that chicken manure increases soil pH.

"The decrease in soil N, P, K, organic carbon and moisture content from 0 to 28 days at every PMPD level might be due to their high demand by microorganisms for sugar phosphorylation, nucleic acid synthesis and other cellular processes" [32,33,34]. "It has also been reported that petroleum hydrocarbon contaminants could destroy inorganic nutrient sources by reacting withthem along with other substances present in soil" [35,32].

Effect of Blends of Poultry Manure and Pig Dung on Total Petroleum Hydrocarbon (TPH) of Spent Engine Oil Polluted Soil: Degradation of TPH in the contaminated soil amended with blends of poultry manure and pig dung (PMPD) might be due to the bacterial consortiums in the PMPD that attacked and degraded the components of the hydrocarbon [30,36].Andrew and Jackson [37] in their study of *in situ* bioremediation techniques reported that it is possible to degrade up to 90 % of hydrocarbon pollutant, during biostimulation. Egbeja et al. [14] reported in their study of microbial degradation,

that higher significant concentration of total petroleum hydrocarbon was recorded in the soil without pig dung applications compared with the least significant ($p < 0.05$) value observed in 100 g pig dung kg^{-1} soil.

Effect of Blends of Poultry Manure and Pig Dung on Hydrocarbon Degrading Bacteria (THDB) Counts and Identification: The decrease in population of total hydrocarbon degrading bacteria from 0 and 28 days in 50 and 100g PMPD kg^{-1} soil recorded in this study might be due to the fact that mineralization of hydrocarbons could have possibly resulted in the production of toxic metabolites which on introduction into the system reduces the growth phase of the microbes [28], when compared to the highly significant increase in THDB count in the control. This is not in consistency with the report of [38], who noted that lower diesel-utilizing bacterial counts in the unamended sample could be as a result of depletion of limiting nutrients. Bada et al. [18] and Egbejaet al. [14] also reported a decline in bacterial population as the biodegradation progressed. Margesinand Schinner [39] reported that "fluctuations in counts of microorganisms might be caused by certain mutual interactions of microorganisms in soil contaminated with spentmotor engine oil".

In this study, "*Enterobacter* species were the most predominantly isolated bacterial species. Its prevalence could be attributed to the fact that it was able to utilize hydrocarbons as the sole source of carbon luxuriantly and has the ability to withstand toxic conditions" [34]. The bacteria had earlier been reported by [40], as efficient hydrocarbon degraders. Generally, soils receiving micro-seepage of hydrocarbon tend to be dominated by bacteria utilizing hydrocarbon in a large number as reported by [41,33]. In the study by [14,17], bacterial isolates of the genera *Enterobacter* and *Pseudomonas* were also reported. Thus, indicating their efficient potentials in the biodegradation of complex hydrocarbons.

The study by [34] revealed that "*Enterobacterssp*IAA-01 caused 78.0% oil degradation after 21days". The efficient ability of the bacteria in degrading hydrocarbon has been reported by other investigators [42,43]. The results of GC analysis further confirmed the biodegradation potential of *Enterobacter* sp. IAA-01 and revealed that the bacteria is capable of utilizing most of the hydrocarbon components especially straight chain alkanes, and cyclo-alkanes [34]. According to [44], the generally accepted pattern of susceptibility of hydrocarbon components to microbial degradation is n-alkane > branched alkanes > low- molecular weight aromatics >polycyclics.

4. CONCLUSION

The results obtained in this study of bioremediation of spent engine oil polluted soil enhanced with blends of poultry manure and pig dung (PMPD), showed the promising potentials of PMPD and the high- level hydrocarbon utilizing bacterial isolate, *Enterobacter sp* in the biodegradation of spent engine oil. An impressive bioremediation efficiency of 50.62% was archived in soil amended with 100g of PMPD blend after 28 days. It is recommended that further studies be carried out to investigate the combined biodegradation efficacy of PMPD blend and *Enterobacter sp*in the biodegradation of spent engine oil over an extended period of time than in this study.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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