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Biostimulation of Used Engine Lubricant Polluted Soil Amended with 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 environment is polluted by hydrocarbons when used motor oils are spilled. Studies were carried out to investigate the bioremediation potential of pig dung in a soil polluted wit h spent engine oil. Top soil (0-15 cm depth) samples were randomly collected from areas with history of spent engine oil pollution within Anyigba, Kogi State, Nigeria. One kilogram of the polluted-soil was measured into each of nine plastic containers. Pig dung collected from the Prince Abubakar Audu University, Anyigba, was standardly prepared and mixed with the soil at the rate of 0, 50 and 100 g kg^{-1} soil in triplicate. 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

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Enterobactersp, *Escherichia coli and Bacillus sp,* with *Bacillus sp.* being 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, 35.52 ± 0.71 and 29.57 ± 2.99 at 28 days respectively. Pig dung and the high level carbon utilizing bacteria, *Bacillus sp,* significantly enhanced the biodegradation process as an impressive 50.54% remediation efficiency was achieved 28 days after amendment in soil treated with 100g of pig dung. It is recommended that a biodegradation strategy that uses pig dung and *Bacillus sp* be employed in the clean-up of used engine lubricant polluted soils.

Keywords: Biostimulation; pig dung; engine lubricant; spent motor oil; contaminated soil; total petroleum hydrocarbon.

1. INTRODUCTION

Spent engine oil contamination of soil has increased dramatically as a result of the increased use of petroleum products worldwide. The environment is polluted by hydrocarbons when used motor oils, such as engine oil, diesel, or jet fuel, are spilled [1, 2]. The bulk of polycyclic aromatic hydrocarbons (PAHs), which are poisonous, mutagenic, and carcinogenic, are responsible for the majority of the hydrocarbon contamination of the air, soil, and water. Spent engine oil include potentially harmful elements such arsenic, zinc, cadmium, and PAH which can also contaminate ground water by penetrating into the soil through various aquifer levels [3, 4].

Today, petroleum hydrocarbons are a significant environmental pollution all over the world since their exploration and usage are linked to economic growth [5, 6]. The management of mechanic shops in Nigeria is subpar, and they can be a constant supply of wasted spent oil ejected from the crank cases of vehicles and motorbikes, which can be unattractive and seriously pollute the environment. Since operators of such sites frequently are unaware of the harmful consequences on the environment, cleanup of mechanic sites is still elusive. A pint of motor oil has the potential to contaminate 100,000 liters of ground water, and there is a chance that it will percolate into the earth [7,8].

“When used as an engine lubricant at high temperatures and pressures within an engine while it runs, used motor oil is similar to unused oil except that it contains extra compounds that accumulate in the oil” [9]. “Metals from worn-out engine parts, such as aluminum, chromium, copper, iron, lead, manganese, nickel, silicon, and tin, can also be found in used motor oil. It's possible to find some of these metals in both surface water and ground water since they can quickly dissolve in water and migrate through the soil. Metals from used oils can therefore accumulate in plants, animals, soil, sediments, and non-draining surface water. Used motor oil contains heavy metals and compounds that are absorbed and transported into various tissues of people, plants, and animals as a result of environmental movement” [10, 11, 12, 13, 14, 15, 16]. These substances can cause major health issues like anemia, tremors, mutagenicity and carcinogenicity which can ultimately lead to death. The accumulation of oil in vehicle repair shops, which has typically gone unattended over time and is now posing a huge environmental risk due to the risk it poses, as was aforementioned, is a major concern in most nations across the world [9].

Current physicochemical treatment technologies are expensive and unfriendly to the environment. Additionally, some of these technologies only transport pollution from one location to another. Recently, a lot of work has gone into finding natural ways to minimize environmental contamination. These methods include phytoremediation (which use plants to remove contaminants by allowing them to bioaccumulate in the tissues of the plants) and bioremediation (which utilizes pollutants using microorganisms) [17]. In mechanic shops all around the country, motor engine oil pollution of the soil has led to a reduction in soil fertility. The biodegradation process can be improved by adding organic or inorganic nitrogen-rich nutrients to the soil [18]. Reclamation of polluted soil is not viable due to the lack of availability for most farmers and the inaccessibility to inorganic fertilizer [19,20]. Organic wastes from plants and animals have been used in oil-polluted soil bioremediation [20]. One of the difficulties pig farmers confront is managing pig dung. Pig dung has not been used much in the rehabilitation of engine oil-polluted soil. The attempt to bridge the gap forms the focus of this study. The objectives of this study were to evaluate the effect of pig dung on the soil's chemical properties and the degradation of total petroleum hydrocarbon in motor oil-polluted soil.

2. MATERIALS AND METHODS

2.1 Samples Collection, Preparation and Experimental Design

"Pig dung was collected from Piggery Unit, Animal House of Prince AbubakarAudu University Teaching and Research Farm, Anyigba, Kogi State, Nigeria. The manure was air dried, ground, mixed, sieved with a 2 mm sieve and stored in polythene bag. Top soil (0-15 cm depth) was collected from a mechanic workshop using a soil auger. The soil was air dried in a clean, well ventilated laboratory, homogenized by crushing and sieved by passing through a 2 mm mesh sieve. One kilogram of soil was weighed into each of the nine containers. The soil samples were then allowed to weather for a period of two weeks before the addition of pig dung at the rate of 0, 50 and 100 g kg^{-1} soil in triplicate. Application rate of pig dung was based on the 50 g chicken manure kg^{-1} soil recommended for the clean-up of polluted soil" [21]. The pig dung was thoroughly mixed with the soil and the nine containers were arranged in a Completely Randomized Design. Soil samples were taken from each container at 28 days for pH, organic carbon, nitrogen, phosphorus, potassium, hydrocarbon degrading bacteria count, hydrocarbon utilizing bacteria and total petroleum hydrocarbon determination.

2.2 Laboratory Analysis

2.2.1Soil Chemical properties and proximate analysis of pig dung

Soil chemical properties were determined after contamination. The pH was determined in distilled water according to [22]. Total nitrogen, potassium and available phosphorus were determined using [23,24,25] respectively. Proximate analysis of pig dung was carried out using the methods described in [26].

2.3 Cultural Characterization

"Serial dilution was carried out on the soil sample as 1 g of the soil was dispersed into 10 ml of sterile distilled water. This was thoroughly mixed after which 1 ml of the mixture was transferred into another 9 ml of sterile distilled water. The process was repeated till dilution factor 10-6 was obtained. Pour plate technique was thereafter used to isolate microorganisms in the soil sample. One ml of the dilution factor 10-6 was transferred into sterile petri dish after which sterile molten nutrient agar was poured and swirled. This was allowed to set and the plates were thereafter inverted in the incubator at 37˚C for 24 hours. After incubation, the cultural characteristics of the colonies such as colour, shape, elevation and opacity were determined" [27].

2.4 Morphological Characterization

"The organisms were identified morphologically using Gram Stain Technique. A loopful of the organism was placed on a clean grease free slide and smeared. This was thereafter heat fixed and the primary stain crystal violet was poured and left for 60 seconds after which it was washed with distilled water. The Mordant Iodine was thereafter poured on the slide for 60 seconds and alcohol was used as decolorizer. The secondary stain Safranin was eventually poured and left for 60 seconds before washing with distilled water. The slides were left to drain-dry and observed under the microscope for their size, shape and Gram stain" [28].

2.5 Biochemical Characterization

A modified method of [28] was used for Gram staining, Catalase test, Urease test, Citrate utilization test, Indole test, Motility test, Coagulase test and Sugar fermentation test

2.6 Determination of Total Hydrocarbon Utilizing Bacteria Count

Total Hydrocarbon Utilizing Bacteria Count was determined in both soil and pig dung. This was carried out on Mineral Salt Medium (MSM) agar as described by [29] and the isolated micro-organisms were identified using Bergey's manual of systemic bacteriology [30].

2.7 Determination of Total Petroleum Hydrocarbon

Ten grams of the petroleum products polluted soil sample was weighed into a clean bottle and 25 ml of dichloromethane was added; the mixture was allowed to stand on a mechanical shaker for a period of 3- 4 hours at a speed of 350 rpm. The procedure was repeated twice and the aliquots was 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 analysed using a Hewlett Packard 5890 series GC system coupled to a mass spectrophotometer VG TRIO 2000 to determine the quantity of total petroleum hydrocarbons. Concentration degraded and percentage degradation was calculated using equations 1 and 2 respectively.

The concentration degraded and percentage degradation was calculated using equation 1 and 2 respectively as described by [10,31].

Concentration degraded = C1 – C2 -- Equation 1

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

2.8 Statistical 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

3.1 Results of Chemical Properties of Used Motor Engine Oil Polluted Soil Using Pig Dung

Application of pig dung (PD) significantly ($p < 0.05$) increased the pH of the contaminated soil compared to the control (without pig dung application) at 28 days (Table 1). The pH of the soil before pig dung application was 6.90 **±** 0.10 while control, 50 and 100g of pig dung were 6.80 **±** 0.10, 7.40 **±** 0.10 and 7.20 **±** 0.10 for 28 days indicating a downward trend. The nitrogen content of the soil (gkg^{-1}) before pig dung application was 1.33 ± 0.08 while control, 50 and 100g of pig dung were 1.13 ± 0.02,1.14 ± 0.02 and 1.26 ± 0.03 for 28 days indicating a downward trend for the experiment. Significantly ($p < 0.05$) lower N, P, K and organic carbon was recorded in 50 and 100g pig dung kg^{-1}at 28 days.

3.2 Total Petroleum Hydrocarbon of Used Motor Engine Oil Polluted Soil

Significantly ($p < 0.05$) higher concentration of total petroleum hydrocarbon was observed in 0g pig dungkg^{-1}soil (control) at the start of the experiment while the value that was recorded in 100g pig dung kg^{-1}soil at 28 days was significantly ($p < 0.05$) lower (Table 2). Total petroleum hydrocarbon of the soil ($mgkg^{-1}$) before pig dung application was 59.78 ± 1.84 while control, 50 and 100g of pig dung were 44.92 ± 2.26, 35.52 ± 0.71 and 29.57 ± 2.99.

3.3 Total Hydrocarbon Degrading Bacteria Count of The Polluted Soil Amended with Pig Dung

The value of total hydrocarbon degrading bacteria (THDB) decreased from the start to the 28 day in 0 (control), 50 and 100g pig dung kg^{-1} soil (Table 3). The total hydrocarbon degrading bacteria were found to be higher in soil amended with pig dung than the control soil.

3.4 Morphological Characteristics of Bacteria Isolated from The Used Motor Engine Oil Polluted Soil

Morphological characteristics of bacteria isolated from the used motor engine oil polluted soil amended with pig dung at 28 days are presented in (Table 4). The size of the bacteria ranged 1-5mm. Most of the bacteria were irregular in shape, grey-white in color, wet consistency, smooth edges, flat elevation and opaque.

3.5 Types and The Relative Abundance of Micro-Organisms in the Polluted Soil

The types and relative abundance of microbial communities in microcosms due to natural attenuation and biostimulation treatment methods recorded in the contaminated soil are presented in Table 5. Five hydrocarbon utilizing bacteria were identified from the polluted soil. The hydrocarbon degrading bacteria identified belong to the genera *Bacillus*, *Staphylococcus*, *Escherichia*, *Pseudomonas* and *Enterobacter. Bacillus* species were the most predominant isolated bacterial species across the treatments.

This study established the pig dung's capacity for cleaning up soil that has been contaminated by used motor engine oil. When compared to the control at 0 and 28 days, it was observed that applying pig dung considerably ($p< 0.05$) raised the pH of the polluted soil. In a comparable experiment, [2] found that the majority of hydrocarbon-utilizing bacteria thrive in a slightly acidic and alkaline media.As reclamation

Table 1. Effects of pig dung application on the soil chemical properties

Pig dung level (g)	Days	pH	Nitrogen (g kg^{-1})	Phosphorus (mg kg^{-1})	Potassium (Cmol kg^{-1})	Organic carbon (g kg^{-1})	Moisture content (g kg^{-1})
0	0	6.90 ± 0.10^{a}	1.33 ± 0.08^{c}	$1.64\times10^{2}\pm2.87^{b}$	1.23 ± 0.03^{b}	65.48 ± 2.91	2.00 ± 0.50^{a}
	28	6.80 ± 0.10^{a}	1.13 ± 0.02^{a}	$1.25\times10^{2}\pm2.6\times10^{1a}$	2.24 ± 0.02	48.29 ± 2.97^{a}	1.65 ± 0.04^{a}
50	0	6.90 ± 0.10^{a}	1.33 ± 0.08^{b}	$1.64\times10^{2}\pm2.87^{b}$	1.23 ± 0.03^{b}	65.48 ± 2.91	2.00 ± 0.50^{a}
	28	7.40 ± 0.10^{b}	1.14 ± 0.02^{a}	$1.25\times10^{2}\pm1.07^{a}$	0.64 ± 0.10^{a}	56.47 ± 2.15^{b}	1.75 ± 0.20^{a}
100	0	6.90 ± 0.10^{a}	1.33 ± 0.08^{b}	$1.64\times10^{2}\pm2.87^{b}$	1.23 ± 0.03^{b}	65.48 ± 2.91	2.00 ± 0.50^{a}
	28	7.20 ± 0.10^{b}	1.26 ± 0.03^{b}	$1.43\times10^{2}\pm2.68^{a}$	1.38 ± 0.10	67.50 ± 0.85	1.87 ± 0.10^{a}

Values are mean ± SD of three replicates. Different superscripts in the same column represent significant difference at $p< 0.05$ (DMRT).

Table 2. Effects of pig dung on total petroleum hydrocarbon (TPH) of spent engine oil polluted soil

Pig dung level (g)	Days	TPH ($mgkg^{-1}$)	TPH Degraded ($mgkg^{-1}$)	Degradation (%)
0	0	59.78 ± 1.84^{d}		
	28	44.92 ± 2.26^{c}	14.86	24.86
50	0	59.78 ± 1.84^{d}		40.58
	28	35.52 ± 0.71^{b}	24.26	
100	0	59.78 ± 1.84^{d}		50.54
	28	29.57 ± 2.99^{a}	30.21	

TPH= Total Petroleum Hydrocarbon

Values are mean ± SD of three replicates. Different superscripts in the same column represent significant difference at $p< 0.05$ (DMRT).

Table 3. Effects of blends of poultry manure and pig dung on hydrocarbon degrading bacteria (thdb) counts and identification

Pig dung level (g)	Days	THDB (CFU g^{-1})
0	0	$2.13\times10^{4}\pm3.51\times10^{2d}$
	28	$9.17\times10^{3}\pm7.37\times10^{2a}$
50	0	$2.13\times10^{4}\pm3.51\times10^{2d}$
	28	$1.54\times10^{4}\pm3.51\times10^{2b}$
100	0	$2.13\times10^{4}\pm3.51\times10^{2d}$
	28	$1.74\times10^{4}\pm1.89\times10^{2c}$

THDB= Total Hydrocarbon Degrading Bacteria

Values are mean ± SD of three replicates. Different superscripts in the same column represent significant difference at $p< 0.05$ (DMRT).

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
0g PD	3-5	Irregular	Grey-white	Dry	Rough	Flat	Opaque
50g PD	3-4	Round	Grey-white	Wet	Smooth	Raised	Opaque
100g PD	2-3	Round	White	Wet	Smooth	Flat	Opaque

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	L	M	Probable organism
0g PD	GNB	-	-	+	-	+	-	-	+	-	+	-	A	A	-	*Enterobactersp*
50g PD	GNB	-	-	+	-	+	+	-	-	-	+	-	A	A	-	*Escherichia coli*
100g PD	GPB	+	+	+	-	+	-	-	-	-	+	-	A	-	-	*Bacillus subtilis*

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, PD- Pig Dung, g- Gram, - = Absent, + = Present, A = Abundant advanced in this study, there was a gradual decline in pH. The addition of various salts and ions from pig dung has been implicated in the reclamation process of oil polluted soil [6]. When the pH of this study was compared with values reported in literature, results revealed that the pH reported in this study was in agreement with the earlier report of [8]. Additionally, the observed pH decrease in this study was consistent with the findings of [32].

Due to the high demand for these nutrients by hydrocarbon degrading bacterial for phosphorylation of sugar, nucleic acid production, and other cellular processes, their quantity in soil decreased from 0 to 28 days at every level of pig dung amendments. Inorganic nutrient sources have been observed to be destroyed by petroleum hydrocarbon pollutants when they react with other elements in the soil [6]. In soil containing 0 (control) and 100g of pig dung $kg^{-1}$of soil, a decrease in the population of hydrocarbon-degrading bacteria was seen from the beginning of the experiment to day 28. This decrease in the total number of bacteria can be attributed to the possibility that the mineralization of hydrocarbons produced toxic metabolites, which, when introduced into the system, shorten the microbes' growth phase [33]. For growth, microorganisms often need sources of mineral nutrients. The number of microorganisms will decline if any necessary nutrient is deficient or becomes scarce, especially the macro-mineral elements [33, 34, 4]. Also observed a decrease in bacterial population as reclamation worked its way forward. The hydrocarbon degrading bacteria species identified in this study belongs to the genera *Enterobacter, Escherichia* and *Bacillus*. Their abundance may be explained by the fact that these species produces spores, which enable microorganisms to survive in hostile environments [31].

Reported [35] the isolation of *Bacillus, Acinetobacter, Staphylococcus* and *Enterobacter* among other bacteria from petroleum contaminated soil. Although from diverse origins, the oil-degrading bacteria obtained for this study have been linked to hydrocarbon biodegradation in the past. Degradation of total petroleum hydrocarbon in the contaminated soil amended with pig dung might be due to the bacterial population in the pig dung that effectively degraded the components of the hydrocarbon. Significantly ($p < 0.05$) higher concentration of total petroleum hydrocarbon was observed in the soil without pig dung amendment. Biostimulation has been reported as an important factor that enhances soil bioremediation [2].

4. CONCLUSION

The ascertained the effects of pig manure on the breakdown of total petroleum hydrocarbons (TPH) and the microbiological makeup. The microcosms with 100 g kg^{-1} of PD showed about 50.54% TPH elimination after 28 days of incubation, while the microcosms without modifications showed just 24.86% TPH removal. When factors like pH need and nutrient availability are taken into account, the results show that PD is successful in hydrocarbon biodegradation.

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 this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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