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Biodegradation of Spent Automobile Engine Oil in Soil Microcosms Amended with Coconut Shell

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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

Food insecurity and low yield have resulted from the detrimental effects of petroleum hydrocarbon pollution on agricultural soils over time. This study was carried out to determine the bioremediation efficacy of coconut shell on microbiological composition and total petroleum hydrocarbon degradation in spent engine oil polluted soil. 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. Coconut shell 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

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identified were *Enterobacter sp* and *Escherichia coli,* with *Enterobacter sp* being the most predominant isolate. The total petroleum hydrocarbon ($mgkg^{-1}$) of the soil on day 0 was 59.78 ± 1.85. After the amendments (at control, 50 and 100 g kg^{-1}), the total petroleum hydrocarbon ($mgkg^{-1}$) values were 44.92 ±2.26, 34.82 ± 1.78 and 31.49 ± 0.87 at 28 days respectively. Coconut shell and the high level carbon utilizing bacteria, *Enterobacter sp,* significantly enhanced the biodegradation process as an impressive 47.32% remediation efficiency was achieved 28 days after amendment in soil treated with 100g of coconut shell. It is recommended that a biostimulation strategy that uses coconut shell and *Enterobacter sp* be employed in the remediation of spent engine oil and petroleum hydrocarbon polluted soils.

Keywords: Biodegradation; coconut shell; spent engine oil; hydrocarbon-degrading bacteria; total petroleum hydrocarbon.

1. INTRODUCTION

Spent engine oil, also known as spent lubricant or waste engine oil, forms under high temperatures and mechanical stress during engine operation [1]. Most commonly obtained after servicing and subsequently draining from automobile and generator engines [2], and much of this oil is spurted into the environment [3].

The unguided throwing away of spent engine oil (SEO) into sewers, water drains, open vacant plots and agricultural lands has become a common practice in Nigeria, especially by motor mechanics [4]. This endangers public health, devastates natural resources, contaminate agricultural lands and soil dependent organisms, as well as surface and groundwater bodies [4]. The environment suffers degradation due to the irregular disposal of spent lubricating oils, especially on land and into water bodies. Moreover, this often results to the accumulation of heavy metals and other pollutants in the environment, and in turn affects economic stability [5-11]. Waste engine oil or spent engine oil (SEO) contains several poisonous and carcinogenic substances such as poly-aromatic hydrocarbons and poly- cyclic benzenes [12,13].

The uncontrolled release of petroleum and its derivative products into the environment, including surface water and groundwater has become a globally serious problem, especially in Nigeria [14,15]. [16], reported that about 20 million gallons of spent oil are generated every year from mechanic workshops across Nigeria, and are wrongly disposed into the environment. However, since the most of the components of this oil are toxic to humans and wildlife, it could have direct impact the food chain, species and their habitats. This may set off a cascade of perturbations that affect the entire food chain. More recently, scientists have aimed their research on elucidating the distribution, fate and behavior of oil and its derivatives in the environment [17,18].

Quite a number of possible remediation technologies used to remediate polluted sites include, but not all of the following; mechanical, burying, evaporation, dispersion, and soil washing [19]. Bioremediation, which exploits the degradation potentials of plants and microorganisms to remove pollutants, is a widespread clean-up technology for petroleum hydrocarbon contaminated soils. It is employed *in-situ* (on contaminated sites), or *ex-situ* (on excavated samples) and has been reportedly cost effective and environmentally friendly [20]. The utilization of organic (crude) wastes derived from plant and animal origin for the bioremediation of petroleum hydrocarbons in polluted soils have been investigated [21,22].

However, there are reasons to further study the processes involved in biodegradation of spent engine oil in contaminated soils. Remediation of spent engine oils is seemingly challenging because they are less biodegradable and less volatile compared to other hydrocarbons. The rapid method of hydrocarbon degradation via the use of microbes can be employed, however, optimum biodegradation can only occur if the right environmental conditions such as pH, temperature, nutrients and relevant microbial consortia are present [23]. This formed the basis of the study. Therefore, the specific objectives of the study were to assess the efficacy of coconut shell on the degradation of total petroleum hydrocarbon in spent motor engine oil polluted soil, soil chemical and microbial properties.

2. MATERIALS AND METHODS

Study Area: The research was carried out in the laboratory within the Department of Animal and

Environmental Biology of Prince Abubakar Audu University, Anyigba, Kogi State, Nigeria, as it provided a suitable setting for the study under standard and controlled conditions.

Coconut Shell Collection and Preparation: samples of coconut shell were obtained from the Prince Abubakar Audu University Teaching and Research Farm, Anyigba, Kogi State, Nigeria. The coconut shell samples were air dried, pulverized, homogenized, sieved with a 2 mm sieve and stored in polythene bag for use, as described by [15,24].

Soil Samples Collection and Preparation: Top soils (0-15 cm depth) were sampled randomly and in triplicates [25], from areas with history of spent engine oil contamination within Anyigba, Kogi State, Nigeria, using a soil helix. The soil samples were air dried in a clean and ventilated laboratory. The samples were crushed and further sieved with a 2 mm mesh [24]. Nine, clean, dry containers of about 3 liters each, were used to collect a kilogram of soil respectively.

The prepared coconut shell was added at the rate of 0 (control), 50 and 100 g kg^{-1} soil in triplicate. The coconut shell samples were mixed with the soil, while the nine containers were arranged using a completely randomized design (CRD) in the laboratory. Furthermore, mixed soil samples were taken from each container at 0 (zero day refers to the day coconut shell 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 [26].

2.1 Laboratory Analysis

Chemical Properties of Soil: The soil chemical properties, including pH, organic carbon, total nitrogen, potassium and available phosphorus were determined in the samples using the methods described in [27].

Cultural Classification of Bacteria: The colonial features such as colony elevation, color, size, opacity, shape, consistency, and edgewere used to classify pure cultures of representative bacteria colonies randomly picked from inoculated plates [27].

Morphological Classification of Bacteria: For 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, as described by [27].

Biochemical Identification of Bacteria: A modified method of [28], was adopted for various biochemical characterizations, including Gram staining, urease test, catalase test, indole test, motility test, citrate utilization test, coagulase test and sugar fermentation test [29].

Determination of Total Hydrocarbon Utilizing Bacteria Count: The total hydrocarbon utilizing bacteria count was performed on a mineral salt medium (MSM) agar as described by [30]; and the isolated microorganisms were identified using Bergey's manual of systemic bacteriology [31].

Determination of Total Petroleum Hydrocarbon: About 10 g of the spent engine oil contaminated soil sample was measured into a clean bottle and 25 ml of dichloromethane was added. The mixture was placed on a mechanical shaker for a period of 3- 4 hours. This procedure was repeated twice and the aliquots were collected and homogenized in a beaker. The aliquots were concentrated on a steam bath, which reduced the extracts to about 5 ml. The concentrate was transferred through a pipette packed with anhydrous sodium sulphate atop a glass wool, to remove moisture and other impurities. A Hewlett- Packed 5890 series Gas Chromatograhy- Mass Spectrophotometer (VG TRIO 2000) was used to analyze and determine the quantity of total petroleum hydrocarbons in the final extract.

The concentration degraded and percentage degradation were calculated using equation 1 and 2 respectively as described by [26,29].

$$\text{Concentration degraded} = C1 - C2 \quad \text{---------------- Equation 1}$$

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

2.2 Data Analysis

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

3. RESULTS AND DISCUSSION

3.1 Effect of Coconut Shell on the Chemical Properties of Soil

In this study, attempt was made to assess the efficacy of coconut shell on total petroleum hydrocarbon degradation, soil chemical and microbial properties of spent engine oil contaminated soil. The effect of coconut shell on the soil chemical properties is shown in Table 1. A non-significant ($p>0.05$) increase from 6.90± 0.10 to 7.20± 0.10 and 6.90±0.10 to 7.10±0.10 in pH was observed in contaminated soil with 50 g and 100g of coconut shell between days 0 and 28 days respectively, as compared with the control (0 g). The total value of Nitrogen (g kg^{-1}) in soil containing 0 g, 50 g and 100g of coconut shell 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 0 g, 50 g and 100 g, with values of 1.12±0.01, 1.17±0.10 and 1.23±0.02 respectively. Total value of Phosphorus in 0 g, 50 g, and 100 g of coconut shell amended soil was1.64×10^2±2.87 on day 0. The total Phosphorus ($Mgkg^{-1}$) was observed to have decreased from 1.64×10^2±2.87 to 1.25×10^2±2.67 and1.64×10^2±2.87 to 1.45×10^2±1.25 at 28 days in soil amended with 50 g and 100 g of coconut shell respectively, indicating a downward trend. There was a significant decrease from 1.23±0.03 to 0.82±0.10 of total potassium ($Colkg^{-1}$) in soil treated with 50 g of coconut shell on day 28. However, level of potassium significantly increased on day 28 from 1.23±0.03 to 2.24±0.02 and 1.23±0.03 to 1.52±0.20 in soil amended with control (0 g) and 100g of coconut shell, respectively. Levels of organic carbon significantly decreased in soil treated with 0 g and 50 g of coconut shell, from 6.5×10^1±2.91to 4.3×10^1±2.97 and 6.5×10^1±2.91 to 5.7×10^1±1.89 on days 0 and 28 respectively. But there was a non-significant increase from 6.5×10^1±2.91to 6.7×10^1±0.66 on day 28 in soil amended with 100g of coconut shell. A downward trend in decrease of moisture content was observed in soil treated with 0g, 50g and 100g of coconut shell, from 2.00±0.50 to 1.65±0.04, 2.00±0.50 to 1.78±0.20 and 2.00±0.50 to 1.86±0.10 respectively, between days 0 and 28 and was non-significant.

3.2 Effects of Coconut Shell Application on the Soil Chemical Properties

The increase in pH as biodegradation progressed, as recorded in this study was connected to the microbial degradation of the contaminant, introducing some ionic and non-ionic substances from the naturally alkaline coconut shell [23]. [32,33], claim that soil pH range between 6.9 and 7.5, as recorded in this study, isgood for mosthydrocarbon utilizing bacteria. The decrease in soil nitrogen, phosphorus, potassium, organic carbon and moisture content from 0 to 28 days at every coconut shell level might be due to the action of microorganisms for sugar phosphorylation, nucleic acid synthesis and other cellular processes [20,34,35]. It was reported that petroleumhydrocarbon contaminants could destroyinorganic nutrient sources by reacting with them along with other substances present insoil [36].

3.3 Effect of Coconut Shell on Total Petroleum Hydrocarbon (TPH) of Spent Engine Oil Polluted Soil

The result for the effect of coconut shell on TPH of spent engine oil polluted soil is shown in Table 2. The coconut shell treatment remediated significantly, the TPH in the polluted soil. The lowest level of TPH ($mgkg^{-1}$) after amendment was recorded on day 28 in soil treated with 100 g of coconut shell, reducing the TPH from 59.78±1.85 to 31.49±0.87 at days 28 indicating that about 28.29 (47.32%) of TPH was degraded. A total of 24.96 (41.75%) of degraded TPH was recorded in soil treated with 50 g of coconut shell, decreasing TPH from 59.78±1.85 to 34.82±1.78 at 28 days. The least percentage of degraded TPH of about18.86 (24.86%) was observed and recorded for the control at 28 days.

The biodegradation of TPH in the spent engine oil polluted soil amended with coconut shell might be due to the microbial (bacterial) communities in the coconut shell, which interfered and broke down the complex components of the hydrocarbon [37,33]. [38], In their study of *in situ* bioremediation techniques, reported that during biostimulation, it is possible to achieve about 90% of hydrocarbon pollutants degraded. [24] reported in their studyof microbial degradation, that significantly higher concentration of total petroleum hydrocarbon was recorded inthe soil without pig dung applications compared with the least significant ($p < 0.05$) concentration observed in 100 g pig dung kg^{-1} soil. Their correlation analysis (r) for the petroleum products biodegradation kinetic process was 0.999, indicating linearity and positive correlations for the decrease in concentration as a function of time.

Table 1. Effects of coconut shell application on the soil chemical properties

Coconut shell (g)	Days	pH	Nitrogen (gkg^{-1})	Phosphorus ($Mgkg^{-1}$)	Potassium ($Colkg^{-1}$)	Organic Carbon (gkg^{-1})	Moisture Content (gkg^{-1})
0	0	$6.90±0.10^b$	$1.33±0.07^c$	$1.64×10^2±2.87^b$	$1.23±0.03^b$	$6.5×10^1±2.91^c$	$2.00±0.50^a$
	28	$6.80±0.10^b$	$1.12±0.01^a$	$1.25×10^2±2.67^a$	$2.24±0.02^d$	$4.3×10^1±2.97^a$	$1.65±0.04^a$
50	0	$6.90±0.10^b$	$1.33±0.07^c$	$1.64×10^2±2.87^b$	$1.23±0.03^b$	$6.5×10^1±2.91$	$2.00±0.50^a$
	28	$7.20±0.10^b$	$1.17±0.10^b$	$1.22×10^2±2.16^a$	$0.82±0.10^a$	$5.7×10^1±1.89^b$	$1.78±0.20^a$
100	0	$6.90±0.10^b$	$1.33±0.07^c$	$1.64×10^2±2.87^b$	$1.23±0.03^b$	$6.5×10^1±2.91^c$	$2.00±0.50^a$
	28	$7.10±0.10^b$	$1.23±0.02^b$	$1.45×10^2±1.25^b$	$1.52±0.20^c$	$6.7×10^1±0.66^c$	$1.86±0.10^a$

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

Table 2. Effect of coconut shell on total petroleum hydrocarbon (TPH) of spent engine oil polluted soil

Coconut Shell(g)	Days	TPH ($mgkg^{-1}$)	TPH Degraded	Degradation %
0	0	59.78 ± 1.85^{d}	14.86	24.86
	28	44.92 ± 2.26^{c}		
50	0	59.78 ± 1.85^{d}	24.96	41.75
	28	34.82 ± 1.78^{b}		
100	0	59.78 ± 1.85^{d}	28.29	47.32
	28	31.49 ± 0.87^{a}		

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

3.4 Effect of Coconut Shell on Total Hydrocarbon Degrading Bacteria (THDB) Counts and Identification

A 100 g of coconut shell treatment of polluted soil significantly decreased THDB count from $2.13 \times 10^4 \pm 3.51 \times 10$ to $1.26 \times 10^4 \pm 1.52 \times 10$ on days 0 and 28 respectively (Table 3). Significantly ($p<0.05$) increased THDB count was observed in the control (0 g). However, there was a recorded non-significant decrease from $2.13 \times 10^4 \pm 3.51 \times 10$ to $1.01 \times 10^4 \pm 2.64 \times 10$ on days 0 and 28 in THDB count for soil amended with 50g of coconut shell.

The morphological characteristics of bacteria isolated from the polluted soil amended with coconut shell at 28 days are presented in Table 4. The size of the bacteria ranged between 2-3mm. Most of the bacteria were round in shape, grey-white in color, wet consistency, smooth edges, flat elevation and opaque.

The types and relative abundance of microbial communities in microcosms due to natural attenuation and bio stimulation 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 *Escherichia*. *Enterobacter* species were the most predominantly isolated bacterial species across the treatments.

The decrease in number of total hydrocarbon degrading bacteria from 0 and 28 days in 50 and 100g coconut shell kg^{-1} soil, as recorded in this study might be due to the mineralization of hydrocarbons, which could have possibly resulted in the generation of toxic metabolites. The introduction these metabolites into the system, however, reduced the growth phase of the microbes [23], when compared to the significant increase in THDB count in the control. [24,25] also reporteda decline in bacterial population as the biodegradation progressed.

In this study, *Enterobacter* species were the most predominantly isolated bacterial species. This could be connected to their ability to utilize hydrocarbons as the only source ofcarbon, and has the ability to withstand toxic conditions. The bacteria had earlier been reported by [39], as well as [40], as efficienthy drocarbon degraders. Large number of hydrocarbon utilizing bacteria tend to dominate soils that receive micro-leaching of hydrocarbons, as reported by [35,41].

The study by [20] revealed that *Enterobacter sp* IAA-01 efficiently degraded78.0% of oil after 21 days of amendment. In addition, the efficient ability of the bacteria in degrading

Table 3. Effects of coconut shell on total hydrocarbon degrading bacteria (THDB) counts and identification

Coconut Shell (g)	Days	THDB ($CFU\ g^{-1}$)
0	0	$2.13 \times 10^4 \pm 3.51 \times 10^{2d}$
	28	$9.16 \times 10^4 7.371 \times 10^{2a}$
50	0	$2.13 \times 10^4 \pm 3.51 \times 10^{2d}$
	28	$1.01 \times 10^4 \pm 2.64 \times 10^{2d}$
100	0	$2.13 \times 10^4 \pm 3.51 \times 10^{2d}$
	28	$1.26 \times 10^4 \pm 1.52 \times 10^{2c}$

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

Table 4. Morphological characteristics of bacteria isolated from the spent engine oil polluted soil amended with coconut shell at 28 days

Isolate Code	Size (mm)	Shape	Color	Consistency	Edges	Elevation	Opacity
CTRL (0 g)	2-3	Irregular	Grey-white	Dry	Rough	Flat	Opaque
CS (50 g)	2-3	Round	Grey-white	Wet	Smooth	Raised	Opaque
CS (100 g)	2-3	Round	White	Wet	Smooth	Flat	Opaque

CTRL – Control; CS – Coconut Shell

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 0 g	GNB	-	-	+	-	+	-	-	+	-	+	-	A	A	-	*Enterobacter sp*
CS 50 g	GNB	-	-	+	-	+	-	-	+	-	+	-	A	A	-	*Enterobacter sp*
CS100 g	GNB	-	-	+	-	+	+	-	-	-	+	-	A	A	-	*Escherichiacoli*

Keys: GR-Gram staining, SP- Spore staining, CA- Capsule staining, CT- Catalase, MO-Motility, IN- Indole, OX- Oxidase,CI- Citrate, 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 hydrocarbon has been reported by other investigators [42,43,44]. The results of GC analysis in the study by [20], further confirmed the biodegradation efficacy of *Enterobacter sp* IAA-01 and revealed that the bacteria has the potential to utilize most of the hydrocarbon components especially straight chain alkanes, and cyclo-alkanes. Moreover, [45] noted that the accepted mode of susceptibility of hydrocarbon components to microbial degradation is n-alkane > branched alkanes > low- molecular weight aromatics >polycyclics.

4. CONCLUSION

In this study, microcosm experiments were carried out to determine the potentials of coconut shell in the bioremediation of spent engine oil contaminated soils. For this study, after 28 days of incubation, approximately 41.75% and 47.32% remediation efficiency was achieved at 50 and 100 g coconut shell kg soil. The results showed that bio stimulation of spent engine oil contaminated soil with coconut shell resulted in the augmentation of petroleum hydrocarbon degradation. Coconut shell is reliable and powerful for bioremediation processes. Therefore, It can be concluded from these results that *Enterobacter* species in this study had considerably high ability of degrading hydrocarbon.

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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