

Influence of Organic Fertilizers produced from SLO Spiked Composting Process on Microbial counts and Hydrocarbon Degradation Rate in Soils.

Abstract

This study evaluated the influence of organic fertilizers produced from spent lubricating oil (SLO) spiked aerobic composting technique on petroleum degradation rate in soils. The compost windrows (F₂ and F₄), consisting of kitchen and agricultural wastes, were spiked with varying concentrations (2% and 4%) of SLO. The resultant organic fertilizers were employed as amendment in pollution simulated potted soils laid out in a complete randomized block design with three replications for 90 days. Results revealed higher counts of hydrocarbon utilizing microbes (HUB: 4.2±0.02×10⁴cfu/g in F₂, 3.0±0.02×10⁴cfu/g in F₄; HUF: 3.9±0.2×10⁴cfu/g in F₂, 2.5±0.02×10⁴cfu/g in F₄) in spiked compost compared to the control, F₀ (HUB: 7.9±0.02×10³cfu/g; HUF: 6.0±0.2×10³cfu/g). Mean microbial count in amended soils reflected a dose-dependent increase which followed the trend: F₂ > F₀ > F₄ for the 5% (3.7×10⁸ cfu/g), 10% (9.2×10⁷ cfu/g) and 15% (6.9×10⁷ cfu/g) levels of fertilizer treatments respectively. There was a significant (P<0.05) reduction in the TPH content of soils after 90days treatment with organic fertilizers. Generally, remediation efficiency followed the order: F₂ > F₀ > F₄, with the highest (11.51%) achieved at 5% F₂ application. Spiking technique was responsible for the higher counts of hydrocarbon utilizing microbes and enhanced bioremediation associated with the use of fertilizers F₂. In line with this, the use of petroleum-spiked organic fertilizers for remediation of petroleum-polluted soils, and further research efforts to perfect compost-spiking as a novel technique in composting is recommended.

Key words: Composting, Spiking, Pollution, Biodegradation, Bioremediation.

1.0 Introduction

Depending on the dose, type of the oil and other factors, petroleum has deleterious effects on biological, chemical and physical properties of the soil. Microbiological components of soil take a downward turn following oil application (Benka-Coker and Ekundayo, 1997; Agamuthu, Tan, and Fauziah, 2013). Worse still, the natural recovery of soil from petroleum oil pollution is slow such that communities affected by this problem are denied utilization of their agricultural lands for very long periods (World Bank (2021). Therefore the need for cost-effective but viable remediation technologies becomes imperative.

Bioremediation, according to Madsen (1991), is “a managed or spontaneous process, in which biological activities, especially microbial catalysis, occur on pollutants, thereby remedying or eliminating environmental contamination”. According to Catallo and Portier (1992), it also refers to the enhancement of the native capability of microorganisms by the addition of oxygen and nutrients to the soil system to support biological growth and improve the degradation. It mainly involves biostimulation where organic or inorganic components were introduced to enhance indigenous microbial growth that directly degrades the contaminants (Chorm, Sharifi, and Motamedi, 2010; Okpashi *et al.*, 2020).

Comment [LP1]: Modify to “Influence of Spent Lubricating Oil spiked Compost on Microbial counts and Hydrocarbon Degradation Rate in Soil”

Comment [LP2]: Delete the period (.); Topics are phrases not sentences. Only sentences require periods at the end.

Comment [LP3]: DELETE

Comment [LP4]: ...compost

Comment [LP5]: The work did not involve the use of different types of soil. So use the singular “soil” and not soils.

Comment [LP6]: Bacterial or fungal?

Comment [LP7]: ...organic fertilizer

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Comment [LP9]: The spiking could have been

Comment [LP10]: ...hydrocarbon degradation

Comment [LP11]: ...it is concluded that petroleum-spiked organic fertilizers can be useful in bioremediation of petroleum-polluted soils. Further research is therefore suggested to perfect the technique.

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More so, Ekpo and Ntekpe (2014), in a deliberate search for an environmentally sound and productive use of solid organic waste, reported the production of organic fertilizers rich in nitrogen and phosphorus, among other nutrients, from organic waste materials using simple aerobic composting technique. The said fertilizers were evidently useful as soil amendments, with great potential in stimulating the population growth of indigenous soil microbes, competing favorably with its synthetic counterparts. It is against these backdrops that this study is designed to convert solid organic wastes materials to organic fertilizers fit for enhanced bioremediation of petroleum oil contaminated sites through a manipulated aerobic composting process.

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2.0 Materials and Methods

Organic Waste Collection

Composting requires the presence of two types of materials, (a) Nitrogenous (materials rich in nitrogen) and, (b) Carbonaceous (materials rich in carbon) materials in a proportion. This proportion is determined by the quality of fertilizer intended (George *et al.*, 1993). A ratio of 1:1 is usually preferred. In this study, kitchen wastes (mainly vegetable, tubers and fruit peelings) and cow dung were used to meet the requirements of nitrogen, while dry garden wastes were employed for carbon requirements.

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Set-up of Composting Process

The separated wastes materials were shredded into workable sizes, and then air-dried to reduce the moisture content to below 60%. Aerobic composting technique of Haydar and Masood (2011) was adopted, and three composting pads (Ft₂, Ft₄, Ft₀) were prepared (plate 1). Windrows Ft₂ and Ft₄ were contaminated with 2% and 4% (w/w) spent lubricating oil (soluble fractions) respectively, as spiking agent. Windrow Ft₀ did not receive any oil spiking. The moisture content of windrows were monitored periodically, and maintained at 60% during the first four weeks. Also, regular turning of the windrows was done for uniform provision of heat, oxygen and moisture until beginning of curing period/maturity, during which the compost temperature ceases to rise and assumed an earthy smell. The matured compost/organic-fertilizer was sieved through a 2.5 mm sieve to achieve the final product.

Comment [LP24]: How was the separation carried out?

Comment [LP25]: ...till the moisture content fell

Comment [LP26]: How was the soluble fractions obtained?

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Plate 1: Organic waste composting Pads

Table 1 : Physicochemical Properties of the Pollutant (SLO)

Parameters	Values
API Gravity	31.5
Density (g/cm ³)	0.82
Nitrogen (ppm)	1000
Pour point (°C)	42.8
Specific Gravity	0.81
Carbon (wt%)	86.0
Hydrogen (wt%)	14

Key:

API = American Petroleum Institute

ppm = Part per million

Field Test Methods

The produced organic fertilizers were subjected to field test through Experiment layed out in Randomized Complete Block Design (Ubom, 2004). Bulk soil taken from an agriculture field was air-

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dried and filtered, by passing through a 2mm sieve. About 300 kg of the filtered soil was placed in a large polythene sheet. The soil was artificially contaminated with 10% (w/w) spent lubricating oil. 30.0kg contaminant were sprayed and mixed thoroughly, such that the whole soil becomes homogeneously contaminated. The contaminated soil was moistened to 20% water holding capacity with distilled water to ensure proper mixing with the contaminant. 10% contamination was adopted to achieve severe contamination (pollution), because beyond 3% concentration, oil has been reported to be increasingly deleterious to soil biota and crop growth (Osuji *et al.*, 2005; Vincent *et al.*, 2011; Agamuthu *et al.*, 2013). 8.0kg each of the polluted soil was placed in thirty (30) clean dry perforated plastic containers (9887.43cm² approx. 9.89L); divided into four (4) groups (A, B, C, and D). in triplicates as shown in Figure 1. These contaminated soil samples were allowed undisturbed for two weeks to stabilize. Group A was amended with varying quantities (5%, 10%, and 15%) of organic fertilizer Ft₀, Group B amended with varying quantities (5%, 10%, and 15%) of organic fertilizer Ft₂, Group C amended with varying quantities (5%, 10%, and 15%) of organic fertilizer Ft₄, and Group D left without any amendment (Control). The moisture contents were adjusted to 60%, using distilled water. Equal rates of tilling (three times a week throughout the duration of experiment) was used to provide the necessary aeration and mixing of nutrients and microbes with the contaminated soil, following the methods of Ayotamuno *et al* (2006); Chorom, Sharifi, and Motamedi, (2010); Agamuthu, *et al* (2013). The remediation experiment lasted for ninety (90) days.

Comment [LP33]: ...on

Comment [LP34]: , and artificially contaminated with spent lubricating oil

Comment [LP35]: Artificial contamination was achieved by adding 30 kg of the spent lubricating oil, and mixing thoroughly till the whole soil became homogeneously contaminated

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Comment [LP40]: Samples of 8 kg of the polluted soil were

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Treatments	Varying Quantity of Fertilizers (%)								
	5			10			15		
A	0	0	0	0	0	0	0	0	0
B	0	0	0	0	0	0	0	0	0
C	0	0	0	0	0	0	0	0	0
D	0	0	0	0	0	0	0	0	0

Figure 1: Field Test Layout

KEY:

A = 8.0 kg of Polluted soil + 5% (0.4 kg); 10% (0.8 kg); and 15% (1.2 kg) of organic fertilizer Ft₀ respectively.

B = 8.0 kg of Polluted soil + 5% (0.4 kg); 10% (0.8 kg); and 15% (1.2 kg) of organic fertilizer Ft₂ respectively.

Comment [LP45]: A under 5: polluted soil + 0.4 kg organic fertilizer (Ft₀), A under 10: polluted soil + 0.8 kg Ft₀, A under 15: polluted soil + 1.2 kg Ft₀.

Comment [LP46]: B under 5: polluted soil + 0.4 kg organic fertilizer (Ft₂), B under 10: polluted soil + 0.8 kg Ft₂, B under 15: polluted soil + 1.2 kg Ft₂.

C = 8.0 kg of Polluted soil + 5% (0.4 kg); 10% (0.8 kg); and 15% (1.2 kg) of organic fertilizer Ft₄ respectively.

D = Polluted soil without any treatment (control).

Comment [LP47]: C under 5: polluted soil + 0.4 kg organic fertilizer (Ft₄), C under 10: polluted soil + 0.8 kg Ft₄, C under 15: polluted soil + 1.2 kg Ft₄.

Comment [LP48]: Then there was no need having triplicates under 5, 10, and 15. Only one set of triplicate is required. Therefore DELETE the 6 circles for D under 10 and 15 in the Figure, and besides the 3 circles for D under 5 insert the following in bracket: (no fertilizer added).

Laboratory Analyses

Analysis (Microbiological and physicochemical) of the various samples including the organic fertilizers produced, spent lubricating oil, and soil samples were undertaken at various times. Soil analyses were done before and after contamination, and after remediation. TPH measurement in soil samples were performed following the method of Vincent *et al* (2011), and Agamuthu *et al* (2013); while chromatographic and mass spectral analysis was carried out following the method described by Odu *et al* (1989). Enumeration and Identification of Soil Bacteria and Fungi were performed following standard protocols described by Cruickshank *et al.*, (1975); Harrigan and McCane (1976); Barnett and Hunter (1980); Holt *et al.*, (1994); Cowan and Steel (1997); APHA (1998); Barrow and Feltham (2003); Cheesbrough (2010).

Comment [LP49]: ...amended compost/organic-fertilizer

Comment [LP50]: ...carried out

Comment [LP51]: ...experimental duration

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Biodegradation rate (hydrocarbon loss)

Average biodegradation/loss rates (mg kg⁻¹ day⁻¹) of hydrocarbons under different treatments were estimated according to Yeung, Johnson, Xu (1997) as:

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$$\Delta HL = (HC_i - HC_e) / \text{Time}_{inc}$$

Where,

ΔHL = the average hydrocarbon loss (mg kg⁻¹day⁻¹)

HC_i = the initial hydrocarbon content in soil (mg kg⁻¹)

HC_e = the hydrocarbon content when the experiment ended (mg kg⁻¹)

Time_{inc} = the degradation time (days)

Remediation Efficiency (R.E)

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The Remediation Efficiency (R.E) which shows (in percentage) the effectiveness of the organic fertilizer amendments (nutrients) relative to the un-amended soils in reducing the total hydrocarbon content (THC) of soils treated with same quantity of spent lubricating oil was calculated thus:

$$R.E = \frac{THC_i - THC_t}{THC_i} \times 100$$

Where:

THC_{ci} = total hydrocarbon content in control soil under a given oil loading.

THC_{ai} = total hydrocarbon content in an amended plot under a given oil loading.

Statistical Analysis

Treatment means were subjected to two way analysis of variance and significant differences separated using Least significant differences test. The statistical package for social sciences (SPSS, Version 18.0) was employed for this purposed as described by Ubom (2004).

Ethical Considerations

There was no conflict of interest. Where this study interferes with the interest of others, due permission was sought and obtained before any progress was made. Direct lifting of other researcher's reports was avoided. All materials and authors consulted in the course of this study are duly acknowledged using reference list.

Comment [LP59]: Take this to the ending, just before the reference list

Comment [LP60]: FALSE! You have TEN (10) "Not in Reference List (NRL)" in your work.

3.0 Results

Comment [LP61]: Your Results section is meaningless. All Tables and Figures in Results section must have preceding texts describing them. Also, Results sections are usually divide into sub-sections concurrent with the sub-sections in the materials and methods sections (MMS). Example: in MMS you may have "Determination of bacterial populations in experimental units", then in the Results section you should have "Bacterial populations in experimental units".

Table 2: Counts of the Various Microbial groups in Different Organic Fertilizers

Organic fertilizers	Microbial Groups (cfu/g)			
	THBC Mean ± SD	HUB Mean ± SD	TFC Mean ± SD	HUF Mean ± SD
Ft ₀	5.9 x10 ⁷ ± 0.02	7.9 x10 ³ ± 0.02	8.0 x10 ⁴ ± 0.5	6.0 x10 ³ ± 0.02
Ft ₂	3.6 x10 ⁷ ± 0.01*	4.2 x10 ⁴ ± 0.02*	6.8 x10 ⁴ ± 0.05*	3.9 x10 ⁴ ± 0.02*
Ft ₄	3.4 x10 ⁷ ± 0.2*	3.0 x10 ⁴ ± 0.02*	5.1 x10 ⁴ ± 0.2*	2.5 x10 ⁴ ± 0.02*

Key: THBC = Total heterotrophic bacterial counts; TFC = Total fungal counts; NUB = Nitrate utilizing bacteria; PSC = Phosphate solubilizing bacterial counts; SD = Standard deviation from mean; HUB = Hydrocarbon Utilizing Bacteria; HUF = Hydrocarbon Utilizing Fungi; * = Significance at P < 0.05

Table 3: Mean Microbial Counts in Soils Receiving Different Organic Fertilizer Treatments during Remediation

Time (week)	Microbial Groups	Microbial Counts per Fertilizer per Treatment Level										
		Controls		ScF ₀ (%)				ScF _{t₂} (%)			ScF _{t₄} (%)	
		S ₀	Sc _{t₀}	5	10	15	5	10	15	5	10	15
0	THBC (cfu/g)	2.6×10 ⁸	6.1×10 ⁷	2.8×10 ⁷	2.9×10 ⁷	3.2×10 ⁷	2.2×10 ⁷	2.8×10 ⁷	1.9×10 ⁷	1.2×10 ⁷	2.3×10 ⁷	1.6×10 ⁷
	TFC (cfu/g)	2.3×10 ³	1.7×10 ³	1.1×10 ³	1.4×10 ³	1.2×10 ³	2.0×10 ³	1.9×10 ³	2.0×10 ³	5.0×10 ⁴	1.4×10 ⁵	8.0×10 ⁴
	HUBC (cfu/g)	7.8×10 ³	4.6×10 ⁴	1.0×10 ³	2.6×10 ³	3.0×10 ³	5.6×10 ⁴	4.3×10 ⁴	1.3×10 ⁴	2.2×10 ⁴	3.6×10 ⁴	4.0×10 ⁵
	HUFC (cfu/g)	8.0×10 ³	1.7×10 ⁴	1.1×10 ³	1.3×10 ³	2.0×10 ³	2.9×10 ⁴	3.0×10 ⁴	1.8×10 ⁴	1.4×10 ⁴	2.0×10 ⁴	6.0×10 ³
2	THBC (cfu/g)	2.4×10 ⁸	3.2×10 ⁷	4.0×10 ⁷	4.8×10 ⁷	6.0×10 ⁷	9.8×10 ⁷	6.2×10 ⁷	1.8×10 ⁶	1.7×10 ⁷	5.4×10 ⁷	6.8×10 ⁷
	TFC (cfu/g)	2.0×10 ⁵	6.3×10 ⁴	2.2×10 ⁵	2.8×10 ⁵	2.4×10 ⁵	7.4×10 ⁴	4.3×10 ⁴	1.4×10 ⁵	2.0×10 ⁴	4.0×10 ⁴	2.1×10 ⁴
	HUBC (cfu/g)	7.3×10 ³	5.2×10 ⁴	6.2×10 ³	7.6×10 ³	6.2×10 ³	6.9×10 ⁴	7.2×10 ⁴	1.0×10 ⁴	2.9×10 ⁴	6.0×10 ⁴	8.1×10 ⁷
	HUFC (cfu/g)	1.8×10 ³	4.8×10 ³	2.2×10 ³	2.7×10 ³	4.1×10 ³	9.0×10 ³	8.9×10 ³	1.9×10 ⁴	1.6×10 ³	7.0×10 ⁴	5.0×10 ³
4	THBC (cfu/g)	8.8×10 ⁷	1.6×10 ⁷	1.4×10 ⁸	9.0×10 ⁷	1.1×10 ⁸	1.6×10 ⁸	8.2×10 ⁷	1.6×10 ⁷	1.2×10 ⁸	7.0×10 ⁷	5.0×10 ⁷
	TFC (cfu/g)	1.9×10 ⁵	2.8×10 ⁴	4.4×10 ⁵	5.0×10 ⁵	5.2×10 ⁵	1.3×10 ⁵	3.5×10 ⁴	1.0×10 ⁵	1.1×10 ⁵	2.5×10 ⁴	8.0×10 ³
	HUBC (cfu/g)	5.4×10 ³	1.2×10 ⁵	1.2×10 ⁴	1.5×10 ⁴	1.0×10 ⁴	2.0×10 ⁵	1.1×10 ⁵	1.6×10 ⁴	3.5×10 ⁵	1.0×10 ⁵	3.1×10 ⁵
	HUFC (cfu/g)	3.8×10 ²	1.0×10 ⁴	4.1×10 ³	5.0×10 ³	7.6×10 ³	1.1×10 ⁴	6.7×10 ³	2.0×10 ⁴	1.0×10 ⁴	4.0×10 ⁴	4.6×10 ³
6	THBC (cfu/g)	6.7×10 ⁷	1.0×10 ⁸	1.6×10 ⁸	1.8×10 ⁸	2.0×10 ⁸	1.9×10 ⁸	1.2×10 ⁸	1.5×10 ⁷	2.0×10 ⁸	8.2×10 ⁷	4.2×10 ⁶
	TFC (cfu/g)	3.2×10 ⁴	1.9×10 ⁴	4.8×10 ⁵	5.2×10 ⁵	5.6×10 ⁵	3.4×10 ⁵	3.4×10 ⁴	3.2×10 ⁵	2.6×10 ⁵	2.2×10 ⁴	3.0×10 ³
	HUBC (cfu/g)	4.9×10 ³	2.6×10 ⁵	3.6×10 ⁴	3.0×10 ⁴	2.6×10 ⁴	3.6×10 ⁵	1.6×10 ⁶	1.3×10 ⁴	1.9×10 ⁵	2.3×10 ⁵	2.8×10 ⁵
	HUFC (cfu/g)	1.8×10 ²	2.3×10 ⁴	1.4×10 ⁴	1.5×10 ⁴	2.0×10 ⁴	4.0×10 ⁴	1.8×10 ⁴	2.1×10 ⁴	1.6×10 ⁴	4.4×10 ⁴	7.0×10 ⁴
8	THBC (cfu/g)	5.9×10 ⁶	1.6×10 ⁸	1.5×10 ⁸	1.8×10 ⁸	1.9×10 ⁸	2.0×10 ⁸	2.4×10 ⁷	2.6×10 ⁷	1.3×10 ⁸	1.2×10 ⁷	6.8×10 ⁷
	TFC (cfu/g)	2.8×10 ⁴	9.2×10 ³	4.2×10 ⁵	5.0×10 ⁵	5.2×10 ⁵	3.3×10 ⁵	1.6×10 ⁴	3.0×10 ⁵	2.8×10 ⁵	1.3×10 ⁴	2.1×10 ⁴
	HUBC (cfu/g)	3.2×10 ³	3.5×10 ⁵	3.2×10 ⁴	2.9×10 ⁴	2.8×10 ⁴	4.2×10 ⁵	2.1×10 ⁵	1.8×10 ⁴	2.0×10 ⁵	1.5×10 ⁵	4.0×10 ⁴
	HUFC (cfu/g)	1.6×10 ²	3.6×10 ⁴	1.5×10 ⁴	1.5×10 ⁴	2.1×10 ⁴	3.8×10 ⁴	1.5×10 ⁴	1.1×10 ⁴	1.5×10 ⁴	1.0×10 ⁴	6.0×10 ⁴
10	THBC (cfu/g)	6.4×10 ⁵	1.2×10 ⁷	8.0×10 ⁷	9.2×10 ⁷	9.0×10 ⁷	3.7×10 ⁸	1.8×10 ⁷	1.9×10 ⁷	1.4×10 ⁸	8.9×10 ⁶	6.9×10 ⁷
	TFC (cfu/g)	2.3×10 ⁴	1.8×10 ⁴	3.0×10 ⁵	3.2×10 ⁵	3.8×10 ⁵	6.0×10 ⁵	1.8×10 ⁴	8.4×10 ⁵	4.2×10 ⁴	1.1×10 ⁴	1.1×10 ⁴
	HUBC (cfu/g)	1.8×10 ³	1.5×10 ⁵	3.0×10 ⁴	2.4×10 ⁴	2.6×10 ⁴	3.8×10 ⁵	3.0×10 ⁵	8.2×10 ⁴	1.8×10 ⁵	1.0×10 ⁵	3.0×10 ⁵
	HUFC (cfu/g)	1.6×10 ²	1.6×10 ⁴	1.1×10 ⁴	1.0×10 ⁴	2.0×10 ⁴	5.0×10 ⁴	1.1×10 ⁴	2.0×10 ⁴	2.1×10 ⁴	3.9×10 ³	8.0×10 ⁴
12	THBC (cfu/g)	4.1×10 ⁵	5.4×10 ⁶	6.0×10 ⁷	7.8×10 ⁷	7.4×10 ⁷	2.9×10 ⁷	1.4×10 ⁷	1.2×10 ⁷	3.0×10 ⁷	5.6×10 ⁶	4.2×10 ⁶
	TFC (cfu/g)	1.2×10 ⁴	1.1×10 ⁴	2.6×10 ⁵	2.2×10 ⁵	3.0×10 ⁵	5.1×10 ⁵	1.6×10 ⁴	1.6×10 ⁵	2.2×10 ⁴	1.1×10 ⁴	1.0×10 ⁴
	HUBC (cfu/g)	1.0×10 ³	1.2×10 ⁵	2.2×10 ⁴	2.1×10 ⁴	1.8×10 ⁴	2.2×10 ⁵	2.4×10 ⁵	7.6×10 ⁴	8.8×10 ⁴	6.4×10 ⁴	3.8×10 ⁴
	HUFC (cfu/g)	1.3×10 ²	1.3×10 ³	2.3×10 ³	6.8×10 ³	1.4×10 ⁴	3.1×10 ⁴	1.2×10 ⁴	1.6×10 ⁴	1.1×10 ⁴	3.4×10 ³	1.9×10 ⁴

Table 4: Total Petroleum Hydrocarbon Biodegradation Analysis after 90 days Treatment.

Parameter	SC ₀ (Control)	Treatments								
		SctFt ₀ 5	SctFt ₀ 10	SctFt ₀ 15	SctFt ₂ 5	SctFt ₂ 10	SctFt ₂ 15	SctFt ₄ 5	SctFt ₄ 10	SctFt ₄ 15
TPH ₀	56.219	56.219	56.219	56.219	56.219	56.219	56.219	56.219	56.219	56.219
TPH ₉₀	51.99	50.249	49.005	51.741	49.751	53.234	52.985	50.751	56.651	56.776
BDR (mgkg ⁻¹ day ⁻¹)	0.047	0.066	0.080	0.050	0.072	0.033	0.040	0.061	-0.149	-0.127
R.E (%)	7.52	10.62	12.83	7.97	11.51	5.31	5.75	9.73	-0.77	-0.99

Key:

TPH₀ = Total Petroleum Hydrocarbon Content in Contaminated Soils before Bioremediation Treatments

TPH₉₀ = Total Petroleum Hydrocarbon Content in Contaminated Soils after 90 days Bioremediation Treatments

BDR = Biodegradation Rate (Hydrocarbon loss)

R.E = Remediation Efficiency

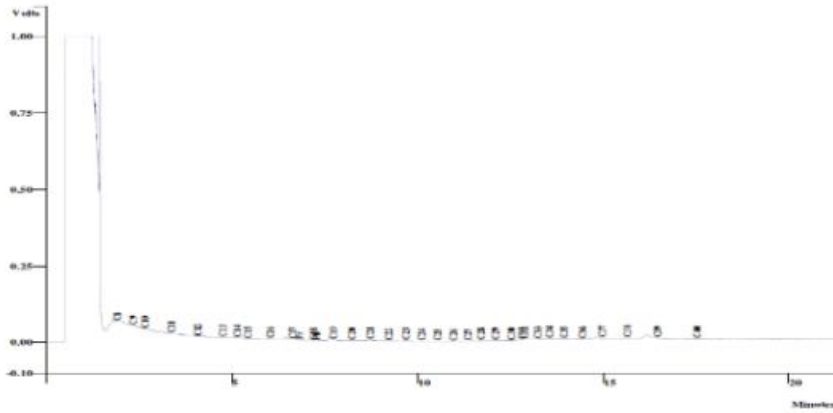


Figure 2: Chromatogram of the Pristine Soil (without any contamination)

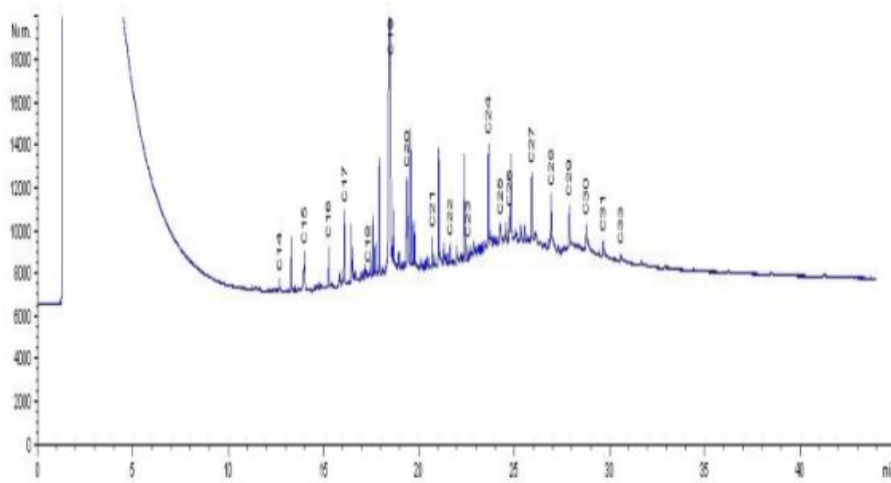


Figure 3: Chromatogram of a 10% (w/w) Spent Engine Oil-contaminated Soil after Stabilizing for two week

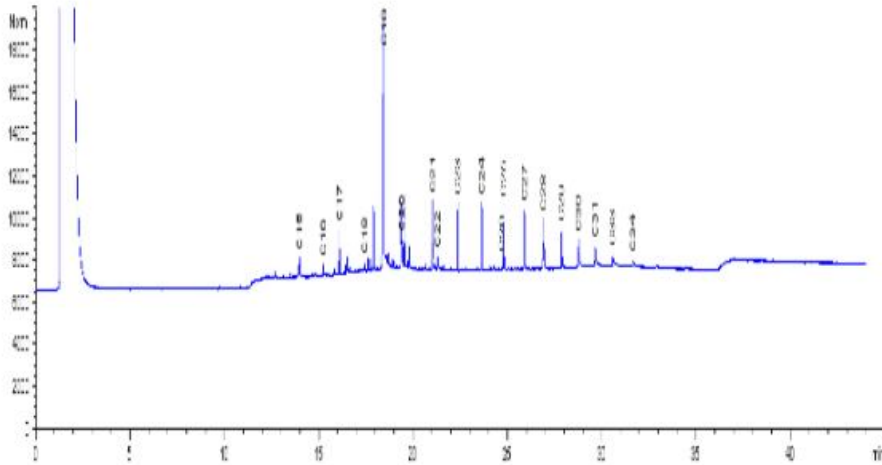


Figure 4: Chromatogram of Contaminated Soil, Treated with 5% F_{t_0} for 90 days.

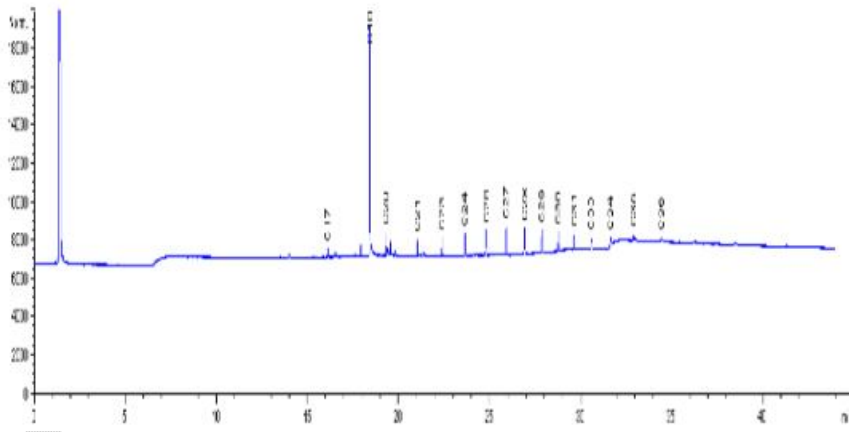


Figure 5: Chromatogram of contaminated soil treated with 5% F_{t_2} for 90 days.

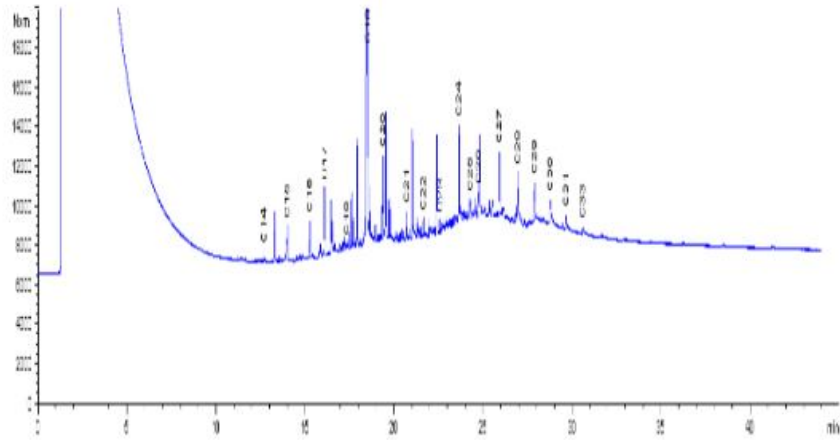


Figure 6: Chromatogram of Contaminated Soil, Treated with 5% Ft₄ for 90 days.

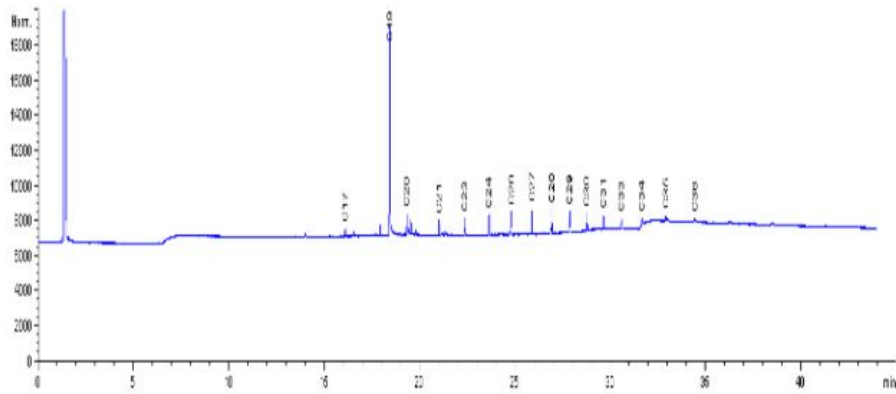


Figure 7: Chromatogram of Contaminated Soil, left without any Treatment with Organic Fertilizer for 90 days.

4.0 Discussion

Comment [LP62]: Your Results section is meaningless, therefore I am unable to review your Discussion section

Composting is a microbiological process that depends on growth and activity of mixed populations of bacteria, actinomycetes, and fungi that are indigenous to the wastes being composted. It is a viable means of transforming various organic wastes into products that can be used safely and beneficially as biofertilizers and soil conditioners. In this study, population of different microbial groups, including total aerobic heterotrophic bacteria counts (THBC), hydrocarbon utilizing bacteria (HUB), total fungal counts (TFC) and hydrocarbon utilizing fungi (HUF) revealed higher counts of HUB, and HUF in organic fertilizers from composting process spiked with SLO (Ft₂ and Ft₄) compared to the unspiked (control) composting (Ft₀). This observation suggest strongly that the spent lubricating oil (SLO), apart from being potent soil pollutant, also serve as agent of natural selection that drives mutations among microbial groups. Most microbes that were able to thrive in the spiked composting processes are those that had evolved the ability to survive the toxic effects of SLO, a typical hydrocarbon product.

Bio-stimulation, occasioned by the addition of different types and concentrations of organic fertilizers to the polluted soil, resulted in a significant increase in population of hydrocarbon degrading bacteria and fungi within the first few weeks. This is due mainly to the fact that organic fertilizers provided nutrients for increased cell growth and enhanced biodegradation rate. The SLO also supported rapid microbial growth since hydrocarbon is an energy source to some bacteria and fungi. The reduction in population of the hydrocarbon degraders, observed in later weeks, could be due to the fact that the organisms have exhausted the available nutrient supplies present in the batch system. Also, mineralization of hydrocarbons could have possibly accumulated the system with toxic metabolites which resulted in reduced growth phase of the microbes. The findings of Amadi and Odu (1993); Akpoveta *et al.* (2011) who reported an initial gradual increase in bacterial population following the application of petroleum hydrocarbon, but a decline as the biodegradation progressed supports these observations.

Comment [LP63]: NRL

Comment [LP64]: NRL

Notably, higher counts of hydrocarbon degraders were observed in contaminated soils treated with SLO-spiked compost compared to those treated with pristine compost. This may not be unconnected with the higher counts of hydrocarbonoclastic bacteria and fungi observed in the organic fertilizers produced from SLO-spiked composting processes as against lower counts in non-SLO-spiked composting process. This suggest strongly that the SLO served as a source of natural selection

or mutagen that drives evolution of microbes capable of hydrocarbon degradation during the composting processes in the compost bins that were spiked with SLO.

Biodegradation of spent lubricating oil (SLO) in soil, as revealed by chromatographic analysis, at the end of study (90 days) showed higher biodegradation rate (BR) in soil amended with 5% of the organic fertilizers spiked with lower concentration of SLO compared to the control soil treatment. At the end of 90 days, polluted soils amended with spiked organic fertilizers showed highest percentage of SLO biodegradation, followed by soil amended with pristine organic fertilizers, compared to the unamended (control) soil that showed lowest biodegradation of SLO. These observation correlates positively with the increased counts of HUB and HUF earlier observed in the different organic fertilizers. However, it was observed that BR reduces with increase in quantity of spiked organic fertilizers applied. For instance, at 5% application of spiked organic fertilizer (Ft₂), BR was 0.072 mgkg⁻¹day⁻¹, but reduces to 0.033 mgkg⁻¹day⁻¹ at 10% application. This may be due to carryover of toxicants in the spiked fertilizers. Conversely, the remediation efficiency (RE) of pristine organic fertilizers increased with increase in quantity of fertilizer applied. At 5% application, RE was 10.62%, and 12.83% at 10% application. However, at 15% application, the RE took a downward turn to 7.97%. These observations are well supported by the findings of Emmanuel *et al.* (2021); Agamathu, *et al* (2013); Akpoveta *et al.* (2011); and Chorom *et al.* (2010) who reported increased counts of bacteria and fungi in soils following addition of organic amendments to polluted soils and a corresponding improvement in biodegradation of spent lubricating oil (SLO) in soils.

Comment [LP65]: NRL

Comment [LP66]: NRL

However, it was also observed that organic fertilizers from composting process spiked with elevated concentration of SLO (Ft₄) was grossly inefficient as soil amendments, since its application resulted in an increased TPH concentration in soils. This suggested that the compost spiking technique should be done with very low concentration of the contaminant to avoid build-up of contaminant in compost (organic fertilizer).

5.0 Summary and Conclusion

Comment [LP67]: DELETE

Whereas there exist a dire need to re-use or recycle the huge proportion of organic wastes generated by agricultural and food processing activities to reduce the volume of organic wastes that ultimately get to the dump-sites, curb their potential risk of pollution and environmental nuisance.

Similarly a growing expanse of wastelands occasioned by petroleum contamination is begging for remediation. This study manipulated aerobic composting technology to convert organic wastes into organic fertilizers fit as agents in bioremediation technology to enhance the decontamination of soils polluted with petroleum hydrocarbon compounds. Technique employed included the spiking of the composting piles with varying concentration of the contaminant to induce adaptation among microbes inhabiting the organic fertilizers. Based on the findings of this study it is safe to conclude that:

- (i) Organic wastes, which constitute a nuisance to the environment, are rich sources of nutrients, which can be converted into different types of organic fertilizers through manipulated aerobic composting technique.
- (ii) Spiking of Composting process with low concentration of hydrocarbon compounds increased viable count of hydrocarbon degrading microbes in the resulting compost (organic fertilizers).
- (iii) Application of low quantity of spiked compost to polluted soils stimulates the growth of hydrocarbon degrading microbes, with a corresponding increase in hydrocarbon degradation rate in treated soils.
- (iv) Hydrocarbon-spiked organic fertilizers (Ft_2), and pristine organic fertilizers (Ft_0) are effective agents for bioremediation of petroleum polluted soils.

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