Original Research Article

Microbial Population Dynamics and Plant Performance in Spent Lubricating Oil (SLO) Polluted Soils Amended with SLO-spiked Organic Fertilizers.

Comment [H1]: Microbial and Plant Assessment in Spent Lubricating Oil (SLO) Cultivated Soils Amended with Organic Fertilizers

Abstract

This study evaluated the changes in microbial population and performance of plant in spent lubricating oil polluted soils amended with different organic fertilizers. The fertilizers were produced from organic waste materials using aerobic composting technique; pollution was simulated in potted soils; soil toxicity were determined using *Zea mays* L. as test crop; microbial counts and physicochemical properties of the test soils were determined using standard microbiological and chemical protocols respectively. Apart from significant($P<0.05$) decrease in population of total heterotrophic bacteria (THB) and total fungal counts (TFC) (2.6×10^8 to 6.1×10^7 cfu/g and 2.3×10^5 to 1.7×10^5 cfu/g respectively), and increase in populations of hydrocarbon utilizing bacteria (HUB) andhydrocarbon utilizing fungi (HUF) (7.3×10^3 to 4.6×10^4 cfu/g and 8.0×10^3 to 1.7×10^4 cfu/g respectively) following contamination of soil with SLO at pollution level., results also revealed increase (improvements) in counts of all microbial groups at the end of remediation treatments. Mean microbial count in soils amended with different levels of fertilizer treatments (5%, 10%, and 15%) reflected a dose-dependent increase as follows: $Ft_2 > Ft_0 > Ft_4$ for the 5% (3.7×10^8 cfu/g), 10% (9.2×10^7 cfu/g) and 15% (6.9×10^7 cfu/g) respectively. At 5% application, post remediation pH increased following the order: $Ft_0 > Ft_2 > Ft_4$ (6.00, 5.34, and 4.90 respectively). The test crop, *Zea mays* L. recorded 100% and 62.5% germination in amended and unamended soils respectively. Leave length and chlorophyll index of *Z. mays* L. grown on remediated soils ranged between 35.10±0.40 – 52.85±0.05 (at 5% treatments); 32.60±0.10 – 56.55±0.35 (at 10% treatments); and 35.35±0.15 – 42.45±0.25 (at 15% treatments), compared with 30.30±0.80 – 50.55±0.75 (for PS) and 18.05±0.85 – 25.50±0.70 (for unamended CS). All test crops yielded except those grown on unamended soils. Conclusively, application of organic fertilizers to SLO polluted soils increased population of different groups of soil microbes, leading to increased breakdown of the pollutant and reduced soil toxicity.

Comment [H2]: Start off with an introductory statement, indicating a problem to be addressed and justification for this research.

Comment [H3]: This is far too long a sentence, please recast.

Comment [H4]: Not necessary, expunge the word conclusively.

Key words: Spent lubricating oil, soil, environment, contaminants, pollution, bioremediation, germination

1.0 Introduction

Environmental stress resulting from various waste streams and petroleum products is a source of concern, especially in developing countries as it contaminates various ecosystems namely soil, water and air. Going byBamiro and Osibanjo (2004), Nigeria generates well over 200 million litres of spent lubricating (automobile engine) oil per annum. This waste oil, which rarely have any secondary application, is disposed unguardedly into gutters, water drains, open vacant plots and farms by auto

mechanics and allied artisans (Echiegu*et al*., 2021), causing more widespread pollution than crude oil spillage (Odjegba and Sadiq, 2002).

Petroleum hydrocarbons are not easily degraded and can adversely affect soil productivity and cause different degrees of harm to microorganisms (Mambwe*et al*. 2021). The entry of petroleum-based substances into the soil has caused a dramatic increase in soil organic carbon content, resulting in an imbalance between soil C content and N content (He *et al*. 2021; Zhang *et al*. 2018; Wen *et al*. 2017). This imbalance destroys the habitat for soil microbial life and changes the number and diversityof soil microbial populations (Zheng*et al*. 2021; Dong 2020). Furthermore, microorganisms play a very important role in decomposition of plant and animal residues; participate in biogeochemical cycling thus, maintaining soil ecosystem functions and promote soil C, N, S, P, and other nutrient (Pan *et al*. 2012; Li *et al.* 2017); and regulate soil material and energy cycle (Iyobosa*et al.* 2021; Kong *et al*. 2021; Wang *et al*. 2021).

Moreso, petroleum oil contaminants contain potentially phyto-toxic polycyclic aromatic hydrocarbons (PAHs), as well as a vast array of heavy metals. According to Kirk *et al.* (2005), in petroleum contaminated soils, plant growth is typically limited by nitrogen and phosphorus as a result of the overabundance of carbon from the petroleum hydrocarbons. They further stated that due to the hydrophobic nature of the contaminants, water and water-soluble nutrients are often limited. In the same vein, the effect of oil on seed germination has been shown to be inhibitory due to unfavourable soil conditions (Agbogidi, 2010; Adewole and Moyinoluwa, 2012). It was reported that upon drying, the soils contaminated with oil became too hard to allow germination. Also, the reduced oxygen content of the soil due to the blockage of pores in the soil and increased water stress on the seed imposed negative effects on germination. The impacts include loss of soil productive capacity, growing expanse of waste-lands, with its far-reaching economic implications.

Bioremediation is “a managed or spontaneous process, in which biological activities, especially microbial catalysis occurs on pollutants, thereby remedying or eliminating environmental contamination”. 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 of contaminants (Babak, 2013). It mainly involves biostimulation where organic or inorganic components were introduced to enhance indigenous microbial growth that directly

degrades the contaminants. This study constitutes part of the search for an ideal bioremediation technology for petroleum polluted soils.

2.0 METHODS

2.1 Organic Fertilizer Production

Organic fertilizers were produced following the aerobic composting technique of Haydar and Masood (2011). Three composting pads (A, B, C) consisting of varying quantities of nitrogen-rich and carbon-rich organic materials in desired proportions, as described by George*et al*. (1993), were constructed and spiked with 0%, 2% and 4% SLO respectively. All factors affecting composting process, viz: moisture content, temperature, pH, aeration/oxygen supply, and particle size were kept at optimum, according to the methods described by Parr *et al*. (2010); Ekpo and Ntekpe (2014).

2.2 Microcosm Set-up / Field Test

The potency of produced organic fertilizers as amendment on SLO polluted soils was tested through Randomized Complete Block Design experiment as described by Hadeel (2021). Bulk soil taken from an agriculture field was air-dried and filtered, by passing through a 2mm sieve. 300.0kg of the filtered soil was placed in a large polythene sheet. The soil was artificially contaminated with 10% (w/v) spent lubricating oil. 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; Akpoveta*et al*., 2011; Agamuthu*et al*., 2013). 8.0kg each of the polluted soil was placed in thirty one (31) clean dry perforated plastic containers of 9887.43cm^2 (approx. 9.89L) capacities; divided into four (4) groups (I, II, III, and IV) in triplicates as shown in Plate 1.0.

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Plate 1.0: Polluted Potted Soil Samples during Remediation Treatment

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These polluted soil samples were allowed undisturbed for two weeks to stabilize. Groups **I, II, & III** were amended with varying quantities (5%, 10%, and 15%) of organic fertilizers **C**, **A &**, **B** respectively. The moisture contents were adjusted to 60%, using tap 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); Choron*et al*. (2010); Agamuthu *et al*. (2013). The remediation experiment lasted for ninety (90) days.

2.3 Laboratory Analyses

Physicochemical Analysis

The soil samples were air-dried, crushed with mortar and passed through a 2mm sieve and stored in polythene bags prior to analysis. Soil physicochemical characteristics such as soil texture, pH, total organic carbon (TOC), total organic matter (TOM), Carbon/Nitrogen ratio, total nitrogen, total phosphorus, soil conductivity (ECe) and heavy metals [vanadium (v), lead (Pb), nickel (Ni), cadmium (Cd), and chromium (Cr)] were determined before contamination, one week after contamination, and after remediation.Soil texture was determined according to Bouyoucos (1975) while bulk density test followed the core method of Blake and Hartge as described by Ntekpe (2014).

The pH of each sample was determined by means of glass electrode pH meter (McLean, 1982). Carbon contents of the fertilizer samples were determined using the Walkely and Black (1934).Nitrogen contents of the fertilizer and soil samples were determined by the Kjeldhal method. Phosphorus (P) was estimated using the Bray P-l method (Jackson, 1979; Bowman, 1988). Exchangeable Cations (Ca^{++}, Mg^{++}, K^{+}, and Na^{+}) were determined by flame photometry methods of AOAC (2005). Particle Size Distribution was determined by the hydrometer method as described by Udo*et al*. (2009). The Electrical Conductivity of the soil samples was determined using the Conductivity meter, after the method of AOAC (2005). Exchangeable acidity was determined with one normal potassium chloride solution following the methods of Udo *et al.* (2009). Organic Matter was determined by the dichromate wet oxidation method as described by Nelson and Sommers (1996). Effective Cation Exchange Capacities (ECEC) was determined by the method described by Lute (1986). The summation method described by Jackson (1979) was adopted to determine the effective cation exchange capacity.

Enumeration of Microorganisms

Total Aerobic Heterotrophic Bacteria (TAHB) in organic fertilizers and soil samples at various intervals were enumerated by surface plating technique on nutrient agar fortified with nystatin (anti-fungal drug), as described by Cheesbrough (2010).

Hydrocarbon utilizing bacteria (HUB) in the fertilizer and soil samples were enumerated using oil agar (OA) having the following composition:1.8g K_2HPO_4, 4.0g NH_4Cl, 0.2g $MgSO_4.7H_2O$, 1.2g KH_2PO_4, 0.01g $FeSO_4.7H_2O$, 0.1g NaCl, 20g agar, 1ml used engine oil in 1000ml distilled water, according to the method of Zajic, as reported by Agamuthu*et al*. (2013). The OA plates were incubated at $30^{o}C$ for 5 days before the colonies were counted.

The fungal load in the organic fertilizer and soil samples were determined by surface plating technique, using Sabouraud Dextrose Agar (SDA), fortified with streptomycin to discourage bacterial growth, as described by APHA (1998).

2.4 Agronomic Parameters

Plant toxicity test using maize (*Zea mays* L.) was conducted on the thirty (30) potted-soils laid out in Plate 1.0 above. Some growth parameters and yield of maize (*Zea mays* L.) were assessed. Relative chlorophyll content (chlorophyll index) of *Zea mays* planted on various treatment were determined by light transmittance/absorbance, using the FT Green LLC, USA chlorophyll meter, model: PN: 0131, SN: 0903-00100145 class B according to the methods of Huang *et al*. (2022).

Statistical Analysis

Treatment means were obtained from each groups and this value were subjected to two-factor analysis of variance to assess significant differences within and between the groups. Significant differences were separated using Least Significant Differences test (LSD). The **s**tatistical package for social sciences (SPSS, Version 18.0) was employed for this purposed as described by Okon, *et. al.*(2019) and Mbong, *et. al.* (2022).

Comment [H7]: *et al*

2.5 Ethical Considerations

Interest and concerns of all participants were respected to avoid conflicts. Due permissions were sought and obtained, as deemed necessary, in the course of this research. The materials and authors cited in this report are duly acknowledged using the reference list.

3.0 RESULTS

Comment [H8]: Results weren't explained at all, you need to adequately state the results you obtained and in this case show the level of significance the treatments used had on the study. Since you used a statistical package.

Table 1.0: Mean Microbial Counts in Soils Receiving Different Organic Fertilizer Treatments during Remediation

Time (week)	Microbial Groups (cfu/g)	Microbial Counts per Fertilizer per Treatment Level										
		Controls		SctC (%)			SctA (%)			SctB (%)		
		S_0	Sct_0	5	10	15	5	10	15	5	10	15
0	THBC	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	2.3×10^{5}	1.7×10^{5}	1.1×10^{5}	1.4×10^{5}	1.2×10^{5}	2.0×10^{5}	1.9×10^{5}	2.0×10^{5}	5.0×10^{4}	1.4×10^{5}	8.0×10^{4}
	HUBC	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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	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}

Key: S_0 = Pristine soil; Sct_0 = Polluted soil without any treatment (control); SctC = Polluted Soil amended with pristine organic fertilizer; SctA = Polluted Soil amended with organic fertilizer from 2% SLO spiked composting process; SctB = Polluted Soil amended with organic fertilizer from 4% SLO spiked composting process

Table 2.0: Physicochemical Properties of Soil Samples before Contamination, after Contamination, and during Remediation with Different Organic Fertilizers

Parameter		Week	S_0	Sct_0	Samples									Textural Class
					SctC (%)			SctA (%)			SctB (%)			
					5	10	15	5	10	15	5	10	15	
pH		0	5.45	4.70	4.70	4.70	4.70	4.70	4.70`	4.70	4.70	4.70	4.70	-
		4	5.40	4.40	5.80	5.76	5.90	4.38	4.00	4.34	4.62	4.45	4.50	-
		8	5.42	5.00	6.68	6.00	6.70	5.80	4.80	4.83	5.02	5.12	4.80	-
		12	5.44	4.90	6.00	5.67	5.66	5.34	5.18	4.80	4.90	5.00	4.67	-
N (%)		0	0.26	0.04	0.08	0.06	0.07	0.08	0.07	0.06	0.05	0.04	0.04	-
		4	0.20	0.06	0.11	0.11	0.10	0.12	0.10	0.09	0.06	0.06	0.05	-
		8	0.16	0.07	0.12	0.11	0.12	0.18	0.12	0.11	0.09	0.08	0.07	-
		12	0.17	0.07	0.14	0.13	0.14	0.21	0.12	0.13	0.09	0.08	0.07	-
Exchangeable Bases (Cmol/kg)	K	0	0.10	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	-
		4	0.11	0.07	0.10	0.11	0.09	0.11	0.10	0.06	0.08	0.07	0.05	-
		8	0.09	0.05	0.14	0.15	0.14	0.16	0.12	0.08	0.09	0.08	0.06	-
		12	0.09	0.04	0.14	0.17	0.18	0.20	0.16	0.13	0.09	0.10	0.08	-
	Mg	0	6.28	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	3.10	-
		4	6.29	3.00	3.16	3.22	3.25	3.30	3.24	3.14	3.11	3.14	3.09	-
		8	6.22	3.06	3.28	3.37	3.39	3.63	3.37	3.26	3.14	3.21	3.12	-
		12	6.19	3.16	3.38	3.49	3.51	4.04	3.58	3.47	3.20	3.21	3.19	-
	Ca	0	5.40	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	-
		4	5.40	3.10	4.00	4.10	4.04	4.10	3.80	3.90	3.80	3.60	3.30	-
		8	5.30	3.10	4.20	4.10	4.06	4.30	3.80	3.70	3.40	3.40	3.30	-
		12	5.20	3.00	4.20	4.30	4.06	4.30	4.10	3.50	3.40	3.40	3.20	-
	Na	0	0.11	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	-
		4	0.10	0.05	0.08	0.11	0.09	0.07	0.08	0.07	0.08	0.07	0.06	-
		8	0.11	0.06	0.10	0.12	0.10	0.11	0.09	0.09	0.08	0.08	0.07	-
		12	0.12	0.09	0.10	0.12	0.10	0.12	0.09	0.10	0.10	0.08	0.08	-
Available P ($mgkg^{-1}$)		0	57.97	54.30	54.30	54.30	54.30	54.30	54.30	54.30	54.30	54.30	54.30	-
		4	56.82	50.10	53.30	55.10	54.80	55.10	54.81	54.00	53.90	52.56	51.59	-
		8	56.78	46.50	50.20	55.58	56.00	56.70	55.12	54.80	52.00	50.67	49.20	-
		12	57.52	47.00.	51.00	56.20	56.20	56.70	55.14	55.12	51.34	48.40	47.10	-
Organic Carbon (%)		0	9.12	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	17.97	-
		4	8.65	16.78	17.70	17.80	19.78	18.10	18.20	18.10	19.00	19.20	19.20	-
		8	7.80	17.20	18.20	17.80	17.20	17.20	18.20	18.20	18.20	18.80	19.20	-
		12	7.10	17.10	18.10	18.00	17.10	16.50	18.30	17.50	18.10	18.00	19.50	-
Exchangeable Acidity (Cmol/kg)		0	1.86	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	-
		4	1.75	1.42	1.42	1.48	1.48	1.52	1.46	1.46	1.47	1.44	1.42	-
		8	1.75	1.43	1.43	1.52	1.46	1.58	1.46	1.48	1.46	1.45	1.40	-
		12	1.71	1.43	1.43	1.52	1.49	1.61	1.50	1.48	1.46	1.45	1.38	-
EC (ds/cm)		0	0.054	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	0.034	-
		4	0.045	0.032	0.035	0.037	0.038	0.042	0.036	0.036	0.035	0.034	0.032	-
		8	0.044	0.032	0.037	0.038	0.039	0.046	0.038	0.038	0.037	0.036	0.035	-
		12	0.045	0.036	0.037	0.038	0.040	0.050	0.039	0.038	0.037	0.036	0.035	-
ECEC (Cmol/kg)		0	13.75	8.66	8.73	8.73	8.73	8.73	8.73	8.73	8.73	8.73	8.73	-
		4	13.65	7.65	8.76	9.02	8.95	9.16	8.84	8.65	8.53	8.32	7.88	-
		8	13.47	7.70	9.15	9.26	9.15	9.78	8.84	8.61	8.17	8.22	7.95	-
		12	13.31	7.72	9.25	9.60	9.34	10.27	9.43	8.68	8.25	8.24	7.93	-
Particle Size Analyses (%)	SA	Sand					63.20							
		Silt					19.14							Sandy loam
		clay					16.03							
	SB	Sand					66.20							
		Silt					19.16							Sandy loam
		Clay					16.55							

Key: S_0= Pristine soil; Sct_0 = Contaminated soil without treatment; SctC = Contaminated soil amended with pristine fertilizer; SctA = Contaminated soil amended with 2% SLO-spiked fertilizer F_2; SctB = Contaminated soil amended with 4% SLO-spiked fertilizer; SA = Soil particle size before contamination; SB = Soil particle size after remediation.

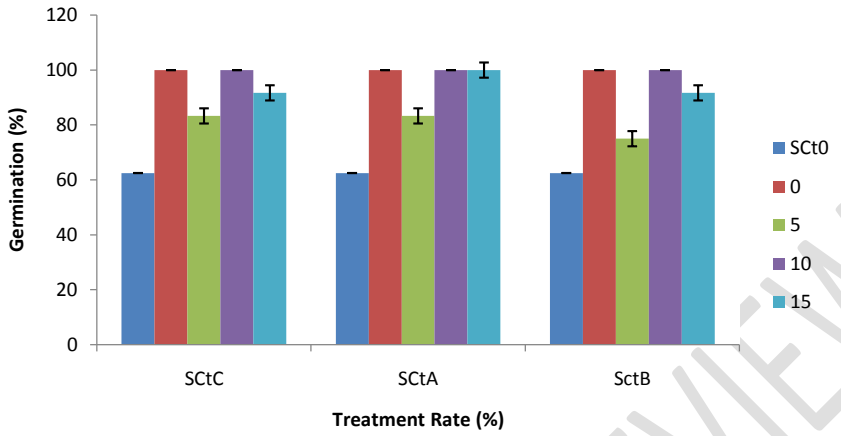

Figure 1.0: Germination rate of *Zea mays* L. Grown on SLO Contaminated Soil, Amended with Different Organic Fertilizers

Comment [H9]: Each figure should be self-descriptive, how long was the cultivation before recording germination?
You should indicate the key for each of the figures. Legends needs to be spelt out.
Applicable to all tables and figures, indicate period from cultivation to when parameter of interest is taken or recorded. For instance, plant height after 5 weeks of cultivation etc.

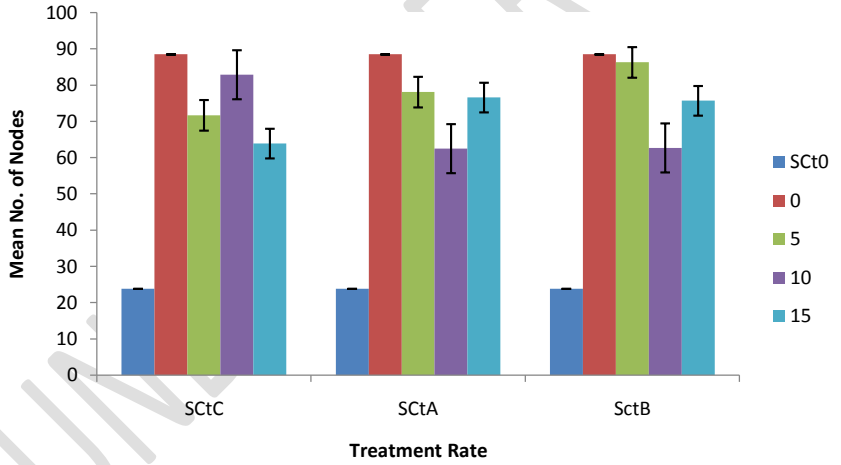

Figure 2.0: Growth of *Zea mays* L. Grown on SLO Contaminated Soil, Amended with Different Organic Fertilizers

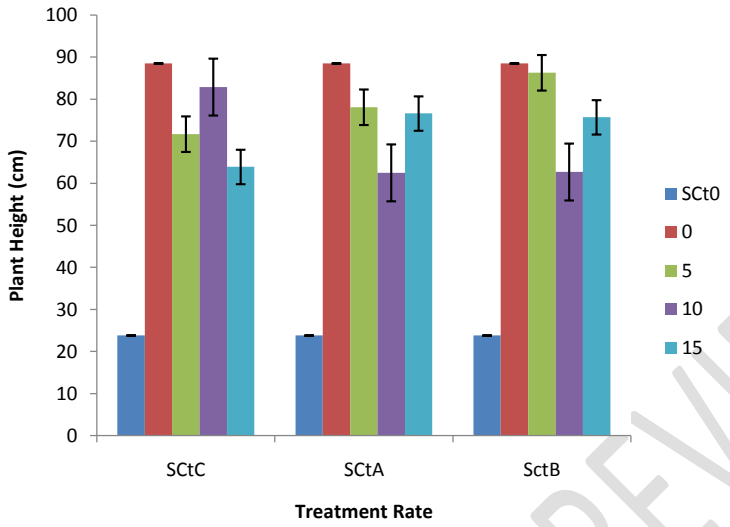

Figure 3.0: Height of *Zea mays* L. Grown on SLO Contaminated Soil, Amended with Different Organic Fertilizers

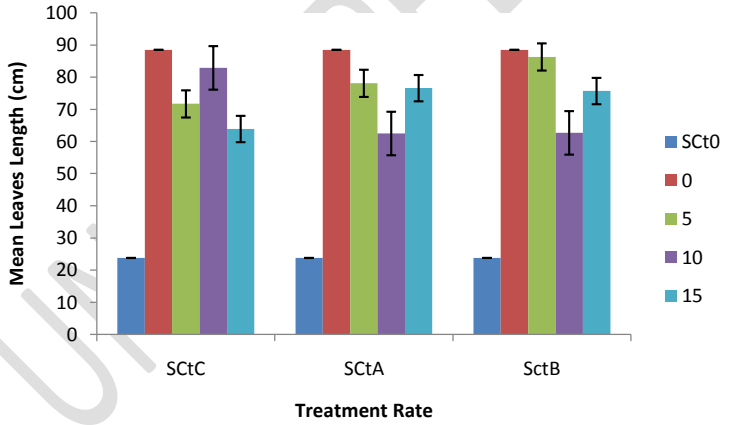

Figure 4.0: Leaves Length of *Zea mays* L. Grown on SLO Contaminated Soil, Amended with Different Organic Fertilizers

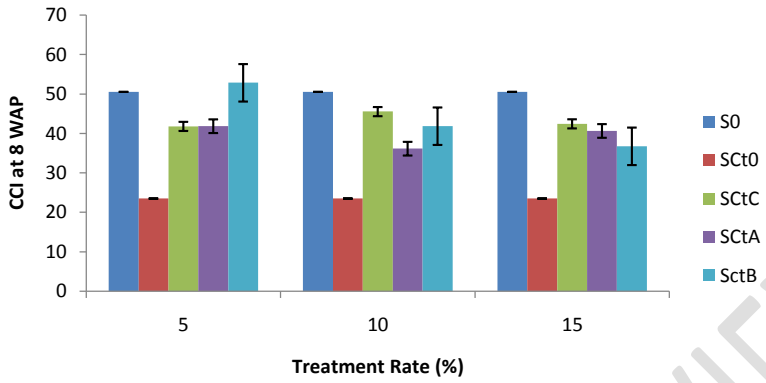

Figure 5.0: Chlorophyll Indices of 8 weeks old *Zea mays* L. plants Grown on SLO Contaminated Soil, Amended with Different Organic Fertilizers

Key: SctC = Contaminated Soil amended with pristine compost (control); SctA= Contaminated Soil amended with compost spiked with 2% spent lubricating oil; SctB= Contaminated Soil amended with compost spiked with 4% spent lubricating oil; Sct_0 = Contaminated soil without any amendment; So = Pristine Soil; CCI = Chlorophyll Concentration Index.

Table 3.0: Yield Per Treatment of *Zea mays* L. Grown on Spent Lubricating oil Polluted Soil Amended with Different Organic Fertilizers

Treatment Code	Treatment Rate (%)	Yield				
		Number of Cobs	Size of Cobs			Total dry Weight of Cobs (g)
			Big	Medium	Small	
SctC	5	4	-	1	3	150
	10	6	-	-	6	110
	15	3	1	-	2	150
SctA	5	3	-	-	3	62
	10	4	-	-	4	56
	15	5	-	1	4	154
SctB	5	4	1	-	3	186
	10	3	-	1	2	100
	15	5	-	1	4	160
Sct_o	-	-	-	-	-	-
S_o	-	4	-	-	4	120

Key:
SctC = Contaminated Soil amended with pristine compost
SctA= Contaminated Soil amended with compost spiked with 2% spent lubricating oil
SctB= Contaminated Soil amended with compost spiked with 4% spent lubricating oil
Sct_o = Contaminated Soil without any amendment – negative control
S_o = Pristine Soil – positive control

4.2 Discussion

Soil health is naturally restored by the biodegradation activities of its indigenous microbial communities after pollution by petroleum hydrocarbons. However, rapid biodegradation depends on the population and types of microorganisms present in the impacted environment. Moreso, biodegradation of hydrocarbons can be enhanced through methods such as, biostimulation (addition of nutrients and/or other factors to promote the growth of indigenous microbes) or bioaugmentation (introduction of known hydrocarbon degraders to the system). Spiking of composting process was done to pre-expose microbes in the fertilizer to the expected contaminant and therefore boasts their ability to degrade SLO.

Contamination of the study soil with spent lubricating oil (SLO), led to a marked decrease in the population of the different microbial groups, as observed in Table 1.0. This was due, in part to the toxic effect of oil on soil microorganisms, as well as by indirect effects connected with

changes in the physicochemical properties of the soil, especially pH alteration. This observation agrees with those of Tang *et al*. (2012) and Lang Arica-Fuentes *et al*. (2014). According to Tamames*et al*. (2010);Bodelier (2011), soil microbes are one of the most diverse on Earth with a very rich microbial pool. The composition of the community at a specific point in time depends highly on environmental conditions, such as pH, organic carbon content, redox potential, moisture, phosphorous and nitrogen content, while fewer influencing factors include the soil texture, temperature, plant community composition, and other biotic factors.

However, despite the fall in populations of other microbial groups, hydrocarbon utilizing bacteria (HUB) and hydrocarbon utilizing fungi (HUF) counts increased markedly. This may be due to the overabundance of carbon from SLO, which provides alternative source of carbon for the growth of bacteria and fungi that can utilize it. This observation is in tandem with those of Ijah*et al.* (2008), and Ekanem*et al*. (2017), who reported increased growth of hydrocarbon utilizing microorganisms in soil following spent engine oil contaminations.

Comment [H11]: Check spaccing

Two weeks into the remediation experiment, it was observed that counts of all microbial groups increased significantly. However, the level of increase attained were treatment dependent. The general increase in microbial populations is due to biostimulation achieved by the addition of nutrient from the fertilizer, and aeration occasioned by routine tilling of the soil. Additionally, the increase in populations of microbes in soil treated with SLO-spiked fertilizers may be due to bioaugmentation, since organic fertilizers from SLO-spiked composting process is believed to habour higher counts of hydrocarbonoclastic microorganisms, consequently polluted soils treated with spiked organic fertilizer recorded the highest counts of hydrocarbon degrading bacteria and fungi. This, expectedly, would lead to increased biodegradation of hydrocarbons in the treated samples.

Comment [H12]: Paragraphing style?

SLO pollution on the soil, as reported in Table 2.0, caused a reduction in pH, conductivity and phosphorus content respectively. These observations were similar to the findings of Akpoveta*et al*. (2011). Reduced pH portends increased acidity which constitutes a serious challenge in agricultural soils since most metal cations, including Hg, Cd, Cu, Ni, Pb and Zn, are readily soluble and available in the soil at low pH (Ntekpe, 2014). The resulting

increased acidity possibly stems from the fact that hydrocarbons harbour many free cations which make them possess properties of a weak acid. As earlier reported by Akpoveta*et al.* (2011), the reduced conductivity could be due to the non-polar nature of SLO resulting in a reduced ionic movement in the soil. Hydrocarbons can act as electron acceptors or oxidizing agents due to the presence of oxygen in them thus producing a reducing environment. This, possibly, may cause oxidation of free phosphorus in the soil to phosphates resulting in the reduced phosphorus content observed in contaminated soils.

The effects of spent lubricating oil polluted soils amended with different organic fertilizers at 5% (w/v) application rate on the germination percentage of *Zea mays* L (Figure 1.0), shows an increase in germination percentage of test crop with increase in the quantities of fertilizer added to polluted soils. Treatment of polluted soils with different organic fertilizers showed significant increase ($p<0.05$) in the germination percentage. Also there were variations in germination percentage among the different levels of fertilizer application with the highest (100%) observed at 10% in all three organic fertilizers. The unamended spent lubricating oil polluted soil had the least germination percentage (62.5%).The low and delayed germination of *Zea mays* L. seeds observed in unamended (non-remediated) soils may be indicative of the degree of soil degradation. Ekundayo*et al.* (2001) and Kirk*et al.* (2005) reported that germination of seeds was delayed in petroleum oil polluted soils due to the phyto-toxic effect of hydrocarbons and oxygen shortage occasioned by blockage of soil pores by oil.

There was significant ($p< 0.05$) increase in growth parameters of *Zea mays* in all the treatments amended with organic fertilizers in the study (Figure 2.0 - 4.0). While there was a significant ($p< 0.05$) decrease in growth parameters of the *Zea mays* in the 0g treatment (SCt_0) of organic fertilizer, also there was variation in plant heights among the different fertilizer application levels, but did not follow any direct proportion to the quantity applied. Plant heights in the amended soils were significantly ($p< 0.05$) higher than the unamended soil.

The number of nodes (Figure 2.0) and leaf length (Figure 4.0) of the *Zea mays* in the spent lubricating oil polluted soils amended with organic fertilizer differed from each other according to the type of organic fertilizer applied. Generally, plants on soil amended with petroleum oil-spiked compost presented longer leaves compared to those from soils amended with pristine

compost. It was observed that the type/quantities of compost had significant effect on the leaf length with the highest effect observed at 5% for $Ft_{2,}$ and Ft_4(86.3±0.2cm and 78.1±2.6cm respectively),whileFt_0recorded the longest leaves (82.9±2.6cm) at 10% application. There was a proportionate increase in the number of nodes as the quantity of the fertilizer increased while there was a significant $p< 0.05$ reduction in the number of nodes in maize grown on the contaminated soils (Sct_0) that receive no organic fertilizer treatment.

There was significant $p< 0.05$ increase in the chlorophyll concentration in the leaves of *Zea mays* in all the treatments amended with organic fertilizers in the study (Table 3.0). While there was a significant decrease in the chlorophyll concentration in the leaves of the *Zea mays* in the 0g treatment (Sc) of organic fertilizer, also there was variation in the chlorophyll concentration in the leaves of plants among the different fertilizer application levels, but did not follow any direct proportion to the quantity applied. Chlorophyll concentration in the leaves of plants from the amended soils were significantly ($p<0.05$) higher than those from the unamended soils (Sc).

Moreso, Yield of *Zea mays* (Table 3.0) in all the treatments amended with organic fertilizers in this study also showed significant ($p< 0.05$) variations. However, there were nil yields of the *Zea mays* in control (Sct_0) soils. The highest yield (186g) was recorded in plants from polluted soils amended with Ft_2 at 5% application level. However, with the exception of plants from the unamended soils (Sc), all other treatments yielded fruits of varying cob sizes and total dry weight.

Comment [H13]: Be consistent with your paragraphing style.

The principle that makes organic fertilizers useful and important in soil fertility maintenance is their impacts on soil nutrients supplies, moisture holding capacity and structural characteristics. Also there existed a positive correlation between the population of microorganisms in soil and the state of such soil. Higher population of microorganisms portends better soil conditions. This implies that a bubbling microbial population in soil led to increased degradation of the contaminant (SLO), with a corresponding detoxification of the soil. As observed in this study, *Zea mays* grown on soils with teaming populations of bacteria and fungi performed better in all indices. In this study, organic fertilizers applications successfully improve the fertility of soils. According to Bremmer (1996), soils are described as fertile if they have the capacity to supply,

in the right quantities and proportions, all the essential nutrients (Nitrogen, Phosphorus, Potassium, Calcium, Magnesium, sulphur, Iron, Manganese, Zinc, Copper, boron, Molybdenum, Chlorine, Carbon, Hydrogen and oxygen) required by plants for healthy growth and development. In agreement with the findings of Murdinah *et al* (2008); Haydar and Masood (2011), the produced fertilizers showed great capacity to boost the supply of key plant nutrients in soils.

CONCLUSION

Comment [H14]: What is your final summation on the different treatments used on the plant and biodegradation process. What can be inferred from this research?

Despite the high concentration of SLO in soil; its known toxicity, and slow rate of decomposition, the soil microbial community not only sustained its quantity but also adapted to the new quality of the environment. Interestingly, the bacterial communities of the study soils showed not only common patterns of response to SLO pollution but also similar biostimulation response following organic fertilizer treatment. SLO pollution of soils also impacted negatively on the soil physicochemical properties, including notable plant nutrients. This study demonstrated that soils heavily polluted by spent lubricating oil have the potential for biological restoration: the counts of bacteria and fungi increased in response to organic fertilizer treatments and surpasses those in unpolluted soils. Also, soil physicochemical properties and plant nutrients improvement correlated positively with increase in microbial counts in soils. The performance of *Zea mays* in this study affirms the phyto-toxicity nature of SLO and the hydrocarbon breakdown achieved by increased microbial population in impacted soils. This suggests strongly, that spiking of composting process with low concentration of a certain contaminant would yield organic fertilizer that may be efficiently deployed as novel bioremediation strategy.

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Comment [H15]: Italicize then ,