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Remediation of a Model Petroleum Hydrocarbon-polluted Soil after Amendment with Nutrient-rich Sludge Obtained from a Beverage Effluent Treatment Plant in Benin City, Nigeria

Beckley Ikhajiagbe[1] and Chinenye C. Chijioke-Osuji[2*]

[1]Department of Plant Biology and Biotechnology, University of Benin, Nigeria.
[2]Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.

Authors' contributions

This work was carried out in collaboration between both authors. Author BI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BI and CCCO managed the analyses of the study. Author CCCO managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The present study investigated the bioremediation of a petroleum hydrocarbon-polluted soil after substrate amendment with nutrient-rich sludge (NRS). Sun-dried top soil was measured into buckets and thoroughly mixed with waste engine oil (WEO) on a weight basis to obtain 5% w/w oil-in-soil. The oil-polluted soil in the buckets were divided into 4 sets of separate treatments including polluted soil only (unamended), polluted soil + 10 g NPK fertilizer, polluted soil + 5%w/w NRS in soil, and polluted soil + 25%w/w NRS in soil. The fifth set of treatments was oil-polluted NRS only (5%w/w). The control was unpolluted soil, unamended. Decrease in heavy metal components of soil was highest in the 25% NRS-amended soil. Total PAH (TPAH) in NPK-amended soils was higher (923.90 mg/kg) when compared to the unamended polluted soil (458.58 mg/kg); this indicated a lower bioremediation efficiency of 38.66% in the former, compared to 69.55% in the

**Corresponding author: E-mail: chinenyechijioke_osuji@yahoo.com;*

latter. Remediation efficiency in the 25% NRS-amended soil (TPAH=260.12 mg/kg) was 82.73%. Phenathrene was totally remediated in the oil-polluted NRS. *Micrococcus varians* was the most prevalent bacteria species obtained in the present study, followed by *Bacillius subtilis*, both being hydrocarbon degraders. The fungi species present were *Aspergillus niger, Penicillium sp. Fusarium solani, Mucor* sp. and *Trichoderma* sp. Phytoassessment of the study using *Vigna unguiculata*, showed improved plant growth response in the NRS-amended oil-polluted soils.

Keywords: Bioremediation; nutrient-rich sludge; natural attenuation; substrate amendment; polyaromatic hydrocarbon.

1. INTRODUCTION

The economic development brought about by petroleum exploration and exploitation in many developing countries like Nigeria, has been accompanied by environmental and socio-economic problems. The demand for petroleum and petroleum products has been on the increase recently; and this is particularly hinged on the ever increasing global population. This apparently constitutes a major source of environmental pollution in the areas concerned.

Attention is gradually being given to pollution caused by oil spills, but no known information yet exists on remediative measures on a large scale that has been earmarked for cleaning up pollution due to the refined products such as engine oil and diesel as well as oily wastes. These kinds of spill attributed to indiscriminate disposal of oil wastes, aside from major crude oil spills from accident or vandalized oil pipelines, have been known to have detrimental impact on agricultural crops [1]. Lubricating oils contain low concentrations of polycyclic aromatic hydrocarbons (PAH); however, waste engine oil (WEO) contain considerably higher concentrations of PAH [2]. They also contain more heavy metals than the unused lubricants [3].

When soil is impacted by oil, conditions necessary for improved plant growth become significantly impaired [4]. Soils become affected with insufficient aeration [5]. Nutrient immobilization resulting from the use of carbon materials as an energy source by microbes results in an increase in nitrogen demand and thus a decrease in available nitrogen in the soil occasioned by increasing oil concentration in soil [6]. It therefore becomes imperative that measures need to be taken to address oil pollution problems. Bioremediation is a very efficient and ecofriendly measure to tackle this problem. For efficient bioremediation, however, soil amendments or additives, such as sawdust, manure, and fertilizers, are added to enhance microbial activities, and also to improve the soil physical properties including water- and nutrient-holding capacity, aeration and water infiltration. Most soil amendments, particular the organic amendments help to increase soil organic matter content and to tie up nitrogen in the soil [7]. The effectiveness of a soil depends on how mostly effective it is thoroughly mixed into the soil. In the present study, nutrient-rich sludge (NRS) has been used as amendment material.

Sludge, among others, is the settled suspension obtained from conventional industrial processes. Once stabilized, the organic carbon in the sludge is desirable as a soil conditioner. There are several treatment methods for sludge; stabilization, thickening, dewatering, drying and incineration. However, incineration of sludge is highly disregarded because of air pollutants in the emissions. Sludge incineration is also associated with the high cost of supplemental fuel, making it less attractive and less commonly constructed means of sludge treatment and disposal. In some developing countries, after centrifugation, the sludge is then completely dried by sunlight, after which the nutrient rich sludge (NRS) are then provided to farmers to use as a natural fertilizer. This method has reduced the amount of landfill. This is common practice in a very popular beverage industry in Benin City, Nigeria, from where the NRS used in the present study was obtained.

Given the fact that the distribution of NRS to farmers in many developing countries is indicative of its rich nutrient status, the present study aims to investigate its effects in the remediation of an oil-polluted soil, thereby enhancing value addition for the re-use of NRS, which is ordinarily supposed a waste.

The aim of the study was to investigate the bioremediation of a petroleum hydrocarbon-polluted soil after substrate amendment with nutrient-rich sludge (NRS) from the breweries and NPK fertilizers. It is acknowledged that bioremediation of crude oil polluted soil is inherently difficult due to the low nutrient deficiency especially regarding low nitrogen and high carbon content. Beverage industries produce a range of effluents and sludge which can be mixed to provide a nutrient rich source for microbial decomposition of contaminated soils.

2. MATERIALS AND METHODS

Top soil (0-10 cm) was collected using a hand trowel and carefully sun air-dried to constant weight. Thereafter 10 kg of the soil dried soil was measured separately into buckets, having 5 perforations made by a 2 mm diameter nail. Waste engine oil (WEO) was poured into each bucket of soil and thoroughly mixed on a weight basis to obtain 5% w/w oil-in-soil. The polluted soils were then prepared for a second round of treatment, where each bucket would be amended with NRS (nutrient-rich sludge) on weight basis. The set up was divided into 4 sets. To the first set, 10 kg polluted soil was amended with 10 g NPK (15:15:15) fertilizer [8]. The second set consisted only of 10 kg oil-polluted soil (no amendment). To the third set, 10 kg polluted soil was amended with 5% w/w NRS in soil. The fourth set was amended with 25%w/w NRS in soil. The fifth treatment was oil-polluted NRS only (5%w/w), whereas the control treatment was unpolluted soil with no NRS amendment. There were 5 replicates per treatment.

Assuming homogeneity of the plot, the treatments were randomized. Having previously determined the soil's water holding capacity to be 211 ml/kg soil, the moisture requirements for the polluted soils were met by wetting weekly with 1000ml distilled water [9]. This entire setup was kept for a period of two months in a well ventilated screen house.

2.1 Soil Physicochemical Analyses

Soils were dried at ambient temperature (22-25°C), crushed in a porcelain mortar and sieved through a 2-mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored in polythene bags for subsequent analysis. The <2 mm fraction were used for the determination of selected soil physicochemical properties and the heavy metal fractions as well as PAH [1]. Total organic carbon (TOC) and total organic matter (TOM) contents were determined according to [10,11] respectively.

2.1.1 Extraction of micronutrients in soils by hydrochloric acid method

Ten (10) g of soil was weighed into a 250 ml plastic bottle. 100 ml of 0.1 m HCl was added, stopper, and then shaken for 30 minutes. The mixture was filtered through Whitman filter paper No.42. And then Fe, Cu, Mn, Zn, Cd, Cr, Pb, Ni, and V were determined in the filtrate by Atomic Absorption Spectrometry.

2.1.2 Determination of polyaromatic hydrocarbon contents

Determination of Polyaromatic Hydrocarbon Contents of Polluted Soil by Gas Chromatography (GC) was carried out according to the method described in [1]. A 10 g sample was extracted with methylene chloride (DCM). The extract was filtered through anhydrous sodium sulphate to remove any trapped water molecule. This was followed by a clean- up/ fractionation of the sample extract into Aliphatic and Aromatic (PAH) components. Finally, the components were concentrated using a rotary evaporator for GC analysis, using FID as detector. Model of GC used was AGILENT 6890.

The GC analysis began by first injecting 1 μL of the sample extract into the GC, and the results calculated as follows:

$$\text{Sample (mg/kg)} = \frac{\text{Area} \times \text{F.vol} \times 1000}{\text{Rf} \times \text{Wt}}$$

Where,

Rf = Response factor = Total Area / Total Concentration, obtained from instrument calibration with standards.

Area is obtained from the chromatogram output.

F.vol is the final volume of the concentrated extract (in ml).

Wt is the initial weight of the homogenized sample (in grams).

2.2 Identification of Soil Microorganisms

The isolation of bacterial and fungal oil degraders, heterotrophic bacterial and fungal counts and identification of soil microorganisms were carried out according to methods described by [12,13].

2.3 Ecotoxicological Quotients

The following parameters were computed [1]:

2.3.1 Contamination factor (CF)

$$CF = \frac{\text{Concentration of pollutant}}{\text{Pre-contamination Concentration}}$$

When CF > 1: Contaminant level is significantly higher than values in undisturbed soil, and hence may pose an ecological threat to resident organisms
When CF = 1: Contaminant alone is not likely to cause ecological risk
When CF < 1: Harmful effects are not likely

2.3.2 Hazard quotient (HQ)

$$HQ = \frac{\text{Measured concentration}}{\text{Selected screening benchmark.}}$$

When HQ > 1: Harmful effects are likely due to contaminant in question
When HQ = 1: Contaminant alone is not likely to cause ecological risk
When HQ < 1: Harmful effects are not likely

Screening benchmarks are available at [14].

2.3.3 Toxic equivalency (TEQ) for polycyclic aromatic hydrocarbons (PAH)

$$TEQ = \Sigma T_i \times TEF$$

Where TEQ = Toxic Equivalency
T_i = PAH concentration in soil
TEF = Toxic Equivalency factor (Cal-EPA, 2005)

2.4 Phytoassessment

The success of remediation at two months was assessed by sowing cowpea (*Vigna unguiculata* cv. Ife Brown). The plants were observed for the following parameters; number of days taken for seedling emergence, percentage emergence, height of emergents, fresh weight of emergents, dry wt. of emergents, percentage survival of emergents, first day of noticed chlorosis, day of noticed necrosis in plant and survival at flowering. Analysis of variance in completely randomized design was done using the SPSS-15 statistical software, and means were separated by using the Least Significant Difference.

3. RESULTS

Table 1 shows the physiochemical properties of soil before amendment with waste engine oil (WEO). Throughout the study, this would be referred to as the pre contamination reference. pH of the soil was 6.11%, total organic matter of the soil was 6.61%, compared to 0.12% of total nitrogen (Table 1).

Two months after soil was amended with waste engine oil, total organic matter content of the soil was 0.76%, compared to 0.69% at 1 week after pollution. Total organic matter in two months old polluted soil amended with NPK was 0.58% as against 1.02% when it was amended with 5% NRS, and 1.16% when amended with 25% NRS. This implied that soil amendment with NRS significantly enhance total organic matter (Fig. 1).

Table 2 shows heavy metal content of the soil at two months after exposure to waste engine oil and soil amendment with NPK and NRS respectively. Fe in the polluted soil only was 1497.34 mg/kg at 1 WAP, compared to 1326.42 mg/kg at 2 MAP. When polluted soil was amended with NPK, Fe in soil was 1202.61 mg/kg and 903.50 mg/kg in 5% and 25% NRS-amended soils respectively. The range of values for Zn in soil was 12.6 mg/kg to 16.4 mg/kg, the lowest value being obtained from 25% NRS-amended soil. Cu, Cr, Cd, Pb, Ni and V were not detected in oil-polluted NRS, indicating that these heavy metals were entirely remediated. Total hydrocarbon content (THC) of soil at 1 WAP was 3425.63 mg/kg. This concentration however decreased two months later to 2426.6 mg/kg in the unamended soil, and further to 1136.42 mg/kg when polluted soil was amended with NPK and 763.48 mg/kg in 25% NRS polluted soil. Contamination factor (CF) for heavy metals in the polluted soils at two months after exposure to the various treatments are presented in Table 3. Results show that CF was less than 1 (<1) in 25% NRS-amended soil for Fe, compared to valued obtained during 1 week of pollution (1.499 mg/kg) in the polluted soil irrespective of the type of amendment applied, CF was significantly greater than unity (CF>1) for Zn, Cr, V, and THC, the implication being that contamination in the soil attributed to these heavy metals was due to exogenous application of waste engine oil [1].

Table 1. Physical and chemical properties of soil and nutrient-rich sludge (NRS) before waste engine oil contamination

Parameters	Units	Soil	NRS
pH	-	6.11	5.49
Electrical conductivity	μs/cm	301	NA
Total org. matter	%	0.61	NA
Total nitrogen	%	0.12	5.21
Exchangeable acidity	cmol/kg	0.22	NA
K	cmol/kg	1.43	NA
Ca	cmol/kg	15.26	28.03
Mg	cmol/kg	10.97	45.42
P	mg/l	153.00	493.21
Clay	%	7.90	NA
Silt	%	13.90	NA
Sand	%	78.20	NA
Fe	mg/kg	998.80	32.32
Mn	mg/kg	16.71	1.23
Zn	mg/kg	12.12	NA
Cu	mg/kg	4.98	NA
Cr	mg/kg	2.08	NA
Cd	mg/kg	N.D	NA
Pb	mg/kg	N.D	NA
Ni	mg/kg	3.60	NA
V	mg/kg	0.76	NA
Total hydrocarbon content	mg/kg	224.06	NA

ND: Not detected ($\leq$0.001 mg/kg); NA: Not available

Hazard Quotient (HQ) expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern. HQ for Fe in the polluted soil was greater than 1 in all the treatments applied apart from the 25% NRS-amended soils; the implication being that Fe in these soils could still pose ecological risks (Table 4). HQ in Mn ranged from 0.1- 0.3. HQ was greater than 1 in Cr and V irrespective of soil amendment applied. Obviously, the presence of soil amendments enhanced growth of weeds on the oil-polluted soil. The presence of plants in oil-polluted soil also amounts to enhanced remediation.

Table 5 and Fig. 2 shows polyaromatic hydrocarbon (PAH) contents of the polluted soil as affected by soil treatments. At 1 WAP, total PAH was 1506.1 mg/kg, which eventually decreased to 458.58 mg/kg at 2 MAP. Total PAH in NPK-amended soil was higher (923.90 mg/kg) when, compared to the unamended polluted soil (458.58 mg/kg). Total PAH was lowest in the 25% NRS-amended soil (260.12 mg/kg), which also had the highest bioremediation efficiency of 82.73%. Phenathrene was totally remediated when waste engine oil was mixed with only NRS.

Table 6 presents Toxicity Equivalent Concentration (TEC) of carcinogenic PAH component of soil (c-PAH). Total TEC in the polluted soil at 2 MAP was 53.11 mg/kg, compared to 220.11 mg/kg when soil was amended with NPK. However, total TEC reduced to 29.89 mg/kg in 25% NRS-amended soils. Total TEC values exceeded the method B clean up level for benzo(a)pyrene (0.137 mg/kg) [15].

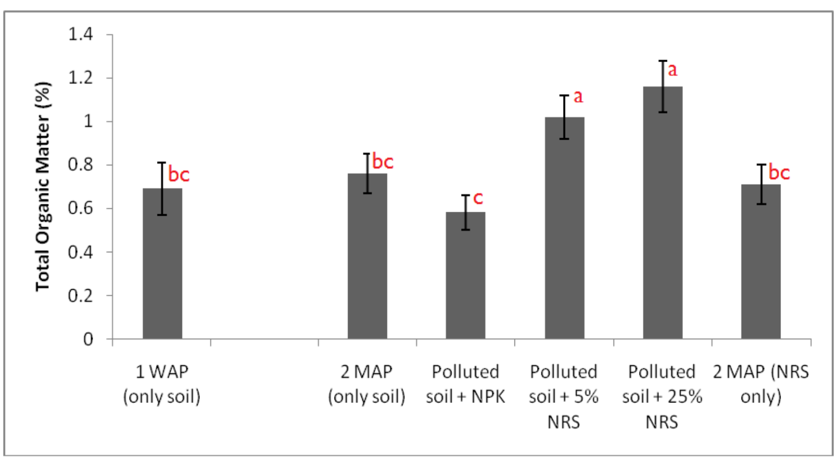

Fig. 1. Organic carbon content of oil-polluted soil subjected to various treatments of soil amendments. MAP, months after pollution; WAP, weeks after pollution; NRS, nutrient-rich sludge. Bars showing similar alphabets do not differ significantly from the other (p>0.05)

Table 2. Heavy metals of soil two months after soil exposure to waste engine oil pollution and soil amendment with nutrient-rich sludges

	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
	mg/kg									
Contaminant reference	998.8	16.7	12.1	4.98	2.08	ND	ND	3.6	0.76	224.06
	1 WAP									
Polluted soil	1097.34	18.4	16.4	5.63	2.83	1.42	1.03	2.95	3.55	3425.63
	2 MAP									
Control (unpolluted)	726.42	9.2	13.9	4.04	1.78	0.65	0.67	1.83	3.08	1882.32
Polluted soil only	1326.42	16.3	14.3	4.86	2.78	1.58	1.03	2.71	3.02	2426.6
Polluted soil + NPK	1202.61	14.2	13.8	3.72	2.18	1.18	0.83	1.83	2.78	1136.42
Polluted soil + 5% NRS	1120.52	14.6	12.1	3.08	2.22	1.02	0.73	1.92	2.42	996.3
Polluted soil + 25% NRS	903.50	11.6	12.6	2.96	1.93	0.96	0.71	1.81	2.48	763.48
NRS only	23.51	1.6	1.4	ND	ND	ND	ND	ND	ND	3.85

WAP Weeks after pollution, MAP months after pollution

Table 3. Contamination factor (CF) of soil two months after soil exposure to waste engine oil pollution and soil amendment with nutrient-rich sludges

	@Fe$^{998.8}$	Mn$^{16.7}$	Zn$^{12.1}$	Cu$^{4.98}$	Cr$^{2.08}$	CdND	PbND	Ni$^{3.6}$	V$^{0.76}$	THC$^{224.06}$
	1 WAP									
Polluted soil	1.098	1.101	1.355	1.130	1.365	ND	ND	0.819	4.671	15.288
	2 MAP									
Control (unpolluted)	0.727	0.550	0.983	0.811	0.855	ND	ND	0.477	2.710	8.762
Polluted soil only	1.328	0.976	1.181	0.975	1.336	ND	ND	0.752	3.973	1.100
Polluted soil + NPK	1.204	0.850	1.140	0.746	1.048	ND	ND	0.508	3.657	5.071
Polluted soil + 5% NRS	1.121	0.874	1.000	0.618	1.067	ND	ND	0.533	3.184	4.446
Polluted soil + 25% NRS	0.904	0.694	1.041	0.594	0.927	ND	ND	0.502	3.263	3.407
NRS only	0.023	0.095	0.115	0	0	ND	ND	0	0	0.016

**CF < 1, contamination in soil is no longer due to exogenous application of waste engine oil [1]. @Values appearing as superscripts are contaminant references (mg/kg) of the respective pollutants*

Table 4. Hazard Quotient (HQ) of soil two months after soil exposure to waste engine oil pollution and soil amendment with nutrient-rich sludges

	Fe$^{@\ 200}$	Mn^{500}	Zn^{50}	Cu^{100}	Cr^{1}	Cd^{4}	Pb^{50}	Ni^{30}	V^{2}
	1 WAP								
Polluted soil	*5.48	0.03	0.32	0.05	*2.83	0.35	0.02	0.09	*1.77
	2 MAP								
Control (unpolluted)	*6.63	0.03	0.28	0.04	*2.78	0.39	0.02	0.09	*1.51
Polluted soil only	*6.01	0.02	0.27	0.03	*2.18	0.29	0.01	0.06	*1.39
Polluted soil + NPK	*5.60	0.02	0.24	0.03	*2.22	0.25	0.01	0.06	*1.21
Polluted soil + 5% NRS	*4.51	0.02	0.25	0.02	*1.93	0.24	0.01	0.06	*1.24
Polluted soil + 25% NRS	0.11	0.00	0.02	ND	ND	ND	ND	ND	ND

*@ Toxicity references are provided in superscripts [14]. *Toxicity is indicated (i.e. HQ > 1). WAP Weeks after pollution, MAP months after pollution.*

Table 5. Polyaromatic hydrocarbon content (mg/kg) of soil after exposure to waste engine oil pollution and soil amendment with nutrient-rich sludge

PAH components (mg/kg)	Polluted soil only	Polluted soil only	Polluted soil + NPK	Polluted soil + 5% NRS	Polluted soil + 25% NRS	Oil-polluted NRS
	(1 WAP)			2 MAP		
Naphthalene	1.7904	1.1726	0.5174	2.5483	1.3876	1.0433
Acenaphthylene	1.8522	0.6211	0.1671	0.7015	0.5653	0.6621
Acenaphthene	1.1998	0.7478	0.4561	0.6879	0.5711	0.3702
Fluorene	1.3446	0.874	0.3579	0.3735	0.2355	0.2370
Phenanthrene	4.0272	2.6880	1.5357	1.7043	0.4171	0
Anthracene	11.4079	10.9662	4.7539	7.4039	1.9375	6.0868
Fluoranthene	24.4623	16.1162	6.5246	4.3703	5.6445	7.0424
Pyrene	77.6378	49.5007	40.3274	23.1479	11.0472	18.3024
Benz(a)anthracene	359.5043	37.5742	190.4658	80.9189	42.5903	59.8425
Chrysene	460.8468	99.7552	363.3801	112.6862	21.9089	187.7656
Benzo(b)fluoranthene	63.0388	33.6508	72.9913	18.4549	35.8387	50.4277
Benzo(k)fluoranthene	139.737	133.1705	124.1892	80.05708	121.4595	181.7257
Benzo(a)pyrene	150.2301	29.1114	49.9230	76.3215	9.1776	41.5562
Indeno(1,2,3-cd)pyrene	90.4585	25.7125	29.4184	30.8406	5.1448	3.8268
Benzo(g,h,i)perylene	118.4962	16.9238	38.8968	25.9619	2.1945	3.0118
TOTAL	1506.034	458.5851	923.9045	466.1789	260.1203	561.9006
Efficiency (%)	-	69.55	38.66	69.05	82.73	62.69

Table 6. Toxicity equivalent concentration (TEC)

PAH components (mg/kg)	Polluted soil only	Polluted soil only	Polluted soil + NPK	Polluted soil + 5% NRS	Polluted soil + 25% NRS	Oil-polluted NRS
	(1 WAP)			2 MAP		
Benzo (a) anthracene [0.1]	35.950	3.755	19.046	8.091	4.259	5.984
Benzo (a) pyrene [1.0]	150.23	29.111	49.923	76.321	9.177	41.556
Benzo (k) fluoranthene [0.1]	13.973	13.317	12.418	8.005	12.145	18.172
Benzo (b) fluoranthene [0.1]	6.303	3.365	7.299	1.845	3.583	5.042
Chrysene [0.01]	4.608	0.997	3.633	1.126	0.219	1.877
Indeno(1,2,3-c,d) pyrene [0.1]	9.045	2.571	2.941	3.084	0.514	0.382
Total TEC (TTEC)	220.109	53.116	95.26	98.472	29.897	73.013

At the start of the experiment, total heterotrophic bacteria in the polluted soil were 2.5 x 105 cfu/g, compared to 3.4 x 105 cfu/g in the control (Table 7). However, two months later, total bacteria counts ranged from 2.2 – 6.1 x 105 cfu/g; the highest bacteria count being obtained from the 25% NRS-amended soil. percentage hydrocarbon degrading bacteria increased with amount of NRS in the oil-polluted soil. percentage hydrocarbon degrading fungi ranged from 63.33 – 81.82%. *Micrococcus varians, Bacillius subtilis, Pseudomonas* sp. and *Clostridium* sp. Were the bacteria isolate obtained from the present study, among these, *Micrococcus varians* was the most prevalent followed by *Bacillius subtilis*, both being hydrocarbon degraders (Table 8). The fungi, *Aspergillus niger, Penicillium sp. Fusarium solani, Mucor* sp. *Trichoderma* sp. were all present in the polluted soil at 2 MAP but *Aspergillus niger* and *Penicillium* sp. and *Fusarium solani* (hydrocarbon degraders) were all present in the amended soil.

Table 9 shows plant species present in the treatment buckets at two months after pollution. Although the control soil has the higher population of plant spp. present, there was none in the unamended soil. However, when soil was amended with NPK, there were a total numbers of 11 plant spp/buckets, compared to 20 in 5% NRS and 55 in oil-polluted NRS treatment. *Eleusine indica* was the prevalent weed species identified in the present study.

Table 7. Total colony counts of bacteria and fungi obtained from waste engine oil-polluted soil exposed to two months of soil amendment with nutrient-rich sludge

	Bacteria			Fungi		
	Total heterotrophic counts (x10^5 cfu/g)	**Total hydrocarbon degraders (x10^5 cfu/g)**	**Percentage hydrocarbon degraders (%)**	**Total heterotrophic counts (x10^5 cfu/g)**	**Total hydrocarbon degraders (x10^5 cfu/g)**	**Percentage hydrocarbon degraders (%)**
Control (unpolluted)	3.4	1.7	50.00	5.2	2.8	53.85
Polluted soil only	2.5	1.8	72.00	2.8	1.2	42.85
Control (unpolluted)	2.8	1.3	46.42	3.0	1.9	63.33
Polluted soil	2.2	1.5	68.18	2.3	1.7	73.91
Polluted soil + NPK	3.8	1.8	47.37	2.2	1.8	81.82
Polluted soil + 5% NRS	4.8	2.5	52.08	2.8	2.0	71.43
Polluted soil + 25% NRS	6.1	3.7	60.66	3.2	2.2	68.75

Table 8. Microbial isolates from waste engine oil-polluted soil exposed to two months of soil amendment with nutrient-rich sludge

Microorganisms	Polluted soil only	Polluted soil + NPK	Polluted soil + 5% NRS	Polluted soil + 25% NRS	Oil-polluted NRS
Bacterial species					
**Micrococcus varians*	+	+	+	+	+
**Bacillus subtilis*	+	+	+	+	-
**Pseudomonas* sp.	-	-	-	+	+
Clostridium sp.	+	+	-	-	+
Fungal species					
**Aspergillus niger*	+	+	+	+	+
**Penicillium* sp	+	+	+	+	-
**Fusarium solani*	+	+	+	+	+
Mucor sp.	+	-	-	-	+
Trichoderma sp.	+	+	+	+	-

**hydrocarbon degraders, + present, - absent. NRS nutrient-rich sludge*

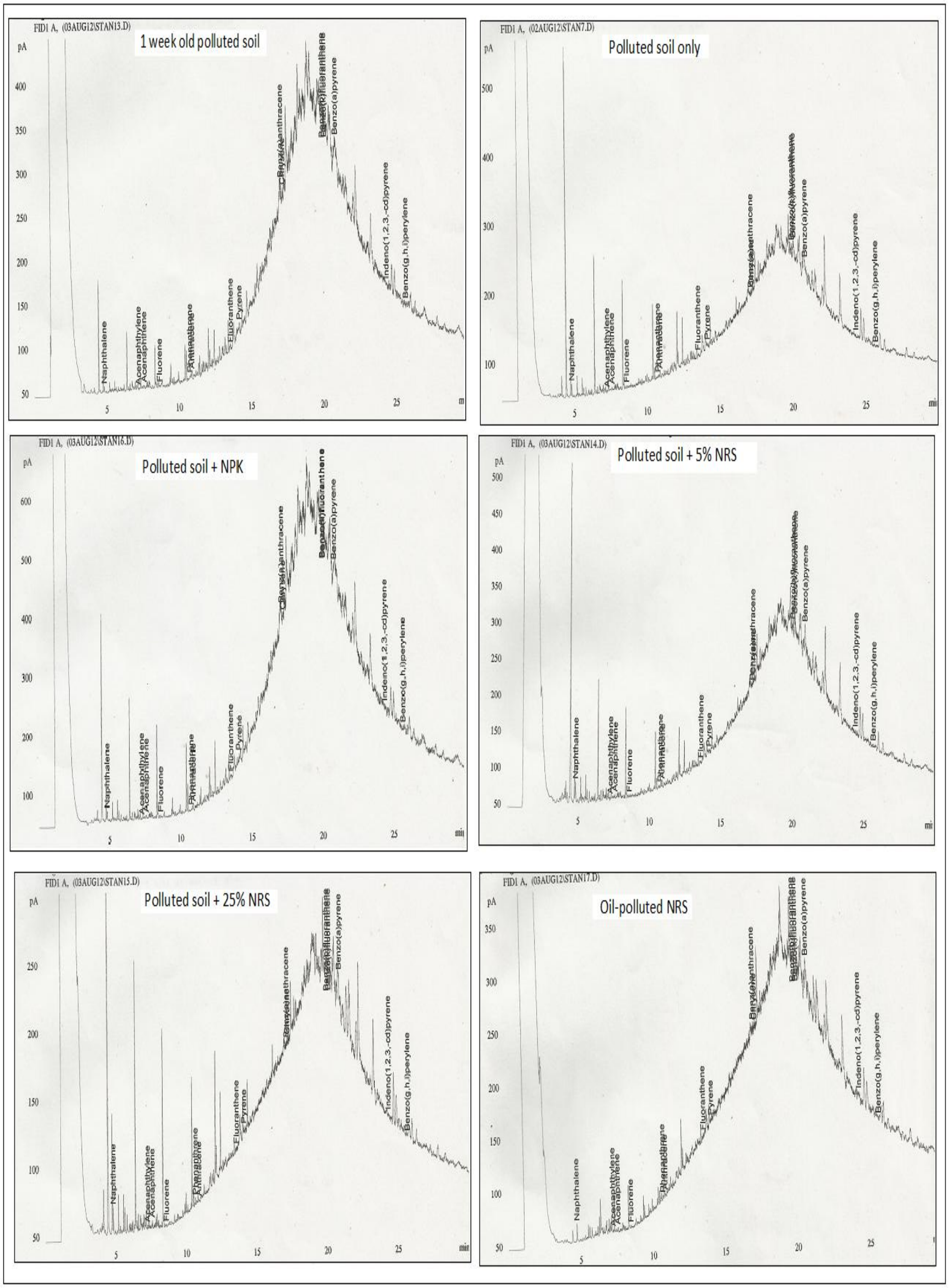

Fig. 2. Chromatograph showing polyaromatic hydrocarbon contents of waste engine oil-polluted soil

Table 9. Plants species present in treatment buckets at two months after pollution

	No. of plant species/bucket	Name of plant identified
Control (unpolluted)	43	*Phyllantus amarus*
	20	*Panicum maximum*
	35	*Eleusine indica*
	05	Unidentified plant species (height <5cm)
Polluted soil only	00	Nil
Polluted soil + NPK	05	*Eleusine indica*
	06	Unidentified plant species (height <5cm)
Polluted soil + 5% NRS	03	*Eleusine indica*
	07	Unidentified plant species (height <5cm)
Polluted soil + 25% NRS	02	*Phyllantus amarus*
	15	*Eleusine indica*
	03	Unidentified plant species (height <5cm)
Polluted NRS	07	*Eleusine indica*
	48	Unidentified plant species (height <5cm)

Table 10. The effects of soil amendment on some growth Parameters of *Vigna unguiculata* (var. Ife Brown) after two months

	Control (unpolluted)	Polluted soil	Polluted soil + NPK	Polluted soil + 5% NRS	Polluted soil + 25% NRS
No. of days taken for seedling emergence	3.83^{b}	5.21^{a}	5.32^{a}	5.85^{a}	5.16^{a}
Percentage emergence at 1 WAS (%)	92.21^{a}	40.51^{c}	52.32^{bc}	58.32^{b}	57.14^{b}
Height of emergents at 9DAS (cm)	15.23^{a}	5.98^{c}	8.72^{b}	9.81^{b}	10.21^{b}
Fresh wt. of emergents at 9DAS (g)	0.786^{a}	0.209^{c}	0.351^{b}	0.345^{b}	0.365^{b}
Dry wt. of emergents at 9DAS (g)	0.253^{a}	0.102^{c}	0.124^{bc}	0.155^{b}	0.169^{b}
Percentage survival of emergents at 2WAS	91.56^{a}	14.09^{c}	18.62^{c}	20.21^{bc}	28.57^{b}
1st Day of noticed yellowing (DAS)	19.14^{a}	7.13^{b}	7.85^{b}	9.14^{b}	9.52^{b}
Day of noticed necrosis in plant (DAS)	0^{c}	10.04^{b}	12.52^{b}	15.25^{ab}	17.85^{a}
Total death at time of flowering	0^{e}	14.08^{d}	18.52^{c}	24.98^{b}	31.95^{a}

Values are means of 10 determinations. Means on the same rows with similar alphabets do not differ significantly (p>0.05) from each other. DAP –days after planting; WAP - weeks after planting.

Results of phytoassessment carried out using *V. unguiculata* showed that although all the plants sown in the oil-polluted soils, whether amended or not, did not grow up to maturity (above Table 10). In spite of the poor performance of the crop in oil-polluted soil, survival of the seedlings was significantly enhanced in the NRTS amended soils than in the non-amended oil-polluted soil. The seedlings in the 25% NRS-amended oil-polluted soils survived for up to an average of 31.95 days after sowing, compared to those in the unamended soils that survived for only 14.08 days.

4. DISCUSSION

Microorganisms abound everywhere, including contaminated site, but for effective remediation, growth of microorganism should be stimulated [16]. In other to stimulate existing microorganisms involved in bioremediation, the environmental condition of the remediation site needs to be optimized. This is usually done by the addition of exogenous nutrient materials to the soil. Soil moisture, pH and organic matter are some of the soil characteristics that are positively enhanced by the amendments; and these also inherently influence microbial population and activities in the contaminated soil [17,18].

As reported earlier, total organic matter in two months old polluted soil amended with NPK was 0.58% as against 1.02% when it was amended with 5% NRS and 1.16% when amended with 25% NRS. This implied that soil amendment with NRS significantly enhance total organic matter (Fig. 1). Soil organisms, including micro-organisms, use soil organic matter as food. As they break down the organic matter, any excess nutrients (N, P and S) are released into the soil in forms that plants can use.

There was decrease in soil composition of heavy metals in amended soils, with the NRS-amended soils showing lower concentrations. Cr and Pb were not detected. There were also significant decreases in total hydrocarbon content in the NRS-amended soils. Natural recovery may, under certain conditions (*e.g.*, through sorption, leaching, volatilization, or oxidation-reduction reactions), effectively reduce the dissolved concentrations and/or toxic forms of contaminants in soil [19-21]. Similarly, the rate of biodegradation of compounds in oil-polluted soils is also greatly affected by the soil's microbial composition. A number of hydrocarbon-degrading microorganisms produce emulsifying agents [19], which help speed up degradation of organic compounds in the soil. [22] have characterized the emulsifying agents produced by strains of *Pseudonomas* and *Corynebacterium*.

Although the present study did not critically investigate the primary role of microorganisms in bioremediation, microorganisms are however the major agents in the degradation of petroleum hydrocarbons [23]. These organisms include bacteria, yeast, filamentous fungi and algae [24,25]. The principal bacteria and fungi responsible for oil degradation in both soils and aquatic environment have been identified as comprising mainly *Pseudomonas, Achrobacter, Bacillus*, *Micrococcus, Nocardia, Actinomyces, Sarcina, Vibrio, Brevibacterium, Flavobacterium, Cylindrocarpon, Fusarium, Penicillium, Aspergillus* and *Morteilla* [26-28,1]. *Micrococcus varians, Bacillius subtilis, Pseudomonas* sp. and *Clostridium* sp. were the bacteria isolates obtained from the present study. The fungi were *Aspergillus niger, Penicillium* sp. *Fusarium solani, Mucor* sp. and *Trichoderma* sp.

Eleusine indica appeared to be the commonest plant spp. identified in the present study area. [29] identified the weed as a recurrent plant species in most oil-polluted mechanic workshops. The possibility therefore exists that it is an oil-tolerant plant and as such a candidate for phytoremediation strategies. As may be deduced from the present study, the importance of soil amendments in remediation strategies is of utmost importance. Remediation was enhanced in the nutrient-rich sludge (NRS) – amended soil than in other treatments applied.

Soil amendment with sawdust has been previously reported to enhance heavy metal bioremediation in waste engine oil-polluted soils [30-32]. Soil amendment or additives, such as sawdust, peat, waste cotton, manure, fertilizers etc, are a necessity for efficient Bioremediation. This is probably because they generally increase micro-organisms' activities in the polluted soils. A soil amendment is any material added to a soil to improve its physical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure [7]. The microorganisms, while growing on the substrate, probably produce enzymes that were used in metabolizing the hydrocarbons in the compost matrix [7,33,34] NRS can act as a soil ameliorant capable of changing pH, moisture content, soil structure and acting as a nutrient source, thereby improving the contaminated soil environment for indigenous or introduced microbial degradative activity. This is even more evident in the results of phytoassessment carried out on the remediated soil after two months. Although results showed that all seedlings in the oil-polluted soil never made it up to maturity, those in the NRS-amended soils survived in the soil for longer times than those in unamended soils.

As may be deduced from the present study, the importance of soil amendments in remediation strategies is of utmost importance. Remediation was enhanced in the NRS-amended soils better than in other treatments. It is important that for increased efficiency in bioremediation, the polluted soils are always amended with materials like sawdust, peat, waste cotton, manure, fertilizers etc. so as to increase the diversity and level of activity of soil microorganisms. Apart from improving the soil's physical properties, such as water retention, permeability, water infiltration, aeration and structure [35], the amendments also add nutrients to the soil. The organisms, while growing on the NRS substrate, probably produce enzymes that were used in metabolising the hydrocarbons in the substrate matrix. The high microbial load in the NRS afforded the population the opportunity to remain high while adapting to and attacking the hydrocarbon substrate.

5. CONCLUSION

After the experiments, it was concluded that soil amendments with nutrient-rich sludge significantly enhance total organic matter which is an indispensable requirement for enhanced microbial action. This was further implicated in the significant decrease in both heavy metals and polyaromatic hydrocarbon contents of Nutrient Rich Sludge amended soils. Since it acts as soil ameliorant, farmers should use nutrient-rich sludge as an amendment to the soil.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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