Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.4, No.2, 2014
www.iiste.org

Effects of Soil Amendments on the Intrinsic Qualities and Development of Soil Seed Bank of a Monitored Naturally Attenuated Petroleum Hydrocarbon-Polluted Soil

Ikhajiagbe, Beckley[1], Chijioke-Osuji Chinenye[2*], Obonodi Nancy[3], Osaruese Usiekhaguere[4].

1. Department of Plant Biology and Biotechnology, University of Benin, Nigeria.
2. Department of Theoretical & Applied Biology, Kwame Nkrumah University of Science & Technology, Kumasi, Ghana.
3. Department of Sci. Laboratory Technology, University of Benin, Nigeria.
4. Department of Plant Biology and Biotechnology, University of Benin, Nigeria.

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

Abstract
A record of the soil seed bank in oil polluted areas is necessary to assess their capacity for tolerance and phytoremediation potentials of such polluted sites. The present study investigated the effect of soil amendments on the development of soil seed bank of a waste engine oil polluted soil. Top soil (0- 10cm) was collected from an area of known soil seed bank and physiochemical parameters. The soil was then contaminated at 5 % w/w oil-in-soil and immediately amended with poultry manure, sawdust and dried leaves of *Vernonia amygdalina* and a combination of any of the amendments. Results showed that at 3 months after pollution (MAP), there was general reduction in heavy metal composition and polyaromatic hydrocarbon (PAH) contents of the soil. This content significantly reduced to 46.85 mg/kg 3 months after pollution using treatment SD. The heavy metal content of Fe was 3250.1 mg/kg. This reduced to 934.5 mg/kg using treatment SD with similar reductions in Mn, Zn, Cu, Cr, Cd, Pb, Ni and V contents. *Euphorbia* spp were the most prevalent weeds. Soil amendment of waste engine oil polluted soil showed the influence on the enhancement of the soil seed bank. Weed diversity was also affected with significant improvement recorded in the polluted soil which probably resulted from enhanced biodegradation activities. Indigenous plant species (particularly *Euphorbia* spp. and *Cyperus* sp.) should be used together with soil amendments in phytoremediation following results from present study.
Keywords: attenuation, bioremediation, heavy metals, petroleum hydrocarbons, soil amendment, soil seed bank.

1. Introduction

The availability of oil in any economy is usually a major boost to its development, but this does not come without the attendant effects on the immediate environment from which oil is explored. Many authors have even showed that oil pollution have now become more prominent in areas where they are used, particularly when such oils are indiscriminately disposed (Atuanya, 1987; Anoliefo and Vwioko, 1995; Anoliefo et al., 2006; Ikhajiagbe, 2010). At the spill sites, areas of industrial production, transportation, refuse burning and associated locations; there is an imbalance in the carbon-nitrogen ratio. In addition, a build-up of essential and non- essential elements, change in physicochemical parameters, structural components and general morphology of the soil and increase in heavy metals concentration available in the soil results (Anoliefo and Vwioko, 1995). The effect on plants and various life forms can be related to its short and long term toxicities, mutagenicity, carcinogenicity, and persistence and dispersion properties. The absorption, translocation and accumulation of heavy metal ions of mercury, lead, Chromium and Cadmium by plants reduce qualitative and quantitative productivity of the species and causes serious health hazards through the food chain and other pathways to other life forms

Although, soil pollution can arise from several sources, pollution as a result of crude oil and crude oil based products, depends both on the type and amount of oil involved, the degree of its weathering or its breakdown, time of the year, species and age of plants involved (Amakiri and Onofeghara, 1984).

The phytotoxic effects of petroleum oil have been been demonstrated by several authors (Udo and Fayemi, 1975; Amakiri and Onofeghara, 1984; Terge, 1984; Ikhajiagbe and Anoliefo, 2010, 2012; Ikhajiagbe et al., 2013b,d). Smith *et al*. (1989) stated that root stress caused by crude oil reduces leaf area through stomata conductance. Epstein (1972) also reported that mineral ions absorbed initially by the roots are finally received by the mesophyll cells thereby causing dehydration in plants. Oil penetrates into plants where it moves in the intracellular spaces and possibly also in the vascular system (Baker, 1970). Hydrocarbons can dissolve in plasmalemma, thus leading to rupture of the plant tissue. The presence of oil in soil creates very unsatisfactory conditions for plant growth; this may be due to insufficient aeration of the soil resulting from the waxy nature of most oil-impacted soils (De Jong, 1980). There is the displacement of air from pore spaces by oil and an increase in demand for oxygen brought about by activities of oil-decomposing micro-organisms (Gudin and Syratt, 1975), which limits normal diffusion processes. Baker (1970) reported that oil penetrated and accumulated in plants

causing damage to cell membranes and leakage of cell contents. Udo and Fayemi (1975) also reported that growth of cereals was adversely affected in oil-polluted soil, resulting in leaf chlorosis and plant dehydration. All these impact on plant and soil usually results in reduction in yield of plants of economic importance (Ikhajiagbe, 2010).

The removal of these pollutants from the areas where they are found is necessary to curb its aggravation. Remediation methods can be physical, chemical or biological (Sarkar *et al.,* 2005). The choice of method for remediation depends on a number of factors, which may include the level of pollution, composition of the pollutant and nature of the area. In the remediation of polluted soil, several methods have been greatly employed. Physical method is the most common. However, this method, as well as the use of chemical agents, does not entirely clean up the environment. Both methods have also been labelled as bot entirely ecofriendly. Consequently, biological methods, which employ biological organisms, are the best alternative. Researches into the use of biological organisms in the remediation process are fast growing. Limitations to the efficiency of these methods still arise, particularly, the slow process.

Ikhajiagbe (2010) reported that the most practice of bioremediation relies on the soil's inherent microbial population, microbial activity and processes. Although, other biological methods may include the sole use of plants in remediation (phytoremediation), Anoliefo and Ikhajiagbe (2011) reported that phytoremediatiove capabilities of plants in oil-polluted soils are better enhanced by their symbiotic interactions with rhizospheric microorganisms. For efficient bioremediation, soil amendments or additives, such as sawdust, are added to increase the activities of micro-organisms. Soil amendments also help to improve the physical such as water retention, permeability, water infiltration, drainage, aeration and structure (Davis and Wilson, 2005), as well as the soil's chemical properties, like pH (Ikhajiagbe et al, 2013a). The goal is to provide a better environment for roots as well as rhizospheric microorganisms.

In the present study, the soil is amended with different materials and then allowed to naturally attenuate, while taking note of development of the soils inherent weeds population form the soil seed bank. The impact of plant presence in oil-polluted soils have been found to be very important in remediation practices; particularly considering the fact that remediation of the soil would be holistically carried out by the resident plants (phytoremediation), the soil's resident microorganisms (microbial bioremediation), as well as the plant-microbial interaction (synergistic bioremediation), not mentioning other physical methods of remediation like volatilization (Ikhajiagbe, 2010). And having considered the effects of soil amendments on remediation, the researchers hope to investigate the effects on soil physiochemical properties, as well as on the remediation of heavy metals and polyaromatic hydrocarbon (PAH) contents of the soil *vis-a-vis* the attendant impact on development of soil seed bank.

1.1 Materials and Methods.

1.1.1 Preparation of materials.

The soil seed bank of a fallow area of land was identified and the top soil (0- 10cm) was collected randomly from a marked plot on the fallow land measuring 50 m x 50 m. The top soil was sun-dried to constant weight and the physiochemical property of the soil was determined before use. Ten (10) kg of soil was measured into 85 cm - diameter perforated bowls. The perforations were made with 2 mm diameter nails at the bottom of each bowl.

Soils were thoroughly mixed with waste petrol-engine oil on a weight basis to obtain uniform 10 % w/w concentration of oil-in-soil.

1.1.2 Soil preparation and amendment.

The soil amendments used for the present study were poultry manure (PO), sawdust (SD), and dried leaves of *Vernonia amygdalina* (LB). All these were initially assayed for their physiochemical properties prior to use. Each bowl received soil amendments at the rate of 1 % on weight basis. For each sample treatment, the weight of the soil amendment used was 100 g. For a combination of 2 amendments, 50g proportions were used; whereas for a combination of three amendments, 33.33 g of each amendment was used. These specifications were made so that, at any given time, each bowl received 1 %w/w of amendment-in-soil.

The soil was exposed to prevailing weather conditions (the late rainy season, December, 2012 to March, 2013). There was observation for weed appearance /presence and microbial composition for a period of 3 months. Afterwards, soils were taken to the laboratory for physiochemical determinations using the methods of Bray and Kurtz (1945a,b), Jackson (1962), and Nelson and Sommers (1982).

1.1.3 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 HCI was added, stopper, and then shaken for 30 minutes. The mixture was filtered through Whatman 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.

1.1.4 Determination of Polyaromatic Hydrocarbon Contents of Polluted Soil by Gas Chromatography (GC).

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:

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

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).

1.1.5 Identification of Soil Microorganisms.

The soil samples were air-dried and sieved through a 2 mm mesh to remove undesirable material. The dilution series for the soil sample was done by transferring 1 gram of the soil to nine (9 ml) millimetres of sterile distilled water in sterile glass containers as blank. The glass containers were shaken for 5 minutes and was taken as 10^{-1} dilution factor, 10 ml were then transferred from the 10^{-1} dilution into another 9 ml blank to obtain a 10^{-2} dilution and same process of transfer was repeated twice to obtain a dilution factor of 10^{-4}.

1.1.6 Heterotrophic Bacterial and Fungal Counts.

The spread plate method was employed in taking the heterotrophic bacteria counts. One (1) ml of the serially diluted portion of 10^{-4} of each soil sample was inoculated onto nutrient agar plates for bacteria and Potato dextrose agar plates for fungal counts. The plates were inoculated at room temperature for 24 hours and 72 hours respectively, for bacteria and fungi growth. After incubation colonies were then counted and the colony forming unit (cfu/g) of the soil samples determined.

1.1.7 Isolation of Bacterial and Fungal Oil Degraders.

Bushnell- Haas (BH) medium ($MgSO_4$, 0.20 g/I; $CaCl_2$, 0.02 g/l; $K_2H,P0_4$, 1 g/l; NH_4NO_3, 1 g/l; $FeCl_3$, 0.05 g/l; KH_2PO_4, 1 g/l; pH 7.0, was used as the enrichment medium with 8 % (v/v) filter sterilized oil as the sole carbon source. The medium was dispensed into in 100 ml Erlenmeyer flasks and autoc1aved at 121 ^{0}C for 15 minutes. Thereafter, 5 g of each soil sample was inoculated into each flask of the medium and incubated at 130 rpm at room temperature in a HY-4 multifunctional shaker (B. Bran Scientific and Instrument Company, England). After 10 days, 1 ml of enriched media was transferred into freshly prepared enrichment media and incubated under the same conditions as described above. Serial dilutions from the third enrichment process were inoculated onto nutrient agar plates and potato dextrose agar plates for oil-degrading bacterial and funga1 counts respectively by methods described by Cowan and Steel (1974) and Cheesebrough (1998).

1.1.8 Computation of selected ecotoxicological statistical parameters.

Selected parameters used in the study were Hazard quotients (HQ) as well as toxicity equivalency concentrations (Ikhajiagbe, 2010); the object being to establish at some point whether concentrations of the contaminants after the remediative experiments could still pose any ecological threat. These were calculated as follows;

Hazard Quotient (HQ)

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

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 Efroymson *et al.* (1997).

Toxic Equivalency (TEQ) for Polycyclic Aromatic Hydrocarbons (PAH).

$TEQ = \Sigma Ti \times TEF$

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

1.1.9 **Bioremediation Efficiency.**

This is regarded as the proportion (%) of contaminant concentration that was bioremediated compared to a measured concentration at a start point. This is calculated as;
Efficiency (%) = Ratio of measured concentration at 3 MAPA and Concentration at 1 WAPA × 100.

1.2 Results.

1.2.1 Identification of soil seed bank.

The rich density of weeds (biodiversity) was identified. This was done to get record of the soil seed bank of the land. The soil seed bank identified is shown in Table 1. Weeds of the family Asteraceae, Cyperaceae, Euphorbiaceae, and Poaceae were very common. These weed species represented the soil seed bank. *Asystasia gangetica, Axonopus compressus, Phyllantus amarus* were the most prevalent weed species found in the area. Table 2 shows the physical and chemical properties of soil, sawdust, poultry, and waste engine oil (WEO) used for the study.

The physiochemical parameters of the soil after 3 months of oil pollution and soil amendment (MAPA) are shown in Table 3. The pH ranged from 5.91- 6.16. Treatment PO+LB recorded the highest pH of 6.16, while PO recorded the lowest pH (5.91). Soil pH in the control was 6.02. Organic carbon and total N in treatment SD were 1.85 % and 0.16 % respectively. Electric conductivity (EC) values ranged from 310 - 390 µS/cm with the highest value in the control (390 µs/cm). The lowest value for soil exchangeable acidity (EA) was recorded in PO+LB (0.50 meq/100g). Range of values for the other physiochemical parameters of soil at 3 MAPA were Cl^- (32.50-46.20 mg/kg), Av. P (8.21- 9.38mg/kg), NH_4N (9.08- 19.57mg/kg), NO_2 (5.28- 10.92mg/kg), NO_3 (7.65-13.41mg/kg) and SO_4 (39.21- 47.00mg/kg) respectively.

Heavy metal contents of oil- polluted soil after amendment at 1 week and 3 months after pollution is shown in Table 4. The control (prior to amendment) recorded a high concentration of Fe (3250.1 mg/kg) compared to Zn (54.21 mg/kg), Mn (23.5 mg/kg), Cu (9.5 mg/kg), Pb (4.68 mg/kg), Ni (4.35 mg/kg), Cr (2.15 mg/kg), V (4.05 mg/kg), and Cd (1.79 mg/kg), respectively at 1 week after pollution (WAPA). A decrease was recorded in the concentrations of all heavy metal present 3 months later. For soils amended with SD, the lowest concentration of Fe was recorded (934.50 mg/kg).There was also decrease in soil content of Mn, including the lowest value obtained though amendment with PO (10.7 mg/kg). Other values were 14.20 mg/kg (PO+ LB), 16.50 mg/kg (SD+LB) and 17.50 mg/kg (PO+SD+LB). There were similar decreases in concentrations Cd, Pb, Ni, and V compared to the control.

Table 1: Record of soil seed bank of the soil used in the present study

S/N	Weeds	Family
1	*Acanthosperum hispidum*	Asteraceae
2	*Andropogon tectorum*	Poaceae
3	*Asystasia gangetica*	Poaceae
4	*Axonopus compressus*	Poaceae
5	*Centrosema pubescens*	Fabaceae
6	*Chromolaena odorata*	Asteraceae
7	*Commelina benghalensis*	Commelinaceae
8	*Cyperus esculentus*	Cyperaceae
9	*Cyperus haspan*	Cyperaceae
10	*Euphorbia hyssopifolia*	Euphorbiacae
11	*Ipomea involucrata*	Convolvulaceae
12	*Kyllinga erecta*	Cyperaceae
13	*Mariscus alternifolios*	Cyperaceae
14	*Oldenlandia herbacea*	Rubiaceae
15	*Oryza barthii*	Poaceae
16	*Panicum maximum*	Poaceae
17	*Phyllantus amarus*	Euphorbiaceae
18	*Sida acuta*	Malvaceae
19	*Talinum triangulare*	Portulacaceae

Table 2: Physical and chemical properties of soil, sawdust, poultry manure, and dried leaves of *Vernonia amaygdalina* before WEO Contamination.

Parameters	Soil	Sawdust	Poultry	WEO	Dried leaves of *Vernonia amaygdalina*
pH	6.11	5.59	6.81	5.98	7.02
Electrical Conductivity (µs/cm)	301	330	NA	NA	NA
Total Org. Matter (%)	0.61	1.99	5.99	NA	NA
Total Nitrogen (%)	0.12	0.15	0.40	NA	0.11
Exchangeable Acidity (meq/100 g soil)	0.22	0.30	8.90	NA	NA
K (meq/100 g soil)	1.43	2.65	0.44	NA	0.15
Ca (meq/100 g soil)	15.26	22.30	1.20	NA	0.68
Mg (meq/100 g soil)	10.97	15.40	0.60	NA	0.14
P (mg/kg)	153.00	112.00	NA	NA	0.72
Clay (%)	7.9	BDL	NA	NA	NA
Silt (%)	13.9	BDL	NA	NA	NA
Sand (%)	78.2	BDL	NA	NA	NA
Fe (mg/kg)	998.8	220	NA	2012.12	98.14
Mn (mg/kg)	16.71	41.70	NA	35.20	BDL
Zn (mg/kg)	12.12	4.80	1.70	59.24	0.03
Cu (mg/kg)	4.98	1.60	0.20	1.70	BDL
Cr (mg/kg)	2.08	1.60	NA	4.11	BDL
Cd (mg/kg)	N.D	N.D	NA	2.06	BDL
Pb (mg/kg)	N.D	N.D	NA	6.25	BDL
Ni (mg/kg)	3.60	0.70	NA	6.01	BDL
V (mg/kg)	0.76	0.64	NA	7.77	BDL
As (mg/kg)	NA	NA	0.60	NA	
THC (mg/kg)	224.06	268.00	NA	3265.22	BDL

NA=Not available; BDL=Below detectable limit (0.0001 mg/kg); THC=total hydrocarbon content.

Table 3: Physico-chemical parameters of soil after 3 months of oil pollution and soil amendment

	pH	EC	Org. C	Total N	EA	Na	K	Ca	Mg	Cl	Av. P	NH_4N	NO_2	NO_3	SO_4
		µs/cm	%		meq/100g of soil					mg/kg					
Control	6.02	390	3.05	0.35	0.7	1.72	0.15	4.92	3.86	41.5	7.05	11.65	5.36	9.09	39.21
PO	5.91	320	4.30	0.39	0.5	1.75	0.10	4.37	3.28	46.2	9.01	19.57	9.25	13.41	46.3
SD	5.97	350	1.85	0.16	0.7	1.80	0.11	4.50	3.38	41.7	8.60	9.67	10.92	8.58	45.1
LB	5.95	336	2.05	0.39	0.6	1.48	0.10	4.16	3.21	32.5	8.21	12.02	7.52	9.20	43.21
PO+SD	6.03	310	3.94	0.37	0.6	1.73	0.13	4.33	3.25	38.2	9.38	12.66	6.73	9.75	42.9
PO+LB	6.16	320	3.17	0.26	0.5	1.75	0.10	4.37	3.28	45.5	9.21	9.08	5.59	8.39	47.0
SD+LB	6.01	340	3.29	0.30	0.6	1.79	0.11	4.46	3.35	40.7	8.60	8.45	5.28	7.65	45.4
LSD (0.005)	0.76	68	0.16	0.09	0.1	0.19	0.04	0.68	0.52	6.01	1.08	3.38	3.54	4.08	12.36

EC: Electrical conductivity, EA: Exchangeable acidity, Org. C: Organic carbon. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

Table 4: Heavy metal contents of oil-polluted soil after amendment at 1 week and 3 months after pollution

Treatments	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
	(mg/kg)								
	1 WAPA								
Control (unamended)	3250.1	23.5	54.21	9.5	4.25	1.79	4.68	4.35	4.05
	3 MAPA								
Control	2006.2	18.2	42.2	5.1	2.08	0.97	2.98	3.15	3.66
PO	993.5	10.7	22.1	2.9	1.09	0.31	1.74	1.99	1.80
SD	934.5	10.9	18.2	2.2	2.67	0.78	2.02	3.52	2.71
LB	1324.1	11.5	20.5	4.2	2.52	0.65	2.96	2.68	1.95
PO+SD	1378.7	12.5	29.9	3.3	1.90	0.16	2.06	3.19	2.67
PO+LB	1272.4	14.2	22.4	4.5	1.74	0.68	2.01	2.85	2.19
SD+LB	1306.2	16.5	28.6	3.8	2.06	0.86	2.25	3.01	1.95
PO+SD+LB	1318.8	17.5	28.6	3.0	2.15	0.72	2.13	1.89	1.58
LSD (0.05)	306.4	5.6	9.5	1.1	0.68	0.09	0.56	0.85	0.49

WAPA: Weeks after pollution and Amendment; MAPA: Months after pollution and amendment. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

Table 5: Hazard quotient to determine toxicity of heavy metal components of waste engine oil-polluted soil up till 3 months after pollution and amendment

Treatments	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
				HQ determining ecotoxicity of metals at 1 WAPA					
Control	16.25	0.24	1.08	0.24	4.25	0.45	0.09	0.15	2.03
				HQ for toxicity against soil microbes and microbial processes at 1 WAPA					
Control	16.25	0.24	0.54	0.10	0.43	0.09	0.01	0.05	0.20
				HQ determining ecotoxicity of metals at 3 MAPA					
Control	10.03	0.18	0.84	0.13	2.08	0.24	0.06	0.11	1.83
PO	4.97	0.11	0.44	0.07	1.09	0.08	0.03	0.07	0.90
SD	4.67	0.11	0.36	0.06	2.67	0.20	0.04	0.12	1.34
LB	6.62	0.12	0.41	0.11	2.52	0.16	0.06	0.09	0.98
PO+SD	6.89	0.13	0.60	0.08	1.90	0.04	0.04	0.11	1.34
PO+LB	6.36	0.14	0.45	0.11	1.74	0.16	0.04	0.10	1.10
SD+LB	6.53	0.17	0.57	0.10	2.06	0.22	0.05	0.10	0.98
PO+SD+LB	6.59	0.18	0.57	0.08	2.15	0.18	0.04	0.06	0.79
				HQ for toxicity against soil microbes and microbial processes at 3 MAPA					
Control	10.03	2.18	0.42	0.05	0.21	0.05	0	0.04	0.02
PO	4.97	0.11	0.22	0.03	0.11	0.02	0	0.02	0.09
SD	4.67	0.11	0.18	0.02	0.27	0.04	0	0.04	0.14
LB	6.62	0.12	0.21	0.04	0.25	0.03	0	0.03	0.10
PO+SD	6.89	0.13	0.30	0.03	0.19	0.01	0	0.04	0.13
PO+LB	6.36	0.14	0.22	0.05	0.17	0.03	0	0.03	0.11
SD+LB	6.53	0.17	0.29	0.04	0.21	0.04	0	0.03	0.10
PO+SD+LB	6.59	0.18	0.09	0.03	0.22	0.04	0	0.02	0.08

WAPA: Weeks after pollution and Amendment; MAPA: Months after pollution and amendment. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

Table 5 shows the values for hazard quotient (HQ) for ecotoxicity of metal components and toxicity to microbial activity and processes. When HQ > 1, it implied that whatever harmful effects recorded were likely due to contaminant in question, otherwise contaminant alone was not likely to cause ecological risk. HQ to show level of ecological toxicity of Fe in the soil prior to amendment was 16.25 at 1 WAPA, the highest value recorded (Table 5). At 3 MAPA, the highest HQ for Fe was recorded for LB treatment (6.69) and the lowest in PO treatment (4.97). Along the treatments, decreasing HQ values indicated decreasing toxicities of the remediated soils. Similarly, HQ to determine toxicity heavy metal components of waste engine oil- polluted soil against soil microbes and microbial processes at 3 MAPA was less than unity for Mn, Zn, Cu, Cr, Cd, Pb and Ni respectively, and indication that no toxicities against microbial processes were recorded within the concentration.

Total PAH at 3 MAPA using the various soil amendments ranged from 46.85- 203.07 mg/kg with the highest value of 203.07 mg/kg recorded when the amendment PO+SD was used, and the lowest value of 46.85 mg/kg in the SD-amended soil, compared to the control (276.33 mg/kg) (Table 6). The treatment combinations of PO+SD+LB showed total remediation of acenaphthylene, 2-bromonaphthalene, acenaphthene, benzo(a)anthracene, chrysene and benzo(b, j, k)fluoranthene. Whereas PO+LB showed complete remediation of acenaphthylene, 2-bromonaphthalene, acenaphthene, fluoranthene, pyrene, benzo(a)anthracene, chrysene and benzo(b, j, k)fluoranthene. Bioremediation efficiency was highest in the SD- (94.38%) as well as the LB-amended soils (94.27%) respectively, compared to 66.86% remediation efficiency in the control.

Table 6: Mean polycyclic aromatic hydrocarbon contents of oil-polluted soil after amendment at 1 week and 3 months after pollution

PAH Content (mg/kg)	1	3 MAPA							
	WAPA	Control	PO	SD	LB	PO+SD	PO+LB	SD+LB	PO+SD+LB
Naphthalene	0.61	BDL	BDL	0.49	0.52	18.38	15.25	16.32	18.42
Acenaphthylene	1.16	1.32	BDL	BDL	BDL	BDL	BDL	BDL	BDL
2-bromonaphthalene	3.91	1.53	BDL	0.15	0.09	18.95	BDL	BDL	BDL
Acenaphthene	2.31	BDL	BDL	0.11	0.36	18.23	BDL	0.35	BDL
Fluorene	11.57	2.59	BDL	0.47	0.59	19.37	15.58	12.05	18.77
Phenanthrene	74.79	44.84	21.18	0.54	0.16	1.12	0.30	1.05	1.63
Anthracene	6.52	BDL	BDL	3.95	2.65	20.54	16.20	13.65	19.54
Fluoranthene	43.38	17.34	35.48	BDL	BDL	19.17	BDL	0.35	18.68
Pyrene	18.01	BDL	BDL	5.16	BDL	19.23	BDL	3.56	19.07
benzo(a)anthracene	33.06	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Chrysene	17.39	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
benzo(b,j,k)fluoranthene	124.13	87.58	118.47	4.42	2.68	0.66	BDL	BDL	BDL
benzo(a)pyrene	62.10	40.58	27.10	25.47	36.54	28.44	19.23	6.98	29.16
indeno(1,2,3-cd)pyrene	29.46	BDL	BDL	6.09	4.21	1.77	0.32	0.15	0.37
dibenzo(a,h)anthracene	336.17	77.90	BDL	BDL	BDL	3.40	2.02	2.38	7.69
benzo(g,h,i)perylene	69.05	2.65	BDL	BDL	BDL	33.81	23.29	19.35	26.71
Total PAH	833.62	276.33	202.23	46.85	47.80	203.07	92.19	76.19	160.04
Efficiency %	-	66.86	75.74	94.38	94.27	75.64	88.94	90.86	80.80

NA: Not available; BDL Below detectable limit of 0.0001 mg/kg. WAPA: Weeks after pollution and Amendment; MAPA: Months after pollution and amendment**.** PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

At 3 MAPA, PEC of PAH compounds in control experiment were given as 12.08 mg/kg (fluoranthene), 82.58 mg/kg (phenanthrene), 277.90 mg/kg (benzo(a) pyrene), 3.74 mg/kg (benzo(g,h, i)perylene) and 1.01 mg/kg (acenaphthylene) (Table 7). Apart from benzo(a) pyrene and phenanthrene, toxicity was implicated for these PAH compounds. By comparing their PEC's against the PECQ's in all levels of pollution, toxicity was not indicated for benzo(a) pyrene in all treatments. Toxicity was indicated for benzo(g, h, i) perylene, indeno (1, 2, 3- cd) pyrene, fluoranthene, phenanthrene, fluorene, acenapthene, and naphthalene in all treatments (PO-PO+SD+LB) although anthracene varied with treatments. In anthracene, toxicity was indicated for using treatment LB (12.80mg/kg), and treatment SD (19.08mg/kg). Toxicity was not indicated for treatment PO+SD, PO+LB, SD+LB and PO+SD+LB with 99.23 mg/kg, 78.26 mg/kg, 65.94 mg/kg and 94.40 mg/kg respectively.

At 1 WAPA and 3 MAPA, TEF, which are toxicity potency factors, were used to evaluate the toxicities of PAH mixtures of the organic pollutant shown on Table 8. At 1 WAPA, the total TEC was 65.29 mg/kg. Other toxicity equivalency concentrations (TEQ) were 0.06 mg/kg (benzo (a) anthracene), 0.18 mg/kg (chrysene), 62.10 mg/kg (benzo(a) pyrene), and 2.95 mg/kg (indeno (1, 2, 3- cd) pyrene). These values exceeded the Method B cleanup level for benzo[a]pyrene (0.137 mg/kg), which are those soil cleanup levels that are to be based on unrestricted land use (Cal-EPA, 2005). At 3 MAPA, TEQ's in treatment PO+SD were 1.84 mg/kg in benzo (a)anthracene and 0.18 mg/kg in indeno(1,2,3-cd)pyrene. These values were higher than benchmark TEF values of the c-PAH of 0.1 mg/kg.

Table 7: Probable effect concentration (PEC) of Polyaromatic hydrocarbon contents of the soil in the present study

PAH content (mg/kg)	1 WAPA	3 MAPA							
		Control	PO	SD	LB	PO+SD	PO+LB	SD+LB	PO+SD+LB
Naphthalene (0.917)	0.67	0	0	0.53	0.57	20.04	16.63	17.80	20.09
Acenaphthylene (1.301)	0.89	1.01	0	0	0	0	0	0	0
Acenaphthene (0.861)	2.68	0	0	0.13	0.42	21.17	0	0.41	0
Fluorene (0.264)	43.80	9.81	0	1.78	2.23	73.37	59.00	45.64	71.10
Phenanthrene (0.543)	137.70	82.58	39.01	0.99	0.29	2.06	0.55	1.93	3.00
Anthracene (0.207)	31.50	0	0	19.08	12.80	99.23	78.26	65.94	94.40
Fluoranthene (1.436)	30.21	12.08	24.71	0	0	13.35	0	0.24	13.00
Pyrene (0.344)	52.35	0	0	15.00	0	55.90	0	10.35	55.44
benzo(a)anthracene (0.192)	172.20	0	0	0	0	0	0	0	0
Chrysene (0.253)	68.74	0	0	0	0	0	0	0	0
benzo(a)pyrene (0.146)	425.30	277.90	185.60	174.50	250.27	194.80	131.70	47.81	200.00
indeno(1,2,3-cd)pyrene (0.183)	161.00	0	0	0.03	23.01	9.67	1.75	0.82	2.02
benzo(g,h,i)perylene (0.708)	97.53	3.74	0	0	0	47.75	32.90	27.33	37.73
Mean PECQ	94.20	64.52	83.11	26.51	41.37	53.73	45.83	21.83	55.20

WAPA: Weeks after pollution and Amendment; MAPA: Months after pollution and amendment**.**Bench mark values indicated in parentheses. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.* Values in parentheses are PEC values for the respective PAH components (Honeywell, 2011)

Table 8: Toxicity equivalency of Polyaromatic hydrocarbon contents of the soil in the present study.

PAH content (mg/kg)	1 WAPA	3 MAPA							
		Control	PO	SD	LB	PO+SD	PO+LB	SD+LB	PO+SD+LB
benzo(a)anthracene (0.1)	0.06	0	0	0.05	0.05	1.84	1.53	1.63	1.84
Chrysene (0.01)	0.18	0	0	0	0	0	0	0	0
benzo(a)pyrene (1.0)	62.10	40.58	27.10	25.47	36.54	28.44	19.23	6.98	29.16
indeno(1,2,3-cd)pyrene (0.1)	2.95	0	0	0.61	0.42	0.18	0.03	0.02	0.04
Total TEC	65.29	40.58	27.10	26.08	37.01	30.46	20.79	8.63	31.04

Benchmark values indicated in parentheses. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

Table 9 shows the microbial composition of waste engine oil-polluted soil at 3 MAPA. In the control, *Achromobacter* sp, *Micrococcus luteus,* M. *roseus, Bacillus pumilis, Pseudomonas* sp and *P. aeruginosa* were the bacterial isolates identified. *Sarcina* sp was isolated from SD, PO+SD, PO+LB, SD+LB and PO+SD+LB treatments respectively in addition to all other species found in the control. Treatment PO+SD+LB recorded the highest number of microbial composition. The percentage hydrocarbon degrading bacteria in the control was 33.3 %. Treatment PO+SD+LB recorded the highest percentage hydrocarbon degrading bacteria (47.92 %).

Fungi isolates identified in the control were *Aspergillus niger, Penicillium notatum, Rhiszopus stolonifer, Mucor* sp, *Geotrichum* sp, *Trichoderma* sp, and S*accharomyces* sp. The percentage hydrocarbon degrading fungi was highest in treatment SD (62.50 %). The first weed emergence was at 25 days after soil was contaminated with oil and amended (DAPA). During this day, only one weed was recorded (Fig. 1). There was no weed emergence in the oil-contaminated soils until the 40th day in the PO+LB-amended soil, and subsequently from the 60th day in nearly all other treatments.

The weeds in PO+SD+LB-amended soil seemed to develop more rapidly in the oil-contaminated treatments. At 100 DAPA, there were a total of 174 weeds per surface area altogether in the control (unpolluted soil), compared to only 5 in the polluted, but unamended soil (Fig. 2). Total number of weeds in the amended soils ranged from 12 – 43, PO+SD+LB being the highest. The distribution of weeds (Table 10) showed that *Chromolaena odorata* and *Euphorbia* sp were jointly most predominant weeds with percentage occurrence of 14.29%. although there were 13.98% of unidentified < 5 cm tall weeds, the least predominant identified weed was jointly *Axonopus compressus* and *Panicum maximum* (5.47%).

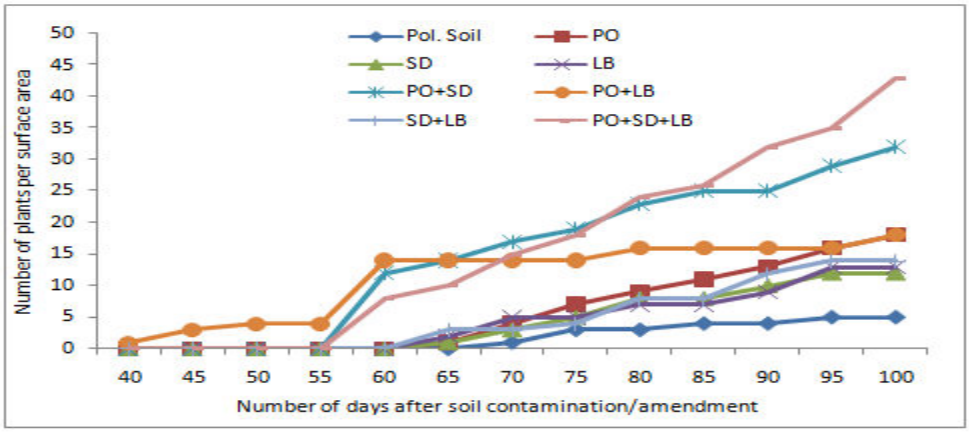

Fig. 1: Development of weeds from soil seed bank of oil-contaminated soil within 100 days of contamination and amendment. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

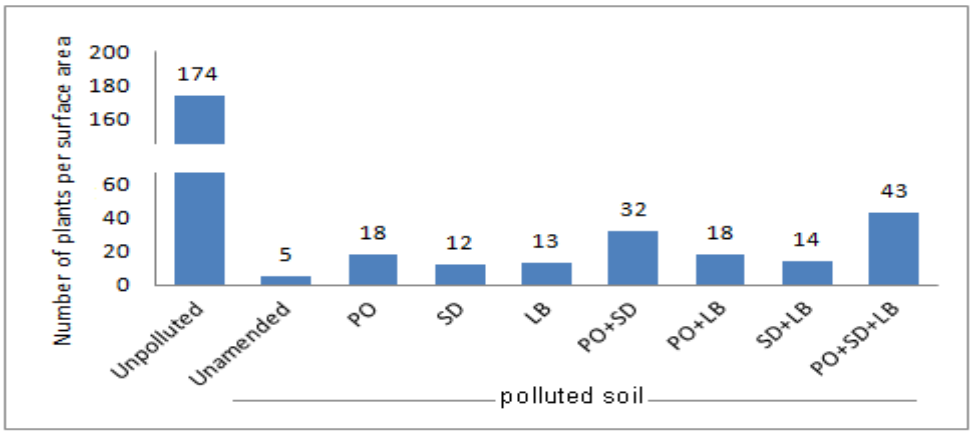

Fig. 2: Total number of weeds that emerged from soil seed bank at 100 days after soil was contaminated with oil and amended as well. PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

Table 9: Microbial composition of waste engine oil-polluted soil at 3 months after pollution

	Bacterial isolates Identified	Bacteria counts ($x10^5$ cfu/g)	Hydrocarbon Bacteria Degraders Counts ($x10^5$ cfu/g)	Percentage hydrocarbon-degrading bacteria (%)	Fungal isolates Identified (x104 cfu/g)	Fungal counts ($x10^5$ cfu/g)	Hydrocarbon Fungal Degraders Counts ($x10^5$ cfu/g)	Percentage hydrocarbon-degrading fungi (%)
Control	**Ac.sp*, **Mi.lu,* Mi.ro, **Ba.pu, Ps.sp*, **Ps.ar*	4.8	1.6	33.33	**As.ng, *Pe.no, Rz.st, Mu.sp, Ge.sp, Tr.sp*, Sm.sp	1.6	0.6	37.50
PO	**Ac.sp*, **Mi.lu,* Mi.ro, **Ba.pu, *Ps.ar*	4.6	1.8	39.13	**As.ng, As.fv* , **As.fm, *Pe.no, *Fu.sn , Rz.st, Mu.sp, Ge.sp*	1.8	0.9	50.00
SD	**Ac.sp*, **Mi.lu,* Mi.ro, *Sc.sp, *Ba.pu , *Ps.ar*	5.1	2.1	41.18	**As.ng, *Pe.no, *Fu.sn , Mu.sp, Tr.sp*	1.6	1.0	62.50
LB	**Ac.sp,* Mi.lu, *Ba.pu , *Ps.ar*	5.0	1.5	30.00	**As.ng, *Pe.no, *Fu.sn , Mu.sp*	2.5	1.3	52.00
PO+SD	**Ac.sp*, *Cl.sp, Sc.sp*, **Ba.pu, *Ba.su*	5.6	1.6	28.57	**As.ng, *Pe.no, *Fu.sn , Mu.sp, Tr.sp*	2.0	0.8	40.00
PO+LB	**Ac.sp*, **Mi.lu,* Mi.ro, *Sc.sp, *Ba.pu*	5.2	2.1	40.38	**As.ng, *Pe.no, *Fu.sn , Mu.sp, Tr.sp*	2.3	1.0	43.48
SD+LB	**Ac.sp*, **Mi.lu,* Mi.ro, *Sc.sp, *Ba.pu , Ps.ar*	4.9	2.0	40.82	**As.ng, As.fv, *As.fm , *Pe.no, *Fu.sn, Mu.sp*	2.9	1.2	41.38
PO+SD+LB	**Ac.sp*, *Cl.sp, Sc.sp*, **Mi.lu,* Mi.ro, **Ba.pu , *Ba.su, Ps.sp, *Ps.ar*	4.8	2.3	47.92	**As.ng, As.fv , *As.fm , *Pe.sp, Fu.sp*	1.8	0.9	50.00

PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.**Hydrocarbon degraders. *Ac.sp= Achromobacter* sp, *Mi.lu= Micrococcus luteus, Mi.ro= M. roseus, Ba.pu= Bacillus pumilis, Ba.su= B. subtilis , Ps.sp= Pseudomonas* sp, *Ps.ar= P. aeruginosa, Sc.sp= Sarcina* sp, *Cl.sp= Clostridium* sp, *As.ng= Aspergillus niger, As.fv= A. flavus, As.fm=A. fumigates, Pe.sp= Penicillium* sp., *Pe.no= Penicillium notatum, Rz.st= Rhiszopus stolonifer, Mu.sp= Mucor* sp, *Ge.sp= Geotrichum* sp, *Tr.sp= Trichoderma* sp, *Sm.sp= Saccharomyces* sp, *Fu.sn= Fusarium solani, Fu.sp= Fusarium* sp.

Table 10: Weed distribution studies of waste engine oil- polluted soil at 3months after pollution

Weeds	Unpolluted soil	Polluted soil	PO	SD	LB	PO+ SD	PO+ LB	SD+ LB	PO+ SD+ LB	Total	Occurren-ce (%)
Acanthosperum hispidum	10	0	0	1	0	4	1	0	4	20	6.08
Axonopus compressus	9	1	1	0	1	2	1	1	2	18	5.47
Chromolaena odorata	19	1	5	2	1	7	3	2	7	47	14.29
Commelina benghalensis	19	1	3	2	3	3	3	3	6	43	13.07
Cyperus haspan	24	0	2	3	2	3	2	3	7	46	13.98
Euphorbia spp	28	1	3	1	1	4	2	2	5	47	14.29
Ipomea involucrata	14	0	0	0	1	2	2	0	3	22	6.69
Panicum maximum	5	1	1	1	2	2	2	2	2	18	5.47
Phyllantus amarus	14	0	1	0	1	2	1	0	3	22	6.69
Unidentified plants (< 5cm tall)	32	0	2	2	1	3	1	1	4	46	13.98
Total	174	5	18	12	13	32	18	14	43	329	100

PO: Poultry manure, SD: Sawdust, LB: Dried leaves of *Vernonia amygdalina.*

1.3 Discussion

The effects of soil amendments on soil physiochemical properties have been previously reported by Ikhajiagbe (2010), Ikhajiagbe and Anoliefo (2010), and Ikhajiagbe and Anoliefo (2012). However, no significant differences were recorded in pH, electrical conductivity, exchangeable acidity, as well as in cationic contents of soil. Electrical conductivity (EC) of the soil was highest in treatment SD (350µS/cm) compared to the control (390µS/cm) indicating an insignificant decrease. Osuji and Nkoye (2007) and Ikhajiagbe and Anoliefo (2010) had previously reported reductions in soil EC as a result of oil contamination. It is very unlikely that the released oil was directly responsible for the observed changes in EC since organic compound, crude oil in this case, have poor electrical current conductivity. Anoxic biodegradation mechanism through direct dehydrogenation might have allowed the anaerobic metabolism of hydrocarbons in the presence of an electron acceptor such as nitrate ion. This presents a high possibility for the observed differences in EC.

There were significant increases in average phosphate contents of soil with or without amendment. Lehtomake and Niemela (1975) reported a low value of N, K and P reserve in petroleum hydrocarbon contaminated soil. This contradicts the discovery in this research. The reduction in the concentration of NO_3^- in the amended contaminated site suggests that the process of nitrification might have reduced nitrite impacted during the incidence of oil spillage, in this case, WEO. According to Odu (1972), oil degrading microbes such as *Azobacter* spp. normally become relatively abundant, while nitrifying bacteria such as *Nitrosomonas* spp. become reduced in number.

Metals are toxic to all biological systems from microbial to plants and animals, with microorganisms affected more, due, in part, to their small sizes and direct relationship with their environment (Giller, *et al*., 1999; Sarret *et al*., 2005). Metal toxicity negatively impacts all cellular processes, adversely influencing metabolism, genetic fidelity and growth. Loss of potential populations in the WEO polluted soil impacts elemental cycling organic

remediation efforts, plant growth and soil structure. The control and optimization of bioremediation processes is a complex system of many factors. These factors encompass the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population, the environment factors such as temperature, pH, the presence of oxygen or other electron acceptors and nutrients (Ashok *et al*., 2010). However, the ultimate goal of soil remediation is to remove toxic elements which encompass heavy metals and PAHs, from the soil or decrease mobility and toxicity within the sample (Amakiri and Onofeghara, 1984; Udo and Fayemi, 1975). Heavy metal remediations in amended oil-polluted soils are made possible by a number of factors ranging from enhanced volatilization and leaching resulting from improved soil porosity (Davis and Wilson, 2005, Ikhajiagbe and Anoliefo, 2010), microbial transformation (Tebo *et al*. 1997) from improved presence of resident microorganisms, as well as plant enhanced and plant-assisted remediations due to presence of weeds that developed from soil seed bank (Ikhajiagbe and Anoliefo, 2012). The present study showed that although there was not much difference among heavy metal remediations in the soil due to soil amendment, PO-amended soil was relatively lower in metal concentrations. This may be perhaps because it appeared to be the richest in nutrients of all the other amendments used (see Table 2). In combinations with other amendments, results were better than when used alone.

Significant decreases in soil total polyaromatic hydrocarbons (PAH) were recorded. Total PAH decreased from 833.62 mg/kg at 1 WAPA to 46.85 mg/kg in the SD-treated soil at 3 MAPA, compared to 276.33 mg/kg recorded in the control at 3 MAPA. The bioremediation efficiency was highest in the SD- (94.38%) as well as the LB-amended soils (94.27%) respectively, compared to 66.86% remediation efficiency in the control. PAH reductions may have resulted from similar mechanisms as the heavy metals reported earlier. The significant impact of sawdust substrate in bioremediation of oil- polluted soil have been previously reported by Ikhajiagbe (2010); Ikhajiagbe and Anoliefo (2012).

Cunningham and Ow (1996), recorded that in phytoremediation, plants together with associated microorganisms are employed for decontamination. This indicated also that amendments were not solely responsible for the decrease in PAH contents, but the possibly in synergism with the weeds that emerged from the soil seed bank.

The present study recorded most prevalent bacteria species as *Achromobacter* sp, *Micrococcus luteus,* M. *roseus, Bacillus pumilis, Pseudomonas* sp and *Sarcina sp* (Table 9). Prevalent fungi were *Aspergillus niger, Penicillium sp, Fusarium solani, Mucor sp* and *Trichoderma sp.* These microorganisms may have been involved in the remediation process indicated by their prevalence. This may signify tolerance to these pollutants. These microorganisms have been previously identified as active members of bioremediation microbial consortia by Cerniglia (1992), Ekundayo and Obuekwe (1997), Yogambal and Karegoudar (1997), Remero *et al*. (2001) and April *et al*. (2000).

The composition of soil seed bank in the soil is important in the present study. A total of 19 different weed species comprising of the families Convolvulaceae, Commelinaceae, Cyperaceae, Euphorbiaceae, Fabaceae, Rubiaceae, Portulacaceae, Malvaceae and predominantly Asteraceae, and Poaceae were identified. The selective enrichment of hydrocarbon degrading microorganisms will be demonstrated in the soils with abundant and unaltered soil seed bank (Ikhajiagbe *et al*., 2013d) indicating their tolerance to oil in association with the synergistic relation existing between them in the present study. The synergy that exists is particularly important in their relationships with plant growth. Some weed species that emerged from the soil seed bank in the oil-polluted soils are likely to be oil tolerant species previously suggested by Anoliefo *et al*. (2006). These plants were identified from an oil polluted auto- mechanic workshop. The capability of a good number of plants for recovery of heavy metals from soils has also been reported (Wong and Chu, 1985, Wong and Lau, 1985, Anoliefo and Vwioko, 2001). The weeds identified to have grown in this study include *Acanthosperum hispidum, Axonopus compressus, Chromolaena odorata, Commelina benghalensis, Cyperus haspan, Euphorbia* spp*, Ipomea involucrata, Panicum maximum, Phyllantus amarus,* and a good number of unidentified plants (< 5cm tall).

It should be reemphasized that remediation occurs by a number of factors including the weed species, microbial composition and the soil amendments. Natural attenuation could have occurred despite the amendments used based on the reliance on natural conditions and behavior of soil microorganisms that are indigenous to soil (Perfumo *et al*., 2007). The natural attenuation process might have been catalysed by the addition of nutrients and other substances, while utilising the indigenous microbial population to remediate the soil through the process of biostimulation might have occurred. There is always a synergistic reaction in the phytoremediation process. Some evidence indicates that microbial community activities recover upon metal remediation. Numerous microbially mediated transformations of metals have been identified (Yamazaki *et al*., 1988; Yogambal and Karegoudar, 1997).

1.4 Conclusion

The present study thus reaffirmed that amendment of soil polluted with waste engine oil offers great

opportunities for increased weed diversity thereby enhancing bioremediation with possible synergism between plants and their associated microorganisms. Inorganic inputs to the soil have its great adverse implications hence it is not encouraged in this study rather the organic inputs of sawdust, poultry manure and dried leaves is encouraged. These amendments are cost effective and show reduced environmental risks. The resident plants and its biodiversity of an area are also very important in phytoremediation process. These plants are better adapted for such areas with reduced risks of competition among plant species since the area has been colonized. In addition to the adaptive mechanisms of resident plants, there are economic implications compared to the use of commercially available seeds. These soil seed banks are abundant, diverse and effective promoters of functioning of the ecosystem invariably the decomposition of contaminants and pollutants in addition to nutrient cycling.

1.5 Acknowledgments
Special thanks to all the undergraduate students in the laboratory who contributed to the success of this study.

References

Amakiri, J. O. and Onofeghara, F. A. (1984). Effect of crude oil pollution on the germination of Zea mays, and Capsicum frutescens. *Environmental pollution*, 35: 159- 167.

Anoliefo, G.O. and Vwioko, D.E. (1995). Effects of spent lubricating oil on the growth of *Capsicum annum* and *Lycopersicon esculentum*. *Environmental Pollution,* 88: 361-364.

Anoliefo, G.O., Ikhajiagbe, B., Okonokhua, B.O. and Diafe, F.V. (2006). Ecotaxonomic distribution of plant species around auto mechanic workshops in Asaba and Benin City: Identification of oil tolerant species. *African Journal of Biotechnology*, 5(19):1757-1762.

Anoliefo, G.O. and Ikhajiagbe, B. (2011). Plant-microbial interaction in the degradation of crude oil in soil: Synergism in bioremediation. *Nigerian Journal of Life Sciences*, 1(1): 40-52.

Anoliefo, G.O. and Vwioko, D.E. (2001). Tolerance of *Chromolaena odorata* (L) K & R. grown in soil-contaminated with spent lubricating oil. *Journal of Tropical Bioscience,* 1(1): 20-24.

April, T.M., Abbot, S.P., Foght, J.M. and Currah, R.S. (2000). Hydrocarbon degrading filamentous fungi isolated from flare pit soils in Northern and Western Canada. *Canadian Journal of Microbiology*, 46: 38-49.

Ashok, K., Balwant, S.B., Vishnu, D.J. (2010). Biosorption of Heavy Metals by four acclimated microbial species, *Bacillus* spp., *Pseudomonas* spp., *Staphylococcus* spp. and *Aspergillus niger*. *Journal of Biology and Environmental science*, 4(12): 97-108.

Atuanya, E.I. (1987). Effect of waste engine oil pollution on physical and chemical properties of soil. A case study of waste oil contaminated Delta Soil in Bendel State. *Nigeria Journal of Applied Sciences,* 5:155-176.

Baker, A.J.M. (1970). The effects of oil on plants. *Environmental Pollution*, 1:27-44.

Bray, R.H. and Kurtz, L.T. (1945a). Soil chemical analysis. *Soil Science*, 59: 39-45.

Bray, R.H. and Kurtz, L.T. (1945b). Determination of total organic and available form of Banks, M.K., Kulakow, P., Schwab, A.P., Chen, Z. and Rathbone, K. (2003). Degradation of crude oil in the rhizosphere of Sorghum bicolor. *International Journal of Phytoremediation*, 5(3): 225-234.

California Environmental Protection Agency (Cal-EPA) (2005). A*ir Toxics Hot Spots Program Risk Assessment Guidelines,* Part II Technical Support Document for Describing Available Cancer Potency Factors. Office of Environmental Health Hazard Assessment, California Environmental Protection Agency. May.

Cerniglia, C.E. (1992). Biodegradation of polycyclic aromatic hydrocarbons. *Journal of Biodegradation*, 3: 351-368.

Cheesebrough, M. (1998). *District Laboratory Practice in Tropical Countries*, part II (Microbiology). Cambridgeshire Tropical Health Technology, Cambridge, UK. 231.

Cowan, S.T. and Steele, K.J. (1974). *Manual for Identification of Medical Bacteria*. 2nd. Ed., Cambridge University Press, Cambridge, UK. 216p.

Cunningham, S.D. and Ow, D.W. (1996). Promises and prospects of phytoremediation. *Plant Physiology*, 110 (3): 715-719.

Davis, J.G. and Wilson, C.R. (2005). Choosing a Soil Amendment. Colorado State University Cooperative Extension and Horticulture. 7: 235.

De Jong, E. (1980). The effect of a crude oil spill on cereals. *Environmental Pollution series A*, *Ecological and biological*, 22(3): 187-196.

Efroymson, R.A., Will, M.E., Suter II, G.W. and Wooten, A.C. (1997). *Toxicological Benchmarks for Screening Contaminants of Potential Concern for Effects on Terrestrial Plants*: 1997 Revision. ES/ER/TM-

85/R3. U.S. Department of Energy, Office of Environmental Management, 123p.
Ekundayo, E.O and Obuekwe, C.A. (1997). Effects of oil spill on soil physicochemical properties of a spill site in a typic paledult of Midwestern Nigeria. *Journal of Environmental Pollution*, 22: 187-196.
Epstein, E. (1972). Mineral Nutrition of plants. Principles and perspective. John Wiley and Sons Inc, New York. p 412.
Giller, K.E., Wittwer, E. and McGrath, S.P. (1999). Assessing risks of heavy metal toxicity in agricultural soils. *Human Ecology and Risk Assessment*, 5: 683–689.
Gudin, C. and Syratt, W.J. (1975). Biological aspects of land rehabilitation following hydrocarbon contamination. *Environmental Pollution*, 8: 107-112.
Honeywell (2011). Draft Onondaga Lake capping, dredging and habitat intermediate design. Honeywell - SYR\446232 - Cap Design\09 Reports\9.1 Intermediate Design Report\Final to DEC - 1-24-11\Final Report 1-24-11\Tables\
Ikhajiagbe, B. (2010). Synergism in Bioremediation: Phytoassessment of Waste Engine Oil Polluted Soils after Amendment and Bioaugmentation. LAP Lambert Academic Publishing, Köln, Germany. 276p
Ikhajiagbe, B. and Anoliefo, G.O. (2010). Impact of soil amendment on phytotoxicity of a 5-month old waste engine oil polluted soil. *Journal of Ecology and Natural Environment*, 2(6): 112-122.
Ikhajiagbe, B. and Anoliefo, G.O. (2012). Weed Biodiversity Studies of a Waste Engine Oil-polluted Soil Exposed at Different Intervals of Natural Attenuation and Substrate Amendment. *Journal of Biological Sciences,* 12: 280-286.
Ikhajiagbe, B., Anoliefo, G.O., Oshomoh, E.O., Ogedegbe, U.A. and Airhienbuwa, N. (2012). Changes in polyaromatic hydrocarbon content of a waste engine oil polluted soil exposed to pH adjustments. *Annual Review and Research in Biology,* 2(3): 66-82.
Ikhajiagbe, B., Anoliefo, G.O., Oshomoh, E.O. and Airhienbuwa, N. (2013a). Changes in heavy metal contents of a waste engine oil polluted soil exposed to soil pH adjustments. *British Biotechnology Journal,* 3(2): 158-168.
Ikhajiagbe, B., Anoliefo, G.O., Jolaoso, M.A., and Oshomoh, E.O. (2013b). Phytoassessment of a petroleum hydrocarbon contaminated soil exposed to two different intervals of monitored natural attenuation using African yam bean [*Sphenostylis stenocarpa*]. *Pakistan Journal of Biological Sciences,* 16 (14): 680 – 685.
Ikhajiagbe, B., Anoliefo, G.O., Oshomoh, E.O. and Agbonrienrien, B. (2013c). Effects of watering regimes on the intrinsic qualities of bioremediated waste engine oil-polluted soil. *Annual Review and Research in Biology,* 3(2): 107 – 123.
Ikhajiagbe, B., Anoliefo, G.O., Chijioke-Osuji, C.C. and Ogedegbe, U.A. (2013d).The Role of Natural Weed Species from Soil Seed Bank in the Natural Attenuation of a Petroleum Hydrocarbon Polluted Soil. *International Journal of Plant & Soil Science,* 2(1): 82-94.
Jackson, M.L. (1962). Soil Chemical Analysis. Prentice Hall, Englewood Cliffs, New Jersey. 276p.
Lehtomake, M. and Niemela, S. (1975). Improving microbial degradation of oil in soil. *Am. Boil*. 4: 126-129.
Nelson, D.W. and Sommers, L.E. (1982). Total carbon, organic carbon and organic matter. In: Methods of soil analysis, Part 2. ASA/SSSA. Madison WI. pp 539 – 579.
Odu, C. T. I. (1972). Microbiology of soils contaminated with petroleum hydrocarbons. In: The extent of contamination and some soil and microbial properties after contamination. *Journal of Institute of Petroleum*, 58: 201- 208.
Osuji, L.C. and Nwoye, I. (2007). An appraisal of the impact of petroleum hydrocarbon on soil fertility: the Owaza experience. African Journal of Agricultural Resources, 2(7): 318-324.
Perfumo, A., Ibrahim, Banat, M., Marchant, R. and Vezzulli, L. (2007). Thermally enhanced approaches for bioremediation of hydrocarbon- contaminated soils. *Chemosphere*, 66: 179-184.
Romero, M.C., Hammer, E., Cazau, M.C. and Arambarri, A.M. (2001). Selection of autochthonous yeast strains able to degrade biphenyl. *World Journal of Microbiology and Biotechnology*, 17: 591-594.
Sarkar, D., Ferguson, M., Datta, R. and Birnbaum, S. (2005). Bioremediation of petroleum hydrocarbons in contaminated soils: Comparison of biosolids addition, carbon supplementation and monitored natural attenuation. *Environmental Pollution*, 136: 187-195.
Sarrett, G., Avoscan, L., Carrière, M., Collins, R., Geoffroy, N., Carrot F Covès, J. and Gouget, B. (2005). Chemical forms of selenium in the metal-resistant bacterium *Ralstonia metallidurans* CH34 exposed to selinite and selenate. *Journal of Applied Environmental Microbiology*, 71: 2331–2337.
Smith, S., Peterson, P.J., and Kwan, K.H.M. (1989). Chromium accumulation, transport and toxicity in plants. *Toxicology and Environmental Chemistry*, 24: 241-251.
Tebo, B.M., Ghiorse, W.C., van Waasbergen, L.G., Siering, P.L., Caspi, R. 1997. Bacterially-mediated mineral formation: insights into manganese(II) oxidation from molecular genetic and biochemical studies. Rev

Mineral 35, 225-266.

Udo, E.J. and Fayemi, A.A. (1975). The effect of oil pollution of soil on germination, growth and nutrient uptake of corn. *Journal of Environmental Quality*, 4: 537–540.

Wong, M.H. and Chu, L.M. (1985). Yield and metal uptake of *cynodon dactylon* (Bermuda grass) grown in refuse- compost- amended soil. *Agricultural ecosystem and environment,* 14: 41-52.

Wong, M.H. and Lau, W.M. (1985). Root growth of *Cynodon* and *Eleusine indica* collected from motor ways at different concentration of Pb. *Environmental resources,* 36: 257-267.

Yamazaki, Y., Hayashi, Y., Hori, N. and Mikami, Y. (1988). Microbial conversion of b -myrcene by *Aspergillus niger*. *Agriculture, Biology and Chemistry*, 52: 2921- 2922.

Yogambal, R.K. and Karegoudar, T.B. (1997). Metabolism of polycyclic aromatic hydrocarbons by *Aspergillus niger*. *Indian Journal of Experimental Biology*, 35: 1021-1023.