TETFUND/UNIBOKKOS/ARJ/3

NATURAL ATTENUATION OF AN OIL-POLLUTED SOIL AFTER EXPOSURE TO COMPOST

[*1,2]Ikhajiagbe, B., [1,2]Anoliefo, G. O and [3]Mshelmbula B. P
[1]Environmentsl Biotechnology and Sustainability Research Group
[2]Dept. of Plant Biology and Biotechnology, Univ. of Benin, Nigeria
[3]Dept. of Biol. Sci., Adamawa State University, Mubi, Nigeria
ikhaj@yahoo.com (+2348037094470)

ABSTRACT

The present study investigated the remediation of an oil-polluted soil (OPS) as well the development of a soil seed bank after exposing it to compost. Top soil was collected from an area of predetermined soil seed bank and was thoroughly mixed with spent lubricating oil (SLO) to obtain a constant 5%w/w concentration. The OPS was immediately amended with compost from nine different sources including sawdust, hay, garden debris, cooked food, uncooked food, and wood ash. After 3, months results showed a decrease in the total petroleum hydrocarbon (TPH) concentration. TPH reduced from 3143.78 - 993.52mg\kg in the sawdust – compost amended soil, indicating a 72% remediation efficiency of 21.74% when no compost was applied. TPH reduced to 2.03 mg/kg in the soil amended with hay-compost (HAC), thus indicating 99.99% remediation efficiency. Comparatively, soil amendment with hay showed significant improvement in oil remediation. Throughout the duration of the study, *Bacillus substilis, Micrococcus luteus, Micrococcus varians, Proteus vulgaris,* and *Pseudomonas* sp. were consistent throughout the study, thus suggesting possible tolerance to oil. The most predominant fungal species was *A. niger.* Enhanced biodegradation may have resulted from the weed diversity shown on the study. Total number of weeds in the amended soil was highest in the composted-hay-amended soil. Weed species of *Fimbristylis ferruginea* and *Sporobolus pyramidalis* prevalent in the study.

KEYWORDS: Bioremediation, compost, hydrocarbons, natural attenuation, soil amendment, weeds.

INTRODUCTION

Oil and gas industries contribute to major industrial pollution and need to provide adequate preventive measures to minimize the environmental pollution have been stressed. In Nigeria, for example, most of the terrestrial ecosystem and shorelines in oil-producing communities are important agricultural land under continuous cultivation (Ikhajiagbe

et al., 2013b). However in non-oil-producing states, most of the agricultural lands are exposed to indiscriminate disposal of spent lubricating oil (SLO). Any contact with SLO results in damage to soil, condition of these agricultural lands, microorganisms and plants, and the resultant effect in the economy and the lives of the populace may be very devastating. Contamination of the environment with petroleum hydrocarbon, consist of aliphatic (cyclic and acyclic), simple aromatics and polynuclear aromatic hydrocarbons (PAHs). This is therefore, a cause of serious concern worldwide (Barker and Gretchen 2002; Ouyang *et al.*, 2005).

The impact of oil in soil is very devastating (Baker, 1970; Gudin and Syratt, 1975; De Jong, 1980; Isirimah *et al*., 1989; Ikhajiagbe, 2010). Plant germinates, develop and grow in soil medium where water, air and nutrient resources supply plant for healthy growth for productive and profitable agriculture. However, Oil-polluted soil could become unsuitable due to number of factors imposed by oil in soil, including reduction in soil oxygen from the soil's waxy nature, impenetration and low infiltration (Ikhajiagbe, 2010), a reduction in the level of available plant nutrients or a rise to a toxic level of heavy metals (Udo and Fayemi, 1975; Wang, 1994), as well as the presence of toxic aliphatic and aromatic compounds (Günther *et al*., 1996).Oil penetrates into plants where it moves in the intracellular spaces and possibly also in the vascular system (Baker, 1970). Several authors (Epstein, 1972; Smith *et al.*, 1989 and Udo and Fayemi, 1975) have also demonstrated experiments on the phytotoxic effects of petroleum oil. Epstein (1972) reported that mineral ions absorbed initially by the roots are finally received by the mesophyll cells thereby causing dehydration in plants. Udo and Fayemi (1975) also reported that growth of cereals was adversely affected in oil-polluted soil, resulting in leaf Chlorosis and plant dehydration. Smith *et al.*, (1989) stated that root stress caused by crude oil reduces leaf area through stomata conductance. All these impact on plant and soil usually results in reduction in yield of plant of economic importance (Ikhajiagbe, 2010).

Since the past decade, soil amendments have been discovered to be one of the various natural means of rehabilitation of oil-polluted soils (Benka-Coker and Ekundayo, 1995; Sutherland *et al*., 1995; Bardos*et al*., 1996; Adedokun and Ataga, 2007; Ikhajiagbe, 2010; Ikhajiagbe and Anoliefo, 2010; 2011). In the bioremediation of oil-polluted soils, soil amendment or additives,

such as sawdust, peat, waste cotton, manure, and fertilizers are usually added to increase activities of soil micro-organisms, as well as to improve the soil's physical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure (Davis and Wilson, 2005; Ikhajiagbe, 2010). While growing on the substrate amendment, the soil microorganisms probably produce hydrocarbon-metabolizing enzymes within the compost matrix (Sutherland *et al*., 1995; Bardos *et al*., 1996; Diaz *et al*., 1996). The high microbial load in the compost at the start of the composting afforded the population the opportunity to remain high while adapting to and attacking the hydrocarbon substrate.

As an emerging arm of bioremediation of oil-contaminated soils, compost technology uses biological system of microorganisms in the compost to sequester or breakdown contaminant in the soil. The use of composted organic matter provides good humus to build up soil quality and some basic plant nutrients and accelerates the abundance and species diversity of soil microbial community (Barker and Gretchen 2002, Ouyang *et al.*, 2005).It provides an efficient, co-friendly, cost-effective solid waste management option (Adekunle and Adekunle 2006). Composting transforms raw organic waste materials into biological stable, humus substances that make excellent soil amendments. This is even a very important way of keeping organic wastes at bay, since by value addition; the organic wastes are put into use in remediation technology as well as in agricultural crop improvement.

In the present study, the soil is amended with different organic waste materials and then allowed to naturally attenuate white taking note of development of the soil's inherent weeds population from the soil seed bank.

MATERIALS AND METHODS

Sources of soil amendments

Sources of compost used in the present study were wood ash (WA) from a bakery, sawdust (SD) from a sawmill, cooked food (CF) and uncooked food wastes (UF) from restaurants in the University of Benin, Benin City, fruit peels (FP) from fruit shops and garden debris (GD) in the University's main campus, hay as well as cow dung from Kara Cattle Market in Aduwawa, Benin City. Measured 3kg of each of the wastes was buried in 95cm deep and left to decompose for 1 month. After 1 month, compost was excavated and ready for use.

Preparation of materials

Soil samples were collected randomly from around the Botanical Garden, University

of Benin, Benin City within a marked plot measuring 10 m × 15 m. the collected soils were pooled together to give a composite sample. Weeds found around this plot and within 5m radius of this plot were identified, and these made up the soil seed bank (SSB) for the present study. Top soil from this marked plot was measured (13kg) into wide bowls (97cm in diameter, 32cm in height) and was thoroughly mixed with spent lubricating oil (SLO) to obtain a constant concentration of 5% w/w oil-in-soil. The bowls were arranged in split plots based on randomized complete block design with 3 replications. The soil was amended separately with compost derived separately from the different organic wastes already collected. One (1) kg of compost was applied to soil in bowls and thoroughly mixed. The entire set up was exposed for 3 months, beside the Botanic garden, University of Benin.

Collection of samples for analyses

The first sample was taken for analysis without amendment immediately after pollution before amendment (APBA). The second set was taken three months after pollution and amendment (3MAPA).Determination of TAH and polyaromatic hydrocarbon (PAH) contents in the soil samples wereachieved according to methods described by Dean and Xiong (2000). The soil samples were also assayed for presence of microorganisms.Isolation and characterization of bacterial and fungal isolates as well as oil degraders were carried out using the methods of Sabba (1995), Cheesebrough (1998) and Taiwo and Oso (2004).

Computation of Contamination Factor

Contamination factor (CF) expresses the ratio between the eventual concentrations of pollutant against its background concentration. Background concentration in this study refereed to concentration of contaminants prior to exogenous applications of SLO (Ikhajiagbe, 2010).

$$CF = \frac{\text{Concentration of pollutant}}{\text{Background/ Concentration}}$$

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 according to Ikhajiagbe *et al*. (2013c) as;

$$\text{Efficiency (\%)} = \frac{\text{Concentration at 3MAPA}}{\text{Concentration at 1DAPA}}$$

Where MAPA = months after pollution and amendment; DAPA = days after pollution and amendment.

RESULTS

The rich diversity of weeds was identified within the experimental plot just before collection of top soil for use (Table 1). Weeds of the family Poaceae and Cyperaceae were very common. *Fimbistylis ferruginea*, *Sporobolus pyramidalis*, and *Eleusinindica* were the most prevalent weed species found in the area.

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

Weeds	Family	*Total individual species	Frequency of occurrence (%)
Andropogon virginatus	Poaceae	1	1.54
Asystasia gangetica	Poaceae	5	7.69
Croton hirtus	Euphorbiaceae	5	7.69
Centrosema pubscers	Fabaceae	3	4.62
Cyperus haspan	Cyperaceae	6	9.23
Chromolina benghanlensis	Commelinaceae	5	7.69
Eleusinindica	Poaceae	6	9.23
Fimbisstylis ferruginea	Cyperaceae	8	12.31
Gomphrina celosoides	Amaranthaceae	4	6.15
Kyllingaerecta	Cyperaceae	3	4.62
Mariscus alterenifolios	Cyperaceae	2	3.08
Pennisetum purpureum	Poaceae	6	9.23
Sporobolus pyramidalis	Poaceae	7	10.77
Tridax proambens	Asteraceae	4	6.15
Total		65	100.00

*The surface area within which plants were surveyed was 10 m × 15 m.

Table 2 shows the total petroleum hydrocarbons (TPH) of materials used for the study. TPH of oil was 35365.61 mg/kg, compared to 16.98 mg/kg in the soil.

Table 2: Total petroleum hydrocarbons (TPH) of materials used for the study

Parameters	Oil	Soil
Nonane (C 9)	4827.21	1.96
Decane(C 10)	5256.98	3.63
Dodecane(C 12)	6443.87	9.65
Tetradecane(C 14)	4734.05	0.64
Hexadecane(C 16)	235.87	<0.001
Octadecane(C 18)	3188.55	0.08
Nonadecane(C 19)	1065.65	<0.001
Eicosane(C 20)	3222.65	0.07
Docasane(C 22)	2000.76	<0.001
Tetracosane(C 24)	856.87	0.95
hexacosane(C 26)	424.98	<0.001
Tricosane(C 30)	1075.76	<0.001
Total Aliphatic Hydrocarbon (mg/kg)	33333.2	16.98
Total Aromatic hydrocarbon(mg/kg)	2032.41	<0.001
TPH (mg/kg)	35365.61	16.98

Just after pollution, TPH was 26,523.76 mg/kg (Table 3). Three months after application of soil amendments, TPH reduced to7274.52 mg/kg in the oil-polluted soil that was amended with sawdust (SDC) and 2.03 mg/kg in the soil amended with hay-compost (HAC), thus indicating 99.99% remediation efficiency. Comparatively, soil amendment with hay showed significant improvement in oil remediation. Although remediation efficiencies for soils amended with compost obtained from wood ash (WAS), cooked food (CFC) and garden debris (GDC) were all above 40%, remediation due to soil amendment with compost from uncooked food wastes (UFC) was less than when no soil amendment (NON) was used. TPH in NON was 20757.01 mg/kg, compared to 21221.42 mg/kg in UFC. Notably, among the wastes obtained for composting, that from uncooked food contained both decaying and freshly disposed wastes. Computation of contamination factor thus showed that HAC totally removed the contaminants from the soil; returning it to its original state (CF, 0.12). Although TPH remediation efficiencies for SDC, WAC, CFC and FPC were all above average, CF was still very greater than unity (i.e. CF >> 1), and hence, contaminants are still assumed to be present in the soil due to the oil initially applied.

Comparatively, hexadecane was the aliphatic hydrocarbon that was significantly

remediated by the treatment applications, where it was undetected in (> 0.001 mg/kg) in SDC, WAC, GDC and HAC, in HAC and SDC respectively after 3 months (Table 3).

Total bacterial count at the first day when compost was mixed with soil (Fig. 2), just before oil was added was 3.7 x 10^5cfu/g in NON, 2.2 x 10^5cfu/g in SDC, 1.2 x 10^5cfu/g in UFC and 2.4 x 10^5cfu/g in FPC. After 1 month total bacterial colony count reduced to 2.5 x 10^5cfu/g in NON, but eventually increased to 5.0 x 10^5cfu/g in the following month. The highest cfu/g was obtained in SDC at 3 month (8.3 x 10^5cfu/g). Bacterial colony-forming units were higher in SDC (7.0 x 10^5cfu/g), CFC and GDC (6.8 x 10^5cfu/g) respectively. Mean fungal colony count was 1.5 x

Table 5 shows the weed diversity studies of spent lubricating oil-polluted soil of different treatments. At 3 MAPA, there were 92 weeds altogether including the control. Total number of weeds in the amended soil ranged from 6 – 26, with HAC being the highest. The distribution of the weed showed that *Sporobolus pyramidalis* (frequency of occurrence, 7.61%), *Eleusineindica* (6.25%), *Fimbristylis ferruginea* (6.25%) and *Pennisetum purpureum* (6.25%) were the predominant weeds respectively. Although, there were 27 species of

respectively, and less than 27 mg/kg in both CFC and UFC. Hexacosane was undetected

10^5cfu/g at start (Fig. 3), and then increased to 5.8 x 10^5cfu/g in 3 months in NON, compared to GDC which also increased from 1.7 x 10^5cfu/g to 4.8 x 10^5cfu/g, making it the amended soil with the most fungal colony-forming units, followed by FPC (4.6 x 10^5cfu/g) .

The most predominant fungal species was *A. niger*. *Pennicillium* sp. was also predominantly present in all treatments but CFC (Table 4). GDC and SDC contained the highest number of individual fungal isolates present; *Aspergillusflavus, A. niger*, *Fusarium solani*, *Penicillium* sp. and *Rhizopus* sp.

unidentified (< 5 cm) tall weeds, the least identified weeds were *Andropogon virginatus* and *Mariscus alternifolios.*

DISCUSSION

The changes in the intrinsic qualities of an oil-polluted soil and development of soil seed bank after exposure different sources of waste, has been investigated. Similar work has previously been reported by Ikhajiagbe *et al.* (2012) and Ikhajiagbe *et al.* (2014). The aim of bioremediation is to remove toxic element which encompasses heavy metals, PAHs, and TPH,

from the soil to decreased mobility and toxicity within the sample (Udo and Fayemi, 1975; Amakiri and Onofeghera, 1984). Barker and Gretchen (2002) and Ouyang *et al*.(2005) reported that composted organic matter provides good humus or build up soil quality and some basic plant nutrients and accelerates the abundance and species diversity of soil microbial community.

Reductions in TPH of oil-polluted soil as a result of composting have been achieved by this study. Comparatively, remediation efficiency ranged from 40.53% - 99.99 %, with HAC being the most effective (99.99%), compared to UFC (40.53%). The reduction in hydrocarbon contents may also have resulted from a number of processes including volatilization, diffusion and microbial degradation in a dissolved state (Kappeler and Wuhrmann, 1978; Jordan and Payne, 1980). These are all processes reported to be synergistically involved in natural attenuation (Ikhajiagbe and Anoliefo, 2011; Ikhajiagbe *et al.*, 2013a, 2014).The performance of such mechanisms of attenuation also depends, to a large extent, on improved soil. Apart from improving water retention in sandy soil and promoting soil structure by increasing the stability of soil aggregates, soil amendments like compost also enhances soil fertility by modifying the chemical, physical and biological properties of the soil (Dick and McCoy, 1993). Maximum degradation efficiency is achieved through maintaining aeration and moisture if necessary and closely monitoring moisture content and temperature.

Bacterial population increased after 3 months of pollution in all treatments including the presence of *Micrococus varians* and *Bacillus subtilis* which were important petroleum hydrocarbon degraders (Das and Mukhjee, 2007), while the presence of *Aspergillus niger* and *Penicillium*sp was also reported as being prevalent among the fungal populations. These microorganisms have been previously identified as active members of bioremediation microbial consortia by Cerniglia (1992); April *et al.* (2000); Ikhajiagbe and Anoliefo (2012); Ikhajiagbe *et al.*, (2014).

Atlas (1981) reported that certain crude oil contain components that are bacteriostatic. These inhibitory effects have also been reported to depend on concentrations; a number of microorganisms like *Pseudomonas* were identified as hydrocarbon degrading microorganisms. No single microorganism has been found to be able to completely degrade a petroleum hydrocarbon molecule.

However, different species or strains of the same species may be capable of degrading different groups of hydrocarbons, found in oil.

The ability of certain microorganisms to degrade petroleum seems to be an adaptive process and is governed by environmental conditions. The presence of petroleum may also affect the microbial community through selection of species (Ibe and Ibe, 1986).

The impact of plants presence in oil-polluted soils have been found to be very important in remediation practices, particularly considering the fact that remediation of the soil could be holistically carried out by the resident plants (phytoremediation), as well as the plant microbial interaction (synergistic bioremediation), not mentioning other physical methods of remediation like volatilization, as already mentioned above (Ikhajiagbe, 2010).

A total of 92 different weed species were discovered in this present study comprising of the families Poaceae, Cyperaceae, Convovulaceae, Asteracreae, Amaranthaceae, Euphorbiaceae, Commelinaiceae and Fabaceae were identified. The weed species that emerged from the soil seed bank even after the same soil had become contaminated with oil are likely to be oil-tolerant species, as previously suggested by Anoliefo *et al.* (2006). The weeds identified were *Andropogon viginatus, Asystasia gangetica, croton hirtus, centroseme pubeseens, sporobolus pyramrdalis, cyperushaspen, Tridax procumbens, chromolina benghanlensis, Eleusin Indi, Fimbrishylis ferruginea, Gomphrina, celosolides, kyllings, Erecta, Mariscus alternifolios, Pennisetum Purpureum, and* a good number of unidentified plants (<5cm tall). The distribution of weeds (Table 2) showed that *Cyperushaspan, Eleusineindica, Fimbristylis ferruginea, Pennisetum purpureum* and *Sprobolus pyramidalus* were jointly predominant weeds with percentage occurrence ranging from 6.52 - 7.61%.

CONCLUSION

The present study reaffirms the position that amendment of oil-polluted soils improves remediative capabilities of the soils, while also increasing weed diversity and their associated micro-organisms. Rather than indiscriminately throw away waste, they could be converted into biofertilizers in the form of compost, and then used to improve the quality of contaminated soils. Although above-average remediation efficiency was obtained from compost derived from cooked-food wastes, but significantly low

when the waste were those of non-cooked foods, research is therefore necessary to find out the reason for the difference.

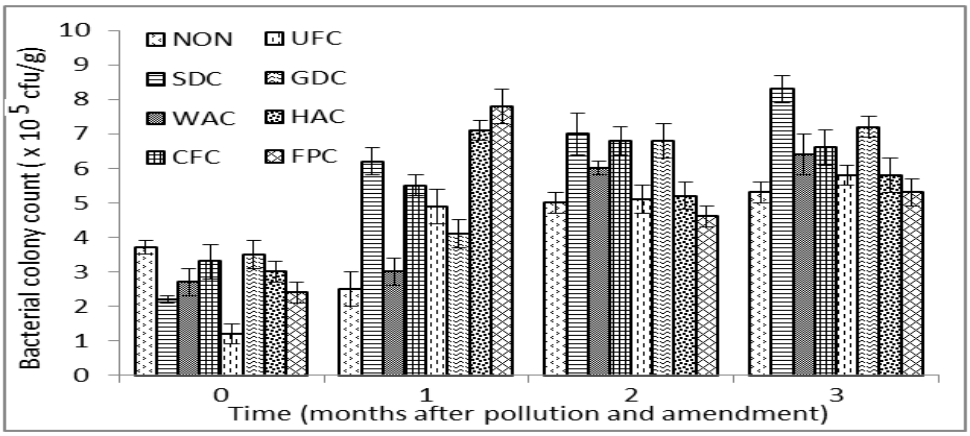

Figure 2: Total bacterial colony count of the oil-polluted soil. SDC –soil amended with Sawdust compost, WAC – with wood ash compost, CFC – with cooked food compost, UFC – with uncooked food compost, GDC – garden debris compost, HAC – with hay compost, FPC – with fruit peel compost, NON – no compost amendment.

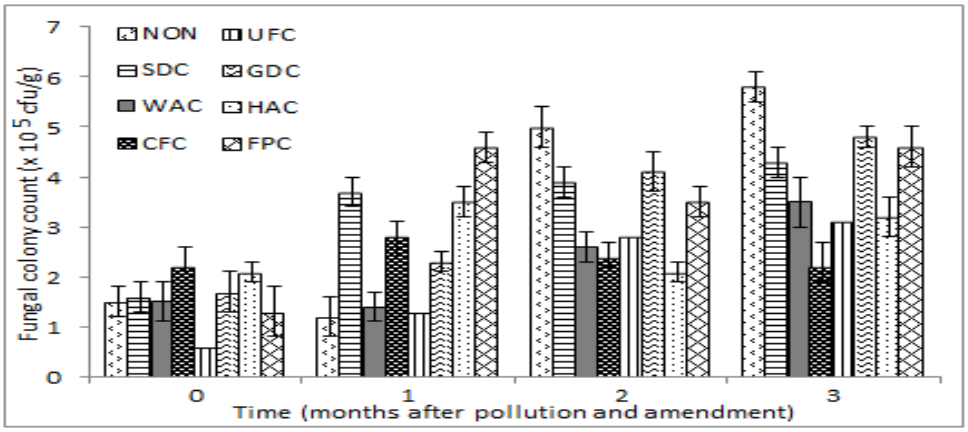

Figure 3: Total fungal colony count of the oil-polluted soil. SDC –soil amended with Sawdust compost, WAC – with wood ash compost, CFC – with cooked food compost, UFC – with uncooked food compost, GDC – garden debris compost, HAC – with hay compost, FPC – with fruit peel compost, NON – no compost amendment.

Table 3: Total petroleum hydrocarbons after 3 months of exposure to various treatments

Parameters		3 months later							
		Unpolluted	Oil-polluted soil						
	APBA	soil	NON	SDC	WAC	CFC	UFC	GDC	HAC	FPC
Nonane (C 9)	3143.78	1.43	2577.90	993.52	1453.68	1834.58	2986.87	1934.67	1.11	2192.82
Decane(C 10)	4326.36	2.63	3547.62	814.67	900.56	1784.64	4254.25	2654.78	0.58	2183.21
Dodecane(C 12)	5526.11	3.75	4531.41	483.16	395.12	856.24	5214.61	4444.26	0.21	4219.23
Tetradecane(C 14)	3276.43	0.15	2686.67	792.32	1403.54	1485.35	2999.84	1943.78	<0.001	1003.27
Hexadecane(C 16)	39.67	<0.001	12.69	<0.001	<0.001	6.67	26.46	<0.005	<0.001	14.21
Octadecane(C 18)	2543.55	0.01	2085.71	1945.39	1305.87	1063.19	2000.52	1352.15	<0.001	1092.12
Nonadecane(C 19)	835.84	<0.001	685.39	711.89	398.46	155.98	809.45	452.69	<0.001	231.32
Eicosane(C 20)	2041.56	0.03	1674.08	486.03	773.21	754.32	1975.31	1322.57	<0.001	94.32
Docasane(C 22)	1053.21	<0.001	863.63	843.26	803.52	632.16	86.45	885.63	<0.001	72.11
Tetracosane(C 24)	783.49	0.13	642.46	100.75	478.34	343.56	523.67	507.35	0.13	231.32
hexacosane(C 26)	237.83	<0.001	195.02	53.96	106.88	<0.001	111.83	103.47	<0.001	98.22
Tricosane(C 30)	303.61	<0.001	248.96	44.26	88.35	54.53	207.29	154.33	<0.001	142.12
TAH (mg/kg)	24111.44	8.13	19751.55	7269.21	8107.53	8971.22	21196.55	15755.68	2.03	11574.27
TVAH (mg/kg)	2412.32	<0.001	1005.46	5.31	10.60	6.54	24.87	18.52	<0.001	16.43
TPH (mg/kg)	26523.76	8.13	20757.01	7274.52	8118.13	8977.76	21221.42	15774.20	2.03	11590.70
TPH remediation efficiency (%)	-	-	21.74	72.57	69.39	66.15	19.99	40.53	99.99	56.30
TVAH remed. efficiency (%)	-	-	95.83	99.97	99.97	99.97	99.89	99.92	99.99	99.93
Contamination factor (CF)	142.07	0.48	1275.01	428.10	477.48	528.34	1248.32	927.89	0.12	711.96

DAP days after pollution; MAP months after pollution; NON no amendment applied; TPH total petroleum hydrocarbon, TVAH Total volatile aromatic hydrocarbon, TAH, total aliphatic hydrocarbon, APBA immediately after pollution before amendment. SDC –soil amended with Sawdust compost, WAC – with wood ash compost, CFC – with cooked food compost, UFC – with uncooked food compost, GDC – garden debris compost, HAC – with hay compost, FPC – with fruit peel compost, NON – no compost amendment. If CF > 1, then inherent contamination is still due to the oil applied, else total remediation of the exogenous contaminant has occurred.

Table 4: Bacterial and fungal species found in the soil after exposure to experimental conditions

Microorganisms	Before oil pollution	**After 3 months**							
		NON	SDC	WAC	CFC	UFC	GDC	HAC	FPC
	Bacterial species								
Athrobacter sp.	-	-	+	+	-	+	-	+	-
Bacillus substilis	+	+	+	+	+	+	+	+	+
Corynebacterium sp.	+	-	-	+	-	+	+	-	+
Micrococcus luteus	+	+	+	+	-	-	+	+	-
Micrococcus varians	+	+	+	-	-	-	+	+	+
Proteus vulgaris	+	-	-	-	+	+	-	-	+
Pseudomonas sp.	+	+	+	+	+	+	+	+	+
	Fungal species								
Aspergillus flavus	-	-	+	-	-	-	+	+	-
Aspergillus niger	+	+	+	+	+	+	+	+	+
Fusarium solani	-	-	-	-	+	-	+	-	+
Geotricum sp.	+	+	+	-	+	-	-	+	-
Penicillium sp.	+	+	+	+	-	+	+	+	+
Rhizopus sp.	+	-	+	-	+	+	+	-	+

+ present, - absent. NON no amendment applied; TPH total petroleum hydrocarbon, TVAH Total volatile aromatic hydrocarbon, TAH, total aliphatic hydrocarbon, APBA immediately after pollution before amendment. SDC –soil amended with Sawdust compost, WAC – with wood ash compost, CFC – with cooked food compost, UFC – with uncooked food compost, GDC – garden debris compost, HAC – with hay compost, FPC – with fruit peel compost, NON – no compost amendment.

Table 5: Distribution of weeds in each 97 cm – diameter pot at 3 months after pollution and amendment

Weeds	NON	SDC	WAC	CFC	UFC	GDC	HAC	Total/ pot*	Occurrence (%)
Adropogon virginatus	0	0	0	0	0	1	0	1	1.09
Asystasia gangetica	1	1	1	2	0	0	0	5	5.43
Croton hirtus	0	0	1	0	0	2	2	5	5.42
Centrosema pubescens	2	1	0	0	0	0	0	3	3.26
Cyperushaspan	5	0	0	0	0	0	1	6	6.52
Chromolina benghanlensis	0	0	0	0	1	1	3	5	5.43
Eleusinindica	0	2	1	0	0	0	3	6	6.52
Fimbristylis ferruginea	5	0	0	0	0	0	3	6	6.52
Gomphrina celosoides	2	0	0	0	0	2	0	4	4.35
Kyllingaerecta	1	0	1	0	0	1	0	3	3.26
Mariscus alternfolics	2	0	0	0	0	2	0	2	2.17
Pennisetum purpureum	2	0	0	0	0	2	2	6	6.52
Sporobolus pyramidalis	3	0	0	0	0	2	2	7	7.61
Tridax procumbens	0	0	2	0	0	0	2	4	4.35
Unidentified plants (< 5cm tall)	5	2	5	3	3	1	8	27	29.35
Total	28	6	11	5	4	12	26	92	-

*Experimental pot was 97 cm in diameter

REFERENCES

Adedokun, O.M. and Ataga, A.E. (2007) Effects of amendments and bioaugmentation of oil-polluted with crude oil, automotive gasoline oil, and spent engine oil on the growth of cowpea (*Vignaunguiculata* L. Walp).*Science Research Essay*, 2: 147-149.

Adekunle, A.A. and Adebambo, O. A. (2007). Petroleum Hydrocarbon Utilization by Fungi Isolated From *Detarium senegalense* (J. F Gmelin) Seeds. *Journal of American Science*, 3:69-76.

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

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: 1757-1762.

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.

Atlas, R.M. (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological Review*, 45: 180-209.

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

Bardos, R.P., Forsythe, S., and Westlake, K. (1996). The co-treatment of municipal and industrial waste. In: de Bertoldi, M., Sequi, P., Lemmes, B., and Papi, T. (eds.) *The Science of Composting.* Blackie Academic and Professional, London. pp767-783.

Barker, A.V. and Gretchen, M.B. (2002) Bioremediation of heavy metals and organic toxicant by composting. *Science world J.* 2: 407-420

Benka-Coker, M.O. and Ekundayo, J.A. (1995).Effect of an oil spill on soil physico-chemical properties of a spill site in the Niger Delta area of Nigeria. *Environmental Monitoring and Assessment*, 36: 93-104.

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

Cheesebrough, M. (I998) *District laboratory practice in tropical countries, part II (Microbiology).* Cambridgeshire Tropical Health Technology, Cambridge, UK

Das, K. and Mukhjee, A.K. (2007) Crude petroleum – oil biodegradation efficiency of *Bacillus subtilis* and *Pseudomonas aeruginosa* strains isolated from a petroleum oil – contaminated soil from North – East India. *Bioresource Technology* 98**:** 1339 – 1345.

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

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

Dean, J. R. and Xiong, G. (2000) Extraction of organic pollutants from environmental matrices: selection of extraction technique. *Trends in Analytical Chemistry*, 19 (9): 553–561.

Diaz, L.F., Savage, G.M., and Golueke, C.G. (1996) Stabilization of hazardous wastes through biotreatment. In: de Bertoldi, M., Sequi, P., Lemmes, B., and Papi, T.

(eds) *The Science of Composting.* Blackie Academic and Professional: London, pp. 1152-1156.

Epstein, E. (1972). *Mineral Nutrition of Plants: Principle and perspective.* John Wiley and Sons Inc, New York. p412.

Gudin, C. and Syratt, W.J. (1975).Biological aspect of land rehabilitation following hydrocarbon contamination. *Environmental Pollution*, 8:107-112.

Günther, T.U., Dornberger, N., Fritsche, V. (1996). Effects of ryegrass on biodegradation of hydrocarbons in soil. *Chemosphere*, 33: 203-215.

Ibe, S.N. and Ibe, E.C. (1986) Control and dispersion potential of oil spills by microbial seeding, In *The Petroleum Industry and the Nigerian Environment. Proceedings of an International Seminar NNPC Nov. 19- 20, PTI Warri Nigeria.* pp. 112-115.

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 the Natural Environment*, 2(6): 112-122.

Ikhajiagbe, B. and Anoliefo, G.O. (2011). Natural attenuation of a 14-month-old waste engine oil polluted soil. *Journal of Soil Science and Environmental Management 2(7)*: 184-192.

Ikhajiagbe, B and Anoliefo, G.O (2012).Weed biodiversity studies of a waste engine oil-polluted soil exposed at a different intervals of natural attenuation and substrate amendment. *Journal of biological sciences*, 12: 280-286.

Ikhajiagbe, B., Anoliefo, G.O.,Chijioke-Oseji, C.C. and Ogedegbe, U.A. (2013a).The Role of Natural Weed species from Soil Seed Bank the Natural attenuation of a Petroleum Hydrocarbon Polluted Soil. *International Journal of Plants and Soil Science*, 2(1): 82-94.

Ikhajiagbe, B.,Anoliefo, G.O., Jolaoso, M.A., and Oshomoh, E.O. (2013b).Phytoassessment of a petroleum hydrogen contaminated soil exposed to two different internals of monitored natural attenuation using African yam bean (*Sphenostylisstenocarpa*). *Pakistan Journal of Biological Sciences,*16(14): 680-685.

Ikhajiagbe, B., Anoliefo, G.O., Okoh, H.U. and Owenaze, I. (2013c). Impact of Organic Mulching on the Enhanced Natural Attenuation of a Petroleum Hydrocarbon Polluted Soil.*Nigerian Society for Experiential Biology Journal* 13(3&4): 32 – 42

Ikhajiagbe,B ,Chijioke- Osuji ,Obonochi,N., Osaruese, U.(2014). Effects of soil Amendments on the intrinsic Qualities and development of soil seed bank of a monitored Naturally attenuated Petroleum Hydrocarbon. *Journal of natural sciences research,* 4(2):59-71

Isirimah, N.O., Zuofa, K., and Longanathan, P. (1989). Effect of crude oil on maize performance and soil chemical properties in the humid forest zone of Nigeria. *Discovery and innovation,* 1: 95-97.

Jordan, B. and Payne, J. R. (1980). *Fate and Weathering of Petroleum Spills in the Marine Environment.* Michigan, Ann Arbor Science Publications Inc., 344p.

Kappeler, Th. and Wuhrmann, K. (1978).Microbial degradation of the water-soluble fraction of gas oil-11.*Bioassays with the Pure Strains of Water Research,* 12:335-342

Ouyang, W,H. Liu, V. Murygona, Y. Yu, Z. Xiu, and S. Kalyyuzhnyi 2005 .comparism of bioaugmentation and composting for remediation of oil sludge; A field – scale study in china proc. *Biochem.* 40;3763-3768

Sabba, R.N.S (1995). *Soil Microbiology: Soil Micro-organism and Plant Growth.* Oxford Publisher, New Delhi. 509p.

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.

Sutherland, J.B., Rafti, F., Khan, A.A., and Cerniglia, C.E. (1995). Mechanisms of polycyclic aromatic hydrocarbon degradation. In: Young, L.Y. and Cerniglia, C.E. (eds). *Microbial Transformation and*

Degradation of Toxic organic Chemicals. Wiley-Liss, New York. pp 269-306.

Taiwo, L.B. and Oso, B.A. (2004). Influence of composting techniques on microbial succession, temperature, and pH in a composting municipal solid waste. *African Journal of Biotechnology*. 3: 239-243.

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.

Vangronsveld, J. and Clijsters, H. (1992). A biological test system for the evaluation metal phyrotoxacity and immobilization by additives in metal contaminated soils. In: Merian, E. and Haerdi, W. (ed). *Metal Compounds in Environment and Life for Science and Technology*. Lett, Northwood, UK.Pp 117-125.

Wang, W. (1994). Rice seed toxicity tests for organic and inorganic substances. *Environmental Monitoring and Assessment*, 29:101-107.