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Natural attenuation of a 14-month-old waste engine oil polluted soil

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The effect of natural attenuation on bioremediation of waste engine oil (WEO) polluted soil was investigated. Soil was polluted with WEO at the rate of 0, 1.0, 2.5, 5.0 and 10.0% w/w oil in soil. The entire set up was left in the open shade for 5 months after pollution (5 MAP) in the first instance, and then for another 9 months (14 MAP), without mechanically disturbing the soil. Soil was carefully irrigated with 200 ml of water twice every week. There was significant reduction in heavy metal concentration of soil as well as total hydrocarbon contents. Total polycyclic aromatic hydrocarbon (PAH) content of soil reduced from 36.95 to 25.45 ppm in the control compared to 538.59 to 456.99 ppm in 10.0% w/w oil in soil. There was total eradication of chrysene, fluoranthene, fluorine and indeno(1,2,3-c,d)pyrene in all levels. Environmental risk factor initially posed by the presence of heavy metals in the soil at 5 MAP was significantly reduced to safe levels at 14 MAP.

Key words: Polycyclic aromatic hydrocarbon (PAH), waste engine oil (WEO).

INTRODUCTION

Industrialization has brought about new problems in the disposal of waste engine oil (WEO). Currently, open vacant plots and farmlands are used as workshops by motor vehicle mechanics, generator engine mechanics, and other artisans who use and dispose of engine oil (Anoliefo and Edegbai, 2000). These plots are usually utilized by these mechanics while waiting full development. A more chaotic situation is the improper disposal of WEO into run-off, gutters and water drains. This eases access of WEO into nearby agricultural lands. The pollution posed by this development becomes widespread when the pollutants are carried by run-off, during rainfall, to nearby farms. The agricultural lands situated near these workshops are the worst hit during rainfalls. They eventually receive water soluble fraction of WEO.

Soil is a key component of natural ecosystems because environmental sustainability depends largely on a sustainable soil ecosystem (Adriano et al., 1998). When soil is polluted, the ecosystem is altered and agricultural activities are affected. The processes, therefore, leading to the eventual removal of these heavy metal and hydrocarbon pollutants from the environment involve the trio of physical, chemical and biological alternatives (Okoh, 2006). Physical and chemical methods, the most widely used procedures for clean-up, are not simple or favourable because they further introduce poisonous contaminants to the environment. The bioremediation technology most suitable for a specific site is determined by several factors, such as site conditions, indigenous microorganism population, and the type, quantity, and toxicity of contaminant chemicals present.

Some treatment technologies involve the addition of nutrients to stimulate or accelerate the activity of indigenous microbes. Optimizing environmental conditions enhance the growth of microorganisms and increase microbial population resulting in improved degradation of

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hazardous substances. However, if the biological activity needed to degrade a particular contaminant is not present at the site, suitable microbes from other locations (exogenous microorganisms) can be introduced and nurtured. Other technologies being demonstrated are phytoremediation, or the use of plants to clean up contaminated soils and ground water, and fungal remediation, which employs white-rot fungus to degrade contaminants. However, inherent in the soil is its ability to biodegrade pollutants in its environment. This ability is a complex process whose quantitative and qualitative aspects depend on the nature and amount of the pollutant present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community (Leahy and Colwell, 1990). The present study however investigates the bioremediation of a 14 month old WEO polluted soil by the soil's own natural ability.

MATERIALS AND METHODS

Soil used in the present study was collected from an area measuring 50 × 50 m marked on a farmland within the University of Benin Campus, Benin City. Top soil (0 to 10 cm), of known physicochemical property, was collected randomly from the marked plot in the morning (7.00 am) and placed on polythene sheets that were spread on an open platform and left in the sun until evening (5.00 pm) for drying. Thereafter, 10 kg soil each was placed into 50 large perforated plastic buckets with 8 perforations made with 2 mm diameter nails per bucket. WEO was obtained as pooled from an auto-mechanic workshop in Ikpoba Hill, Benin City, Nigeria that specializes in repairs of heavy duty trucks/vehicles. The WEO was stored in 50-L jerry cans and a sample was taken as pooled from the jerry cans and analyzed for polyaromatic hydrocarbon composition. Soils in each bucket were carefully poured out onto a flat platform covered with cellophane. WEO with 5 different levels of pollution: 0, 1.0, 2.5, 5.0 and 10.0% w/w WEO were poured into the measured soil, and were thoroughly mixed, before taking back into each bucket. This process was repeated for every bucket. The concentrations were obtained as follows:

0% (Control): No oil, but 'clean' soil only
1.0%:100 g WEO in 10 kg soil
2.5%: 250 g WEO in 10 kg soil
5.0%: 500 g WEO in 10 kg soil, and
10.0%: 1 kg WEO in 10 kg soil

For clarity, the 100 g WEO measured 135.0 ml. The treatment levels 0, 1.0, 2.5, 5.0 and 10.0% were labeled as SP_0, $SP_{1.0}$, $SP_{2.5}$, $SP_{5.0}$, and $SP_{10.0}$ respectively.

The entire set up was left in an open shade for 14 months. Soil was carefully irrigated twice every week with 200 ml of water.

Computation of hazard quotient (HQ)

HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern (COPEC). The hazards quotient is expressed by the following equation:

$$HQ = \frac{\text{Measured concentration}}{\text{Toxicity reference value or selected screening benchmark}}$$

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.

Computation of environmental risk factor (ERF)

The environmental risk factor (ERF) is a pollution index employed to determine environmental risk in order to establish potential threat to aquatic organisms. It is employed following the sequential extraction of heavy metals from sediments. The environmental risk factor (ERF) is expressed by the following equation.

$$ERF = CSQV - \frac{Ci}{CSQV}$$

Where, CSQV = concentration sediment quality value (background/pre-industrial concentration); Ci = heavy metal concentration in the soil fractions; ERF < O = potential ecological threat, and ERF > O = no threat.

RESULTS AND DISCUSSION

There were comparative decreases in NH_4N, NO_2, NO_3, SO_4, P, and the exchangeable bases (Table 1). There was no significant change in soil pH over time. pH ranges were 5.46 to 5.58 from 1 to 14 MAP. The minimal decreases (in pH) observed may be attributed to the degradation of the hydrocarbons, which may have resulted in the release of acidic intermediate and final products that probably lowered pH of the mixture (Alexander, 1999; Eweis et al., 1999). Hambrick et al. (1980) found that at pH values between 5 and 8, mineralization of hydrocarbons in estuarine sediments was highly dependent on oxygen availability. The pH ranges recorded in the present study falls within the range provided by Hambrick et al. (1980). EC was significantly lower in the oil-affected soils than in the control soils. Electrical conductivity at 1 MAP was 293 to 308 us/cm in the polluted soil as against 280 us/cm in the control. At 14 MAP, there was reduction in EC from 189 to 210 us/cm in the polluted soil compared to 139 us/cm in the unpolluted soil. This confirms the previous work of Osuji and Nkoye (2007). It is not likely that the released oil was directly responsible for the observed changes in EC since organic compounds like crude oil cannot conduct electrical current very well. However, it is possible that the anoxic biodegradation mechanism through direct dehydrogenation allowed the anaerobic metabolism of hydrocarbons in the presence of an electron acceptor such as nitrate ion, which may be partially responsible for the observed differences in EC. Concentration of soil potassium decreased with increasing pollution level, with a corresponding decrease in P. Generally, however, there was reduction in concentration of K and P at 14 MAP compared to values at earlier periods, possibly occasioned by remediation

Table 1. Physicochemical parameters of WEO-polluted soil at 1, 5, and 14 MAP.

Time	Code	pH	EC (µS/cm)	TOC	TN	EA	Na	K	Ca	Mg	Cl	P	NH4N	NO$_2$	NO$_3$	SO$_4$
				---%---			------(meq / 100 g of soil)----						------(ppm)-----			
1 MAP	SP$_0$	5.53	280	0.40	0.23	0.14	12.2	1.69	15.04	10.93	1698	110.2	26.3	16.21	32.10	16.2
	SP$_{1.0}$	5.50	293	0.51	0.34	0.23	11.5	1.71	15.23	10.32	1701	98.6	37.6	15.48	33.69	17.0
	SP$_{2.5}$	5.49	279	0.57	0.21	0.28	12.1	1.82	14.91	10.60	1698	102.5	28.5	16.09	43.05	18.3
	SP$_{5.0}$	5.48	295	0.64	0.23	0.24	11.9	1.78	15.21	10.73	1723	98.5	27.9	17.61	45.21	21.6
	SP$_{10.0}$	5.50	308	0.69	0.20	0.23	11.8	1.93	15.44	11.91	1781	98.9	29.3	18.30	48.20	22.3
5 MAP	SP$_0$	5.50	210	0.95	0.12	0.16	10.2	0.76	14.33	9.83	1718	74.1	19.0	14.30	27.8	16.3
	SP$_{1.0}$	5.46	258	0.86	0.18	0.21	10.8	0.79	13.91	8.90	1692	69.8	18.8	12.90	27.2	16.0
	SP$_{2.5}$	5.49	271	0.91	0.21	0.18	10.2	0.85	14.10	8.72	1702	72.3	20.2	13.8	27.2	16.8
	SP$_{5.0}$	5.46	263	0.89	0.16	0.17	10.3	0.85	14.53	9.18	1769	70.1	21.6	13.60	28.0	17.5
	SP$_{10.0}$	5.50	280	0.99	0.13	0.21	9.8	0.83	14.82	9.33	1800	82.6	23.8	15.98	28.4	18.18
14 MAP	SP$_0$	5.58	139	1.24	0.08	0.20	2.40	0.08	8.60	3.61	35.39	2.07	1.04	2.31	9.43	1.01
		+0.90	*-33.81*	*+30.53*	*-33.33*	*+25.00*	*-76.47*	*-89.47*	*-39.98*	*-63.33*	*-97.92*	*-97.21*	*-94.47*	*-83.84*	*-66.08*	*-93.80*
	SP$_{1.0}$	5.60	189	1.60	0.23	0.26	3.96	0.18	9.36	3.86	48.30	2.13	1.13	2.40	12.52	1.43
		+1.82	*-26.67*	*+86.05*	*-27.78*	*+23.81*	*-63.33*	*-77.22*	*-32.71*	*-56.63*	*-97.16*	*-96.94*	*-93.98*	*-81.39*	*-53.97*	*-91.06*
	SP$_{2.5}$	5.62	220	1.56	0.34	0.30	4.11	0.22	10.12	4.69	44.24	1.38	0.65	1.24	15.06	1.57
		+2.37	*-18.82*	*+71.43*	*+61.90*	*+66.67*	*-59.71*	*-74.12*	*-28.23*	*-46.22*	*-97.39*	*-98.09*	*-96.78*	*-90.87*	*-44.63*	*-90.65*
	SP$_{5.0}$	5.58	195	1.39	0.29	0.28	5.00	0.22	8.07	4.01	49.76	1.59	0.98	1.26	12.28	2.03
		+1.82	*-25.86*	*+56.18*	*+81.25*	*+64.71*	*-51.46*	*-74.12*	*-44.46*	*-56.32*	*-97.11*	*-97.73*	*-95.46*	*-90.73*	*-56.14*	*-88.40*
	SP$_{10.0}$	5.55	210	1.49	0.25	0.25	8.87	0.23	8.12	3.77	106.54	1.24	0.31	1.09	13.76	2.69
		+0.91	*-25.00*	*+50.51*	*+92.31*	*+19.05*	*-9.49*	*-72.29*	*-45.21*	*-59.59*	*-94.02*	*-98.50*	*-98.69*	*-93.10*	*-51.55*	*-85.20*

SP: Various levels of WEO-pollution in soil; MAP: Months after pollution; Italicized numbers with +ve and –ve signs represent percentage gains and losses respectively compared to values from those at 5 MAP.

processes. Soils contaminated with petroleum products have been shown to have large increases in nitrogen and phosphate content (Odu, 1972; Amund et al., 1993). Lehtomake and Niemela (1975) reported a low value of nitrogen, potassium and phosphorus reserve in petroleum hydrocarbon contaminated soil. This confirms the discovery in this research. The reduction in the concentration of NO$_3$N in the contaminated site suggests that the process of nitrification might have reduced following the incidence of oil spillage. Oil degrading or hydrocarbon-utilizing microbes such as *Azobacter* spp. eventually become more abundant while nitrifying bacteria such as *Nitrosomonas* spp. become reduced in

Table 2. Heavy metal composition of WEO-polluted soil at 1, 5 and 14 MAP.

Time (MAP)	Code	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
						------(ppm)-----					
1	SP$_0$	898	19.8	38.9	3.8	1.8	ND	0.03	3.0	1.84	565
	SP$_{1.0}$	1159	32.6	46.3	4.6	2.7	0.01	0.58	4.2	2.98	4361
	SP$_{2.5}$	1532	38.7	52.7	5.2	3.2	0.02	1.08	5.8	3.28	6763
	SP$_{5.0}$	1635	38.2	66.5	5.8	3.9	0.03	1.73	5.8	3.73	8480
	SP$_{10.0}$	2963	42.6	78.6	6.7	4.8	0.04	2.30	6.5	4.06	9892
5	SP$_0$	768	18.5	22.8	2.3	1.5	ND	ND	2.5	1.86	362
	SP$_{1.0}$	1039	30.2	36.3	3.2	2.3	0.01	0.45	2.6	2.06	3028
	SP$_{2.5}$	1063	35.6	47.8	3.8	2.6	0.02	0.80	3.2	2.12	4106
	SP$_{5.0}$	1096	36.9	56.3	3.7	2.8	0.03	1.41	4.2	2.48	7010
	SP$_{10.0}$	1389	38.7	68.6	4.2	3.8	0.03	2.08	4.1	3.48	8521
14	SP$_0$	266	30.8	10.6	0.71	0.32	N/D	ND	0.07	0.058	28.5
		-65.36	*+66.48*	*-53.51*	*-69.13*	*-78.67*			*-97.20*	*-96.88*	*-92.13*
	SP$_{1.0}$	429	36.7	18.7	0.73	0.69	N/D	0.20	0.10	0.100	113.74
		-58.71	*-21.52*	*-48.48*	*-77.19*	*-70.00*		*-55.56*	*-96.15*	*-95.15*	*-96.24*
	SP$_{2.5}$	619	33.8	20.8	1.19	0.78	0.018	0.46	0.12	0.097	286.35
		-44.77	*-5.06*	*-56.49*	*-68.68*	*-67.14*	*-60.00*	*-42.50*	*-96.25*	*-95.42*	*-93.03*
	SP$_{5.0}$	785	25.2	23.1	1.31	0.92	0.012	0.78	0.10	0.123	425.98
		-28.38	*-31.71*	*-58.97*	*-64.59*	*-71.58*	*-60.00*	*-44.64*	*-97.62*	*-95.04*	*-93.92*
	SP$_{10.0}$	698	28.1	30.8	1.43	1.08	0.019	0.83	0.13	0.148	608.35
		-49.75	*-27.39*	*-55.10*	*-65.59*	*-71.58*	*-36.67*	*-60.09*	*-96.83*	*-95.75*	*-92.86*

SP: Various levels of WEO-pollution in soil; MAP: Months after pollution. Italicized numbers with +ve and –ve signs represent percentage gains and losses respectively compared to values from those at 5 MAP.

number.

Heavy metal concentration at 14 MAP decreased from original values at earlier periods irrespective of the level of pollution. At 14 MAP, soil concentration of Fe was 266 to 698 ppm as against 898 to 2963 ppm at 1 MAP. Soil concentration of Mn at 14 MAP was 25.2 to 36.7 ppm, 0.6 to 30.8 ppm of Zn, 0.71 to 1.43 ppm of Cu and 0.07 to 0.13 ppm of Ni (Table 2). These value ranges were significant reductions from their original concentration at 1 MAP. THC at 1 MAP was 431 to 9892 in polluted soil as against 565 ppm in the control. This significantly decreased at 14 MAP to 113.74 to 608.35 ppm in polluted soil as against 28.5 ppm in the control. This represents over a hundred percent remediation in hydrocarbon content of soil.

Total PAH content of soil reduced from 36.95 to 25.45 ppm in the control; 130.55 to 103.73 ppm in SP$_{1.0}$; 237.04 to 207.61 ppm in SP$_{2.5}$; and from 538.59 to 456.99 ppm in SP$_{10.0}$ (Table 3). There was total eradication of chrysene, fluoranthene, fluorine and indeno (1,2,3-c,d)pyrene in all levels. The other PAH compounds however showed significant decreases from their original concentrations at 5 MAP. PAH reductions may have resulted from evaporation and microbial degradation in a dissolved state (Jordan and Payne, 1980; Kappeler and Wuhrmann, 1978). Hydrocarbonoclastic microbes play a paramount role in bioremediation of PAH compounds. These organisms have been isolated from heavily oil-polluted deposits or in a variety of soils and water continuously exposed to hydrocarbon for several years (Ibe and Ibe, 1986).

Acalypha ciliata, Acanthospermum hispidum, Ageratum conyzoides, Brachiaria deflexa, Echinochloa stagina, Eragrostis tenella, Erigeron floribundus, Euphorbia heterophylla, Euphorbia hirta, Fluerya aestuans, Gomphrena celosioides, Ipomoea involucrata, Panicum maximum, Paspalum polystachyrm, Phyllanthus amarus, Platosfonca africanum, Spigelia anthelmia, Synedrella nodiflora, and *Tridax procumbens* were weed species that were originally isolated from the farm land from where soil used in the experiment was obtained (Table 4). Of these only *A. ciliata, A. conyzoides, E. heterophylla, E. hirta, P. maximum, P. amarus, S. anthelmia, S. nodiflora*, and *T. procumbens* were present in polluted soils at 14 MAP, and as such adjudged tolerant species. They could be introduced in remediation strategies. There are several plant characteristics that exclude species for possible use in phytoremediation which need consideration. For example, *Tridax procumbeus* is an undesirable invasive weed that can

Table 3. Polyaromatic hydrocarbon (PAH) content of WEO-polluted soil at 5 and 14 MAP.

PAH (ppm)	5 MAP					14 MAP				
	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}
Acenapthene	0.4989	0.8366	1.3013	1.8386	2.4235	0.2067	0.4742	0	0.4821	0.1971
Acenaphthylene	0	1.1164	1.8862	2.4629	3.2847	0	0.4181	0.8628	0.9883	0.6354
Anthracene	0	4.4281	6.1823	8.0082	10.7625	0	0	0	0.4941	2.3954
Benzo(a)anthracene	0	2.5396	2.8650	3.0625	3.5389	0	2.1840	2.6180	0	0
Benzo(a)pyrene	2.6025	2.4925	6.9330	11.1135	13.4380	0.6605	0.8384	0	1.4952	4.5170
Benzo(b) fluoranthene	0	0	0.8593	0	2.1187	0	0	0	0.7216	0.5563
Benezo(g,h,i) perylene	29.4638	89.1187	109.5631	78.0462	100.7342	23.1843	198.2766	140.2351	0	84.7930
Benzo(k) fluoranthene	0	18.2800	70.5652	196.7631	294.4268	0	0	62.4118	211.8732	360.2710
Chrysene	0	0	0.1006	0	0.0735	0	0	0	0	0
Dibenzo(a,h) anthracene	0	0.3895	0.6789	1.0591	1.2879	0	0	0.6649	0	1.1278
Fluoranthene	0	0.9843	14.2890	23.0301	38.4333	0	0	0	0	0
Fluorene	0	0.2876	0.3942	0.4887	0.6623	0	0	0	0	0
Indeno(1,2,3-c,d)pyrene	0	0	0.6672	1.0816	2.0076	0	0	0	0	1.1705
Naphthalene	0.2389	0.2836	0.8369	0.3398	0.2995	0	0.2611	0.1634	0	0
Phenanthrene	1.2828	3.4386	8.0375	11.2681	28.4367	0.1285	0.4014	0.6499	0.7599	0.5687
Pyrene	2.8673	6.3542	11.8793	20.281	36.6667	1.2742	0.4727	0	0.4562	0.4606
Total	36.9542	130.5497	237.0390	358.8434	538.5948	25.4542	103.7265	207.6059	217.706	456.9929

Table 4. Weeds distribution of WEO-polluted soil at 14 MAP.

S/No.	Names of organisms	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}
1	*Acalypha ciliata*	2*	2	0	0	0
2	*Acanthospermum hispidum*	0	0	0	0	0
3	*Ageratum conyzoides*	0	2	0	1	0
4	*Brachiaria deflexa*	3	0	0	0	0
5	*Echinochloa stagina*	3	0	0	0	0
6	*Eragrostis tenella*	2	0	0	0	0
7	*Erigeron floribundus*	0	0	0	0	0
8	*Euphorbia heterophylla*	2	5	3	0	0
9	*Euphorbia hirta*	0	2	0	0	0
10	*Fluerya aestuans*	2	0	0	0	0
11	*Gomphrena celosioides*	0	0	0	0	0
12	*Ipomoea involucrata*	0	0	0	0	0
13	*Panicum maximum*	9	2	0	0	0

Table 4. Contd.

14	*Paspalum polystachyrm*	0	0	0	0	0
15	*Phyllanthus amarus*	2	3	1	0	0
16	*Platosfonca africanum*	0	0	0	0	0
17	*Spigelia anthelmia*	2	0	1	0	0
18	*Synedrella nodiflora*	2	0	0	0	0
19	*Tridax procumbens*	4	4	2	0	0
20	Unidentified plants (<5cm tall)	5	4	3	5	0
	Total	38	24	9	6	0

The entire weeds in the aforementioned table were originally present in the farmland from which experimental soil was collected.
*Values indicate number of weed species present per bucket.

Table 5. Distribution of microorganisms of WEO-polluted soil at 1, 5, and 14 MAP.

S/No.	Names of organisms	5 MAP					14 MAP				
		SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}	SP_0	SP_1	$SP_{2.5}$	SP_5	SP_{10}
1	*Achromobacter* sp.	+	+	+	+	+	-	-	-	-	-
2	*Clostridium* sp.	+	+	+	+	+	-	-	-	-	-
3	*C. perfringens*	-	-	-	-	-	-	-	+	-	+
4	*Sarcina* sp.	+	+	+	+	+	-	-	-	-	-
5	*Micrococcus* sp.	+	+	+	+	-	-	-	-	-	-
6	*M. luteus*	+	+	+	+	-	-	-	-	-	-
7	*Bacillus pumilis*	+	+	+	-	-	+	-	-	-	+
8	*B. subtilis*	+	-	-	-	-	-	-	-	-	-
9	*A. niger*	+	+	+	+	-	+	-	-	-	+
10	*A. Flavus*	+	+	-	-	-	-	-	+	-	-
11	*A. fumigatus*	+	+	-	-	-	+	+	-	-	-
12	*Penicillium* sp.	+	+	+	+	+	-	-	-	-	-
13	*P. notatum*	-	-	+	+	+	+	-	-	-	-
14	*Fusarium* sp.	+	+	+	-	-	-	-	+	-	-
15	*Mucor* sp.	+	+	-	-	-	-	-	-	-	-
16	*Geotrichum* sp.	+	+	+	+	+	-	-	-	-	+
17	*Trichoderma* sp.	+	+	+	+	+	-	-	-	-	
18	*Saccharomyces* sp.	+	+	-	-	-	-	-	-	-	+
19	*Streptomyces* sp.	+	+	-	-	-	-	-	-	-	
20	*Nocardia* sp.	+	+	+	+	+	+	+	+	+	+

replace natural pastures, a characteristic that could cause conflicts with farmers in surrounding of remediation site and additional costs for weed control. Anoliefo et al. (2006) identified a number a plants in an oil polluted auto-mechanic workshop, suggesting therefore that these weeds could have a tolerance for oil. These weeds included *T. procubens*, *A. hispidum*, *E. heterophyllia, E. tenelia, P. maximum*, and *F. aestuans. Eleucine indica, Cynodon dactylon, P. maximum, Euphorbia hirta, Chromolaena odorata* have been previously demonstrated to possess the capability for recovery of heavy metals from soil (Wong and Chu, 1985; Wong and Lau, 1985; Nedelkoska and Doran, 2000; Anoliefo and Vwioko, 1995).

A. sp., *Clostridium* sp., *Sarcina* sp., *Penicillium* sp., *Geotrichum* sp. *Trichoderma* sp., *Bacillus pumilis, Aspergillus niger, Penicillium notatun* and *Nocardia* sp. were dominant species in the present study (Table 5). Yamazaki et al. (1997) reported that *A. niger* and *A. fumigatus* both metabolize terpenes and PAHs. *A. niger* converts the terpene B- myrcene to dihydroxylated derivatives; and there is even a report of the ability of *A. niger* to cleave the rings of naphthalene, anthrcene, and phenanthrene (Yogambal and Karegoudar, 1997). There were more heterotrophic microorganisms than the hydrocarbon degraders (Table 6). Heterotrophic bacterial

Table 6. Total heterotrophic and oil degraders counts of WEO-polluted soil at 1, 5 and 14 MAP.

Sample identity (MAP)	Bacteria			Fungi			Actinomycetes		
5	**Het** ($\times 10^6$ cfu/g)	**Hyd** (10^5 cfu/g)	**% Hyd**	**Het** ($\times 10^5$ cfu/g)	**Hyd** (10^5 cfu/g)	**% Hyd**	**Het** ($\times 10^4$ cfu/g)	**Hyd** (10^3 cfu/g)	**% Hyd**
SP_0	1.21	7.36	60.8	6.38	5.31	8.32	4.97	3.81	7.67
$SP_{1.0}$	0.98	8.19	83.57	4.22	6.16	14.60	3.88	3.06	7.89
$SP_{2.5}$	0.87	6.40	73.56	5.75	6.19	12.00	2.97	2.87	9.66
$SP_{5.0}$	0.56	4.82	86.07	6.09	5.42	8.90	3.23	2.43	7.52
$SP_{10.0}$	0.53	4.38	82.64	4.88	4.98	10.20	2.78	2.09	7.52
14	($\times 10^5$ cfu/g)	($\times 10^4$ cfu/g)	-	($\times 10^4$ cfu/g)	($\times 10^3$ fu/g)	-	($\times 10^3$ cfu/g)	($\times 10^2$ cfu/g)	-
SP_0	0.77	4.03	52.47	5.76	1.23	2.14	2.73	1.96	7.18
$SP_{1.0}$	0.53	2.28	43.43	5.87	1.08	1.84	2.50	2.00	8.00
$SP_{2.5}$	0.47	2.03	43.38	4.99	1.02	2.04	2.86	1.68	5.87
$SP_{5.0}$	0.41	1.67	40.53	5.23	1.06	2.03	3.01	1.78	5.91
$SP_{10.0}$	0.44	1.91	43.51	5.16	0.98	1.90	3.06	2.11	6.90

Het: Heterotrophic; Hyd: Hydrocarbon (oil) degrader.

count ranged from 0.44 to 0.77 $\times 10^5$ cfu/g at 14 MAP compared to 0.98 to 1.23 $\times 10^4$ cfu/g of heterotrophic fungi. There were also more bacteria than fungi, and the actinomycetes, in that order. These microorganisms however reduced in their counts with time. The finding of the presence of higher oil-degrading bacterial populations in contaminated soils corroborates the results of Hubert et al. (1997) and Michalcewicz (1995) that attributed these high microbial populations to the stimulatory effect of additional carbon and energy source in the form of lubricating oil. The population of oil degraders was significantly lower in non-impacted than in impacted soils. Oil degraders isolated by Nkwelang et al. (2008) included *Pseudomonas, Bacillus* and *Actinetobacter* as the major genera of bacteria active in polluted soil. In a study by Ibe and Ibe (1986), a variety of oil degrading microorganisms were isolated from oil polluted samples and among the bacteria, *Pseudomonas*, *Acetobacter*, *Chromobacterium* and *Corynebacterium* were isolated as dominant species. There were decreases in microbial populations with time, as against observation by Atagana (2004) who recorded increases in microbial populations and rapid reductions in hydrocarbon content continued probably due to a decrease in nitrogen levels caused by the initial high microbial activity (Piccinini et al., 1996).

Hazard quotients (HQ) for heavy metals in the polluted soils obtained at 1, 5 and 14 MAP are presented in Table 7. Collectively, HQ's for heavy metals at 1 MAP were higher than at 5 MAP, indicating a reduction in toxicity potential of the heavy metals after 5 months. At 1 MAP, HQ was very high in Fe (4.49 to 14.81), Zn (5.88 to 11.87), and Cr (4.50 to 12.00). However, HQ<1 in Ni (0.22 to 0.48), and at lower concentration of Cu (SP_0 = 0.70, $SP_{2.5}$ = 0.96). This indicated a non-toxic situation. At

5 MAP, however, HQ for Fe reduced from 3.84 in SP_0 to 6.94 at $SP_{10.0}$. Value ranges were 3.44 to 10.36 for Zn, 3.74 to 9.50 for Cr, 1.00 to 3.00 for Cd, 9.00 to 41.60 for Pb and 1.17 to 2.19 for V. all of these presented a HQ>1 situation. However, for Cu and Ni, HQ<1 for all levels at 5 MAP, signifying no toxicity was indicated for Ni and Cu for all levels of pollution from 1 to 5 MAP.

At 14 MAP, HQ ranged from 0.84 to 5.13 in Fe, 2.31 to 7.34 in Zn, 1.02 to 4.45 in Cr, 1.30 to 1.80 in Cd and 4.60 to 19.60 in Pb. The quotients were higher than the provided benchmark and as thus showed possibility for ecological risk. It must however be noted that their general decreases in HQ progressively from 1 to 14 MAP.

Environmental risk factor significantly increased at 14 MAP compared to values at 5 MAP (Table 8). ERF values ranged from -69.30 to -14.97 for Pb and -0.01 for V I SP_{10} at 5 MAP. These values significantly increased at 14 MAP to 7.64 to 32.64 for Pb and 1.70 for V, respectively. The implication was that Pb in the polluted soil and V at SP_{10} were contaminants of ecological risk at 5 MAP, but the threat was ameliorated at 14 MAP, thus attesting for the success of remediation. Natural attenuation affords the soil the opportunity for the allowance of its wealth of biological and physicochemical processes in remediating the soil. The present study has successfully demonstrated that bioremediation can occur by natural attenuation.

Table 7. Hazard quotients for heavy metals in WEO-polluted soil at 1, 5 and 14 MAP.

Code (MAP)	Fe^+	Zn	Cu	Cr	Cd*	Pb*	Ni	V
1								
SP_0	4.49	5.88	0.70^	4.50	ND	9.60	0.22^	1.16
$SP_{1.0}$	5.79	6.99	0.85^	6.75	1.00	11.60	0.31^	1.87
$SP_{2.5}$	7.66	7.96	0.96^	8.00	2.00	21.60	0.43^	2.06
$SP_{5.0}$	8.17	10.04	1.07	9.75	3.00	34.60	0.43^	2.35
$SP_{10.0}$	14.81	11.87	1.24	12.00	4.00	46.00	0.48^	2.55
5								
SP_0	3.84	3.44	0.40^	3.75	0	0	0.20^	1.17
$SP_{1.0}$	5.19	5.48	0.59^	5.75	1.00	9.00	0.18^	1.30
$SP_{2.5}$	5.31	7.22	0.70^	6.50	2.00	16.00	0.24^	1.33
$SP_{5.0}$	5.48	8.50	0.69^	7.00	3.00	28.20	0.31^	1.56
$SP_{10.0}$	6.94	10.36	0.78^	5.50	3.00	41.60	0.30^	2.19
14								
SP_0	0.84	*2.39	0.12	*1.02	N/D	N/D	0.006	0.047
$SP_{1.0}$	*2.11	*2.31	0.22	*1.80	N/D	*4.60	0.040	0.070
$SP_{2.5}$	*3.49	*3.05	0.24	*2.52	0.80	*9.80	0.009	0.060
$SP_{5.0}$	*3.93	*4.61	0.26	*3.02	*1.30	*16.60	0.010	0.075
$SP_{10.0}$	*5.13	*7.34	0.32	*4.45	*1.80	*19.60	0.080	0.192

+ Benchmark used is Efroymson et al. (1997), # Benchmark used is FEPA (1991); * When HQ≥1, implication is the possibility for ecological risk.

Table 8. Environmental risk factor (ERF) of heavy metals in WEO-polluted soil at 1, 5 and 14 MAP.

	Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
5 MAP									
SP_0	1008.23	16.94	29.24	3.31	1.9	N/D	N/D	2.91	0.86
$SP_{1.0}$	1007.97	15.22	28.79	3.08	1.12	N/D	-14.97*	2.88	0.78
$SP_{2.5}$	1007.95	14.91	28.41	2.93	0.99	N/D	-26.64*	2.71	0.72
$SP_{5.0}$	1007.91	14.83	28.13	2.95	0.90	N/D	-46.97*	2.43	0.53
$SP_{10.0}$	1007.62	14.72	27.71	2.82	0.44	N/D	-69.30*	2.46	-0.01*
14 MAP									
SP_0	1008.83	15.81	29.47	3.88	1.99	N/D	N/D	3.85	1.82
$SP_{1.0}$	1008.58	15.48	29.49	3.86	1.85	N/D	7.64	3.57	1.81
$SP_{2.5}$	1008.31	15.48	29.33	3.86	1.72	N/D	16.3	3.57	1.81
$SP_{5.0}$	1008.22	14.95	28.98	3.85	1.62	N/D	27.63	3.56	1.80
$SP_{10.0}$	1007.98	14.94	28.38	3.84	1.36	N/D	32.64	3.32	1.70

ERF<0… There is potential ecological risk; ERF>0…No potential risk. *Table shows that all heavy metals did not pose any potential ecological risk after treatment.

REFERENCES

Adriano DC, Chlopecka A, Kaplan KI (1998). Role of soil chemistry in soil remediation and ecosystem conservation. Soil Science Society of America Special Publication, Madison, WI. pp. 361-386.

Alexander M (1999). Biodegradation and Bioremediation. Academic Press, San Diego. p. 234.

Amund OO, Omole CA, Esiobu N, Ugoji EO (1993). Effects of waste engine oil on soil physico-chemical and microbiological properties. J. Sci. Res. Dev., 1: 65-68.

Anoliefo GO, Edegbai BO (2000). Effects of spent engine oil as a contaminant on the growth of two egg plant specie, *Solanum melongea* (L.) and *S. incanum* (L.) J. Agric. For. Fish., 1: 21-25.

Anoliefo GO, Ikhajiagbe B, Okonokhua BO, Diafe FV (2006). Ecotaxonomic distribution of plant species around auto mechanic workshops in Asaba and Benin City: Identification of oil tolerant

species. Afr. J. Biotechnol., 5(19): 1757-1762.

Anoliefo GO, Vwioko DE (1995). Effects of spent lubricating oil on the growth of *Capsicum annum* and *Lycopersicon esculentum*. Poll., 88: 361-314.

Atagana HI (2004). Bioremediation of creosote-contaminated soil in South Africa by land farming. J. Appl. Microbiol. 96: 510-520.

Efroymson RA, Will ME, Suter II GW, Wooten AC (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. p. 123.

Eweis JB, Ergas SJ, Chang DP, Schroeder ED (1999). Biodegradacion de compuestos concretos principios de biorrecuparacion. McGraw Hill, Spain, pp. 131-147.

FEPA (1991). Guidelines and Standards for Environmental Pollution and Control in Nigeria. Federal Environmental Protection Agency, Lagos. p. 98.

Hambrick GA, Delaune R, Patrick, Jr WH (1980). Effects of estuarine sediment P^H an oxidation reduction potential on microbial hydrocarbon degradation. Appl. Environ. Microbiol., 40: 365-369.

Hubert C, Shen Y, Voordouw G (1997). Comparison of toluene – degrading microbial communities from soil at different concentrations of toluene. Appl. Environ. Microbiol., 63: 3064-3070.

Ibe NS, Ibe EG (1986). Control and dispersion potential of oil spills by microbial seeding. In: The Petroleum Industry and the Nigerian Environment. Proceeding of an International Seminar NNPC Nov. 19-20, PTI Warri Nigeria. pp. 112-115.

Jordan P, Payne JR (1980). Fate and weathering of petroleum spills in the marine environment. Ann Arbor Science Publications, Inc., Ann. Arbor, Mich. p.175.

Kappeler T, 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.

Leahy JG, Colwell RR (1990). Microbial degradation of hydrocarbons in the environment. Microb. Rev., 54: 427 – 450.

Lehtomake M, Niemela S (1975). Improving microbial degradation of oil in soil. Ambio, 4: 126-129.

Michalcewicz W (1995). The influence of diesel fuel oil on the number of bacteria, fungi, actinomycetes and soil microbial biomass. Rocz. Panstw. Zakl. Hig., 46: 91-97.

Nedelkoska TV, Doran PM (2000). Characteristics of metal uptake by plant pecie with potential for phytoremediation and phytominibng. Mineral Egineering, 13: 549 – 561.

Nkwelang G, Kamga HFL, Nkeng GE, Antai SP (2008). Studies on the diversity, abundance and succession of hydrocarbon utilizing micro organisms in tropical soil polluted with oily sludge. Afr. J. Biotechnol., 7(8): 1075-1080.

Odu CTI (1972). Microbiology of soils contaminated with petroleum hydrocarbon I. Extent of contamination and some soil and microbial properties after contamination. J. Inst. Pasteur., 58: 201-208.

Okoh AI (2006). Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. Biotechnol. Mol. Biol. Rev., 1: 38-50.

Osuji LC, Nwoye I (2007). An appraisal of the impact of petroleum hydrocarbon on soil fertility: the Owaza experience. Afr. J. Agr. Res., 2(7): 318-324.

Piccinini S, Rossi L, Bonazzi G, Dall'Orso G (1996). The Emilia-Romaga Experiment in animal manure composting, In: de Bertoldi M, Sequi P, Lemmes B, Papi T (eds) The Science of Composting. Blackie Academic and Professional: London, pp. 1275-1280.

Wong MH, Chu LM (1985). Yield and metal uptake of *Cynodon dactylon* grown o refuse compost amended soil. Agric. Ecosyst. Environ., 14: 41–52.

Wong MH, Lau WM (1985). Root growth of *cynodon dactylon* abd *Eleucine indica* from motor ways at different concentrations of lead. Environ Res., 36: 257–267.

Yamazaki Y, Hayashi Y, Hori N, Mikami Y (1997). Microbial conversion of β-myrcene by *Aspergillus niger*. Agric. Biol. Chem., 52: 2921-2922.

Yogambal RK, Karegoudar TB (1997). Metabolism of polycyclic aromatic hydrocarbons by *Aspergillus niger*. Indian J. Exp. Biol., 35: 1021-1023.