

Full Length Research Paper

Impact of substrate amendment on the polyaromatic hydrocarbon contents of a five month old waste engine oil polluted soil

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The present study investigated the impact of substrate amendment on the bioremediation of waste engine oil-polluted soil. Top soil was collected randomly from a 50 × 50 m marked plot on a farmland, sun-dried and weighed. Ten kilogram's of soil each was placed into perforated 25 L buckets. Waste engine oil (WEO) was prepared at 5 different levels of pollution: 0, 1.0, 2.5, 5.0, and 10.0% w/w, and poured into the measured soil, and thoroughly mixed. The entire set up was left under an open shade for 5 months without mechanically disturbing the soil, after which the polluted soil was amended with sawdust and left for a further 9 months. Significant reductions in polyaromatic hydrocarbon (PAH) content of soils over the experimental period were recorded. Total (100%) remediation of some PAH compounds (benzo[a]anthracene, benzo(a)pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorene, and indeno[1,2,3-c,d]pyrene) was recorded. Total PAH ranged from 1.10 to 6.35 mg/L in the substrate-amended treatment compared to a range of 25.45 to 456.99 mg/L in the unamended soil. Toxicity equivalencies (TEQ) in the unamended soils were generally higher than benchmark values, implying that the cleanup levels were not met. In the amended treatments, however, TEQs were presented for only benzo[a]pyrene (0.7284) in the control and indeno[123-c]pyrene (0.0272) in 2.5% w/w oil in soil treatment. TEQ's of benzo[a]pyrene in the amended 5% w/w oil in soil treatment was 3.1252 mg/L, also higher than the benchmark TEQ of 1.0 mg/L.

Key words: bioremediation, substrate amendment, polyaromatic hydrocarbon.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are important pollutants found in air, soil and sediments. They and their derivatives are widespread products of natural and anthropogenic sources, generated during the incomplete combustion of solid and liquid fuels or derived from industrial activities. In recent times, the major source of PAH pollution are industrial production, transportation, refuse burning, gasification and plastic waste incineration, and most importantly from pollution by waste engine oil. The spill arising from disposal of this waste engine oil (WEO) is becoming a visible problem that needs serious attention. Disposal of WEO is not only attributed to service stations, draining oil from automobile

and generator engines also account for some amounts of WEO dumped into the ecosystem (Anoliefo and Vwioko, 2001). 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, 2001). A more chaotic situation is the improper disposal of WEO into run-off, gutters and water drains (Odjegba and Sadiq, 2002). 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 fractions of WEO.

The fate of polycyclic aromatic hydrocarbons in nature is of great environmental concern due to their toxic,

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mutagenic, and carcinogenic properties. For example phenanthrene is known to be a human skin photosensitizer and mild allergen. It has also been found to be an inducer of sister chromatid exchanges and a potent inhibitor of gap junction intercellular communications. PAHs can sorb to organic-rich soils and sediments, accumulate in fish and other aquatic organisms, and may be transferred to humans through seafood consumption (Mrozik et al., 2003).

Considering the heavy burden imposed on the environment by these pollutants, bioremediation becomes imperative. Because many PAHs are so toxic there is interest in understanding the physicochemical processes and microbial degradation reactions that affect the mobility and fate of these compounds in groundwater and soil sediment system. The biodegradation of PAHs can be considered on one hand to be part of the normal processes of the carbon cycle, and on the other as the removal of man-made pollutants from the environment. The use of microorganisms for bioremediation of PAH-contaminated environments seems to be an attractive technology for restoration of polluted sites. Microbial biodegradation is an effective and inexpensive approach to degrade and remove PAHs and other hydrocarbon compounds from contaminated soils, as long as the correct population of microorganisms is employed and the oily sludge wastes are conducive to the biodegradation of the contaminants. Furthermore, with recent developments in soil amendments as a means of increasing microbial populations and performance, the processes of hydrocarbon biodegradation have advanced substantially.

For efficient bioremediation, soil amendments, such as sawdust, are added to increase activities of microorganisms. A soil amendment is any material added to a soil to improve its physical properties, such as water retention, permeability, water infiltration, drainage, aeration and structure (Davis and Wilson, 2005). The organisms, while growing on the sawdust substrate, probably produce enzymes that were used in metabolizing the hydrocarbons in the compost matrix (Sutherland et al., 1995; Diaz et al., 1996). It is therefore the aim of the study to investigate the impact of substrate amendments with sawdust on the polyaromatic hydrocarbon content a 5 month old WEO polluted soil. The study also hopes to compare probable effect concentrations (CEC) and toxicity equivalent concentrations (TEQ) of the PAH compounds from both amended and unamended polluted soils. The TEQs would be compared against benchmark values provided by Cal-EPA (2005).

The development and the establishment of a toxicity equivalency factor (TEF) are used in the assessment of mixtures containing PAHs. The TEF methodology was developed by the U.S. Environmental Protection Agency (EPA) to evaluate the toxicity and assess the risks of a mixture of structurally related chemicals with a common

mechanism of action. A TEF is an estimate of the relative toxicity of a chemical compared to a reference chemical. Health risk assessment associated with eventual PAHs uptake is often estimated on the basis of the benzo[a]pyrene (BaP) concentrations in air, soil, or water. These media are possible gateways for uptake of these c-PAHs by man (MDH, 2004; Cal-EPA, 2005; Halek et al., 2008). PAHs are toxic pollutants released by various urban combustion sources and BaP is a representative member of the class of PAHs. The Washington State Department of Ecology uses the TEF methodology to evaluate the toxicity and assess the risks for environmental mixtures of dioxins/furans and carcinogenic polycyclic aromatic hydrocarbons (c-PAHs). Cal-EPA (2005) however requires that mixtures of c-PAHs be considered a single hazardous substance when establishing and determining compliance with cleanup levels and remediation levels.

In the present study, compounds having TEF = 0 are not used in calculating TEQ (Cal-EPA, 2005, Halek et al., 2008). Benzo[a]pyrene has a TEF value of 1 and therefore serves as an index PAH for other PAH compounds as well as a representative member of the class of PAHs. With regards to the interpretation of TEQ in line with clean-up levels, Cal-EPA (2005) provides that when the Total TEC for the c-PAH mixture of a particular soil sample exceeds 0.137 mg/kg, the cleanup level for benzo[a]pyrene was not met for that particular soil sample.

MATERIALS AND METHODS

Top soil (0 to 10 cm), of known physicochemical properties (Table 1), was collected randomly from an area measuring 50 × 50 m on a farmland situated in the University of Benin Campus (Lat. 6° 36'; Long. 6° 19'). Thereafter, 10 kg sun-dried soil measured into 25 L buckets with 8 perforations made with 2 mm diameter nails at the bottom of each bucket. WEO was obtained from an auto-mechanic workshop in Ikpoba Hill, Benin City that specializes in repairs of heavy duty trucks/vehicles. The WEO was stored in 50 L jerry cans for use. Oil was added to soil in the buckets and mixed thoroughly to obtain 5 different concentrations on weight basis: 0, 1.0, 2.5, 5.0, and 10.0% w/w oil in soil.

The entire set up was left for 5 months, without mechanically disturbing the soil. Soil was carefully irrigated daily with 400 ml of water. After 5 months, the entire set up was divided into 2 sets of equal buckets. The first set received sawdust prepared by replacing 3 kg of soil from each bucket with 3 kg air-dried sawdust, of known physicochemical properties (Table 2), obtained from *Brachistegia nigerica*. The second set was left unamended. These buckets were left for an additional 9 months. PAH content of soil was determined at 5 MAP and 9 months after (that is, 14 MAP), with or without amendment. The buckets without substrate amendments were labeled "SP" irrespective of their respective WEO-in-soil concentrations, where as those buckets with substrate amendments were labeled "SSP" as well. The levels of pollution were recorded as subscripts of either "SP" or "SSP".

Treatment designations

SP₀: Unamended soil polluted with 0% w/w WEO in soil.

Table 1. Physical and chemical properties of soil and sawdust used before soil contamination.

Parameter	Units	Soil	Sawdust
pH	-	5.58	5.59
EC	µs/cm	300	330
TOC	%	0.41	1.99
Total Nitrogen	%	0.10	0.15
EA	meq/100 g soil	0.20	0.30
Na	meq/100 g soil	10.90	11.10
K	meq/100 g soil	1.65	2.65
Ca	meq/100 g soil	15.60	22.30
Mg	meq/100 g soil	11.30	15.40
Cl	mg/L	1666.00	1347.00
P	mg/L	153.00	112.00
NH ₄ N	mg/L	25.40	6.25
NO ₂	mg/L	15.01	25.99
NO ₃	mg/L	30.75	92.23
SO ₄	mg/L	14.63	14.95
Clay	%	4.4	0
Silt	%	7.8	0
Sand	%	87.8	0
Fe	mg/L	1009	220
Mn	mg/L	17.00	41.70
Zn	mg/L	30.00	4.80
Cu	mg/L	3.90	1.60
Cr	mg/L	2.18	1.60
Cd	mg/L	N.D	N.D
Pb	mg/L	0.03	N.D
Ni	mg/L	3.60	0.70
V	mg/L	1.36	0.64
THC	mg/L	754.00	268.00

ND: Not determined (≤ 0.0001 mg/L).

SP₁: Unamended soil polluted with 1% w/w WEO in soil

SP_{2.5}: Unamended soil polluted with 2.5% w/w WEO in soil

SP₅: Unamended soil polluted with 5% w/w WEO in soil

SP₁₀: Unamended soil polluted with 10% w/w WEO in soil

SSP₀: Soil polluted with 0% w/w WEO in soil, and amended subsequently

SSP₁: Soil polluted with 1% w/w WEO in soil, and amended subsequently

SSP_{2.5}: Soil polluted with 2.5% w/w WEO in soil, and amended subsequently

SSP₅: Soil polluted with 5% w/w WEO in soil, and amended subsequently

SSP₁₀: Soil polluted with 10% w/w WEO in soil, and amended subsequently

Soil analyses

In the laboratory, soils were dried at ambient temperature (22 to 25°C), crushed in a porcelain mortar and sieved through a 2 mm (10 meshes) stainless sieve. Air-dried <2 mm samples were stored

in polythene bags for subsequent analysis. The <2 mm fraction was used for the determination of polyaromatic hydrocarbon contents by gas chromatography (GC) using the by methods of ASTM (1982) and ERI (1999).

Statistical analysis

Bioecological statistical analyses performed, at 5% probability, were probable effect concentrations CEC and TEQ of the PAH compounds (Ikhajiagbe, 2010). TEQs were compared against benchmark values provided by Efromson et al. (1997) and Cal-EPA (2005). The development and the establishment of a TEF are used in the assessment of mixtures containing PAHs.

Computation of probable effect concentration (PEC) of PAH compounds

$$PEC = \frac{\text{Concentration of PAH in soil}}{\% \text{ Total Organic Matter in soil}}$$

Table 2. Polyaromatic hydrocarbon content of waste engine oil that was used for the experiment.

Polyaromatic hydrocarbons	Concentrations (mg/L)
Acenaphthene	2.6202
Acenaphthylene	4.5330
Anthracene	19.6154
Benzo[a]anthracene	1.4686
Benzo[a]pyrene	13.5513
Benzo[b]fluoranthene	1.6875
Benzo(ghi)perylene	190.8163
Benzo[k]fluoranthene	369.9780
Chrysene	3.8984
Dibenzo[a,h]anthracene	4.2598
Fluoranthene	70.8033
Fluorene	3.6777
Indeno[1,2,3-c,d]pyrene	6.6644
Naphthalene	0
Phenanthrene	44.0441
Pyrene	88.2102
Total PAH	825.8485
Total Hyd. content	1662.1365

$$\text{PECQ (PEC Quotient)} = \frac{\text{Concentration of PAH in soil}}{\text{PEC of PAH}}$$

PECQ predicts the presence or absence of toxicity

$$\text{Mean PECQ} = \frac{\text{Sum of individual quotients}}{\text{No of PECs evaluated.}}$$

Where mean PECQ > PEC, toxicity is indicated.

Computation of concentration of toxic equivalency (TEQ) for polycyclic aromatic hydrocarbons (PAH)

$$\text{TEQ} = \sum \text{ETi} \times \text{TEF}$$

Where TEQ = Toxic equivalency, Ti = PAH concentration in soil, TEF = Toxic equivalency factor.

RESULTS

Prior to use as the contaminant, WEO was assessed for PAH compositions (Table 3). Total PAH of WEO was 825.84 mg/L compared to 1662.1 mg/L as total hydrocarbon content, implying that the WEO used was predominantly polyaromatic. Five months after pollution (5 MAP) (Table 4), PAH compounds present in the control, and their respective concentrations were acenaphthene (0.4989 mg/L), benzo[a]pyrene (2.6025 mg/L), benzo[g,h,i]perylene (29.4638 mg/L), naphthalene (0.2389 mg/L), phenanthrene (1.2828 mg/L) and pyrene

(1.2828 mg/L). Soil contained all 16 PAH compounds in both SP_{10.0} and SP_{2.5} treatments.

Nine months after the 5 month old polluted soils received substrate amendment, total PAH in the unamended control experiment (SP₀) was 25.45 mg/L (Table 5). PAH compounds present in SP₀ were acenaphthene (0.2067 mg/L), benzo[a]pyrene (0.6605 mg/L), benzo[g,h,i]perylene (23.1843 mg/L), phenanthrene (0.1285 mg/L), pyrene (1.2742 mg/L). Total PAHs were 113.7265 mg/L in SP_{1.0} and 456.9929 mg/L in SP_{10.0}. This favorably compared with total PAHs of both SP_{1.0} (130.5497 mg/L) and SP_{10.0} (538.5948 mg/L) at 5 MAP. Total PAH ranged from 1.1015 to 6.3475 mg/L in SSP-treatment. There was total (100%) remediation of benzo[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenzo[a,h]anthracene, fluoranthene, fluorine, indeno[1,2,3-c,d]pyrene, and naphthalene in all levels of pollution. Total PAH was highest in SP-treatment. Hence remediation of these compounds was better in the amended treatments.

PEC gives a ratio value of actual concentration of PAH in soil to the percentage total organic matter in soil. PEC of PAH compounds in SP₀ were given as 0.304 (in acenaphthene), 1.587 (in benzo[a]pyrene), and 1.748 (in pyrene) (Table 6). Apart from benzo[g,h,i]perylene and pyrene, 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]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, Benzo[k]fluoranthene and pyrene. Toxicity was indicated for

Table 3. Polyaromatic hydrocarbon content of waste engine oil-polluted soil (unamended) 5 months after pollution.

PAH (mg/L)	SP ₀	SP _{1.0}	SP _{2.5}	SP _{5.0}	SP _{10.0}
Acenaphthene	0.4989	0.8366	1.3013	1.8386	2.4235
Acenaphthylene	0	1.1164	1.8862	2.4629	3.2847
Anthracene	0	4.4281	6.1823	8.0082	10.7625
Benzo[a]anthracene	0	2.5396	2.8650	3.0625	3.5389
Benzo[a]pyrene	2.6025	2.4925	6.9330	11.1135	13.4380
Benzo[b]fluoranthene	0	0	0.8593	0	2.1187
Benzo[g,h,i]perylene	29.4638	89.1187	109.5631	78.0462	100.7342
Benzo[k]fluoranthene	0	18.2800	70.5652	196.7631	294.4268
Chrysene	0	0	0.1006	0	0.0735
Dibenzo[a,h]anthracene	0	0.3895	0.6789	1.0591	1.2879
Fluoranthene	0	0.9843	14.2890	23.0301	38.4333
Fluorene	0	0.2876	0.3942	0.4887	0.6623
Indeno[1,2,3-c,d]pyrene	0	0	0.6672	1.0816	2.0076
Naphthalene	0.2389	0.2836	0.8369	0.3398	0.2995
Phenanthrene	1.2828	3.4386	8.0375	11.2681	28.4367
Pyrene	2.8673	6.3542	11.8793	20.281	36.6667
Total PAH	36.9542	130.5497	237.0390	358.8434	538.5948

SP_x: Soils with substrate amendment; Subscripts of SP represent their respective % concentration of oil in soil.

Table 4. Effects of remediation on polyaromatic hydrocarbon content of a 5-month old waste engine oil-polluted soil at 9 months after substrate amendment with sawdust.

PAH (mg/L)	Without amendment					With amendment				
	SP ₀	SP ₁	SP _{2.5}	SP ₅	SP ₁₀	SSP ₀	SSP ₁	SSP _{2.5}	SSP ₅	SSP ₁₀
Acenaphthene	0.2067	0.4742	0	0.4821	0.1971	0.4256	0	1.2904	0	0
Acenaphthylene	0	0.4181	0.8628	0.9883	0.6354	0.3816	0.3215	1.3362	0.3015	0.3215
Anthracene	0	0	0	0.4941	2.3954	0	1.1218	2.1527	2.2856	0
Benzo[a]anthracene	0	2.1840	2.6180	0	0	0	0	0	0	0
Benzo[a]pyrene	0.6605	0.8384	0	1.4952	4.5170	0.7284	0	0	3.1252	0
Benzo[b]fluoranthene	0	0	0	0.7216	0.5563	0	0	0	0	0
Benzo[g,h,i]perylene	23.1843	108.2766	140.2351	0	84.7930	0	0	0	0	0
Benzo[k]fluoranthene	0	0	62.4118	211.8732	360.2710	0	0	0	0	0
Chrysene	0	0	0	0	0	0	0	0	0	0
Dibenzo[a,h]anthracene	0	0	0.6649	0	1.1278	0	0	0	0	0
Fluoranthene	0	0	0	0	0	0	0	0	0	0
Fluorene	0	0	0	0	0	0	0	0	0	0
Indeno[1,2,3-c,d]pyrene	0	0	0	0	1.1705	0	0	0.2718	0	0
Naphthalene	0	0.2611	0.1634	0	0	0	0	0.7411	0	0
Phenanthrene	0.1285	0.4014	0.6499	0.7599	0.5687	0.2667	0.7869	0	0.6352	0.7800
Pyrene	1.2742	0.4727	0	0.4562	0.4606	0	0	0	0	0
Total	25.4542	113.7265	207.6059	217.706	456.9929	1.8023	2.2302	5.7922	6.3475	1.1015

SP_x: Soils with substrate amendment; SSP_x: Soils without substrate amendment; Subscripts of both SP and SSP represent their respective % concentration of oil in soil.

acenaphthene, benzo[b]fluoranthene, dibenzo[a,h]anthracene, fluorene, indeno[1,2,3-c,d]pyrene, and naphthalene in all the levels of pollution considered (that is, SP_{1.0} - SP_{10.0}).

However, after 9 months following soil amendment,

PEC of PAH compounds in the unamended control experiment (SP₀) were 0.096 (in acenaphthene), 0.309 (in benzo[a]pyrene), 10.834 (in benzo[g,h,i]perylene), 0.060 (in phenanthrene), and 0.595 (in pyrene). Comparable differences were obtained in the amended soils (SSP₀) as

Table 5. Probable effect concentrations (PEC) of polyaromatic hydrocarbon compounds in waste engine oil polluted- soil (unamended) at 5 months after pollution.

PAH (mg/L)	SP ₀	SP ₁	SP _{2.5}	SP ₅	SP ₁₀
Acenaphthene	0.304	0.565	0.829	1.202	1.417
Acenaphthylene	N/A	0.754	1.201	1.609	1.921*
Anthracene	N/A	2.992*	3.938*	5.234*	6.294*
Benzo[a]anthracene	N/A	1.716*	1.829*	2.002*	2.069*
Benzo[a]pyrene	1.587	1.684*	4.416*	7.263*	7.858*
Benzo[b]fluoranthene	N/A	N/A	0.547	N/A	1.239
Benzo[g,h,i]perylene	17.643*	60.215*	67.785*	51.010*	58.909*
Benzo[k]fluoranthene	N/A	12.350*	44.945*	128.663*	172.179*
Chrysene	N/A	N/A	0.064	N/A	0.430
Dibenzo[a,h]anthracene	N/A	0.263	0.432	0.692	0.753
Fluoranthene	N/A	0.665	9.101*	15.052*	22.476*
Fluorene	N/A	0.194	0.251	0.319	0.387
Indeno[1,2,3-c,d]pyrene	N/A	N/A	0.425	0.707	1.134
Naphthalene	0.146	0.192	0.563	0.222	0.175
Phenanthrene	0.782	0.323	5.119*	7.364*	16.629*
Pyrene	1.748*	4.293*	7.566*	13.256*	21.443*
Mean PEC quotient	1.64	1.48	1.57	1.53	1.71

SP_x: Soils with substrate amendment; Subscripts of SP represent their respective % concentration of oil in soil. *Toxicity is not indicated. Where $\frac{\text{mean PECQ}}{\text{PEC}} > 1$, toxicity is indicated for the particular PAH compound (Ingersoll et al., 2002).

Table 6. Probable effect concentrations (PEC) of polyaromatic hydrocarbon compounds of a 5month-old waste engine oil polluted- soil 9 months after substrate amendment.

PAH (mg/L)	Without amendment					With amendment				
	SP ₀	SP ₁	SP _{2.5}	SP ₅	SP ₁₀	SSP ₀	SSP ₁	SSP _{2.5}	SSP ₅	SSP ₁₀
Acenaphthene	0.096	0.172	-	0.202	0.077	0.095	-	.0256	-	-
Acenaphthylene	-	0.151	0.321	0.414	0.247	0.085	0.064	0.265	0.058	0.063
Anthracene	-	-	-	0.027	0.932	-	0.224	0.426	0.440	-
Benzo[a]anthracene	-	0.791	0.973	-	-	-	-	-	-	-
Benzo[a]pyrene	0.309	0.304	-	0.626	1.758	0.163	-	-	0.020	-
Benzo[b]fluoranthene	-	-	-	0.302	0.216	-	-	-	-	-
Benzo[g,h,i]perylene	10.834*	108.071*	52.132*	-	32.993*	-	-	-	-	-
Benzo[k]fluoranthene	-	-	-	23.201*	88.650*	140.183*	-	-	-	-
Chrysene	-	-	-	-	-	-	-	-	-	-
Dibenzo[a,h]anthracene	-	-	0.247	-	0.439	-	-	-	-	-
Fluoranthene	-	-	-	-	-	-	-	-	-	-
Fluorene	-	-	-	-	-	-	-	-	-	-
Indeno[1,2,3-c,d]pyrene	-	-	-	-	0.455	-	-	0.053	-	-
Naphthalene	-	0.095	0.061	-	-	-	-	0.147	-	-
Phenanthrene	0.060	0.145	0.242	0.318	0.221	0.060	0.157	-	0.122	0.152
Pyrene	0.595	0.171	-	0.191	0.179	-	-	-	-	-
Mean PECQ	2.14	2.76	2.69	2.39	2.57	4.48	5.00	5.05	5.19	5.14

SP_x: Soils with substrate amendment; SSP_x: Soils without substrate amendment; Subscripts of both SP and SSP represent their respective % concentration. of oil in soil. *Toxicity is not indicated. Where $\frac{\text{mean PECQ}}{\text{PEC}} > 1$, toxicity is indicated for the particular PAH compound (Ingersoll et al., 2002).

0.095 in acenaphthene (0.095), 0.085 in acenaphthylene, 0.060 in phenanthrene, and 0 in pyrene. In SSP₁₀, PEC of PAH compounds included acenaphthylene (0.063) and

Table 7. Toxicity equivalent concentrations of polyaromatic hydrocarbon compounds in waste engine oil polluted- soil (unamended) at 5 months after pollution.

PAH (mg/L)	SP ₀	SP ₁	SP _{2.5}	SP ₅	SP ₁₀
Benzo (a)anthracene [0.1]	0	0.2540	0.2865	0.3063	0.3539
Benzo[a]pyrene [1.0]	2.6025	2.4925	6.9330	11.1135	13.4380
Benzo[b]fluoranthene [0.1]	0	0	0.0860	0	0.2119
Benzo[k]fluoranthene [0.1]	0	1.8280	7.0565	19.6763	29.4427
Chrysene [0.01]	0	0	0.0010	0	0.0007
Dibenzo[a,h]anthracene [0.1]	0	0.0389	0.0679	0.1059	0.1288
Indeno[1,2,3-c,d]pyrene [0.1]	0	0	0.6672	1.0816	2.0076
TTEC	2.6025	4.6134	15.0981	32.2836	45.5836

SP_x: Soils with substrate amendment; Subscripts of SP represent their respective % concentration of oil in soil. TEF values of c-PAH's are given in bracket.

Table 8. Toxicity equivalent concentrations of a 5 month-old waste engine oil polluted-soil 9 months after substrate amendment.

PAH (mg/L)	SP ₀	SP ₁	SP _{2.5}	SP ₅	SP ₁₀	SSP ₀	SSP ₁	SSP _{2.5}	SSP ₅	SSP ₁₀
Benzo (a)anthracene [0.1]	-	0.2184	0.2618	-	-	-	-	-	-	-
Benzo[a]pyrene [1.0]	0.6605	0.8384	-	1.4952	4.5170	0.7284	-	-	3.1252	-
Benzo[b]fluoranthene [0.1]	-	-	-	0.0722	0.0556	-	-	-	-	-
Benzo[k]fluoranthene [0.1]	-	-	6.2412	21.1873	36.0271	-	-	-	-	-
Chrysene [0.01]	-	-	-	-	-	-	-	-	-	-
Dibenzo[a,h]anthracene [0.1]	-	-	0.0665	-	0.1128	-	-	-	-	-
Indeno[1,2,3-c,d]pyrene [0.1]	-	-	-	-	0.1171	-	-	0.0272	-	-
TTEC	0.6605	1.0568	6.5695	22.7547	40.8296	0.7284	-	0.0272	3.1252	-

SP_x: Soils with substrate amendment; SSP_x: Soils without substrate amendment; Subscripts of both SP and SSP represent their respective % concentration of oil in soil. TEF values of c-PAH's are given in bracket.

phenanthrene (0.152). Apart from benzo[g,h,i]perylene, toxicity was implicated for these PAH compounds. By comparing their PECs against the PECQs in all levels of pollution, toxicity was not indicated for only benzo[b]fluoranthene, and benzo[g,h,i]perylene. Toxicity was indicated for every other PAH compounds at all the levels of pollution considered.

At 5 MAP, TEF, which are toxicity potency factors, were used to evaluate the toxicities of PAH mixtures of the organic pollutant. In SP₁, TEQ's values were 0.2540 (in benzo[a]anthracene), 2.4925 (in benzo[a]pyrene), 1.8280 (benzo[k]fluoranthene), and 0.0389 (in dibenzo[a,h]anthracene) (Table 7). Total toxicity equivalent concentration in SP₀ was 2.6025. Total toxicity equivalent concentrations were also 4.6134 in SP_{1.0}, 15.0981 in SP_{2.5}, 32.2836 in SP_{5.0} and 45.5836 in SP_{10.0} respectively. 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). The implication is that the cleanup level for benzo[a]pyrene (according to Cal-EPA, 2005) was not met for these particular soil samples.

After additional 9 months (with or without substrate amendment), toxicity equivalents (TEQ's) in SP₁ were

0.2184 mg/L in benzo[a]anthracene and 0.8384 mg/L in benzo[a]pyrene (Table 8). These values were higher than benchmark TEF values of the c-PAH of 0.1 mg/L. TEQ's of c-PAH in SP_{2.5} was 0.2618 in benzo (a)anthracene, 6.2412 in benzo[k]fluoranthene, and 0.0665 in dibenzo[a,h]anthracene. In the amended treatments, TEQ's were presented for only benzo[a]pyrene in SSP₀ (0.7284) and indeno[123-c]pyrene in SSP_{2.5} (0.0272). TEQ's of benzo[a]pyrene in SSP₅ was 3.1252 mg/L, also higher than the benchmark TEQ of 1.0 mg/L. Total toxicity equivalent concentration (TTEC) in SP₀ was given as 0.6605 mg/L, and 1.0568 mg/L in SP_{1.0}, 6.5695 mg/L in SP_{2.5}, 22.7547 mg/L in SP_{5.0} and 40.8296 mg/L in SP_{10.0} respectively. These values exceeded the benchmark clean-up level for benzo[a]pyrene (0.137 mg/L) (Cal-EPA, 2005). However, TTEC for the amended soils present did not exceed the cleanup level for benzo[a]pyrene. The implication is that the cleanup level for benzo[a]pyrene according to Cal-EPA (2005) was met for amended soil samples.

DISCUSSION

There were significant reductions in soil PAH compounds.

Reductions were either total (100%) or significantly partial (50 to 98% reductions). Remediation was enhanced in the sawdust-amended polluted soil compared to the remediation ability of the unpolluted (SP) soil treatments. The importance of soil amendments in remediation strategies is of utmost importance. Microorganisms usually present in the sawdust substrate, while growing, probably produce enzymes that were used in metabolizing the hydrocarbons in the substrate matrix (Sutherland et al., 1995; Diaz et al., 1996). Some of these microorganisms associated with sawdust, like *Aspergillus niger* and *Aspergillus fumigatus* (Ikhajagbe, 2010), both metabolize PAHs (Yamazaki et al., 1988), and there is even a report of the ability of *A. niger* to cleave the rings of naphthalene, anthracene, and phenanthrene (Yogambal and Karegoudar, 1997). *A. fumigatus* also produces a cytochrome P₄₅₀ that hydroxylates benzo[a]pyrene (Venkateswarlu et al., 1996). *Micrococcus*, which is also associated with sawdust (Ikhajagbe, 2010) has been reported to be a petroleum hydrocarbon degrading bacteria by Bartha and Atlas (1997).

The phytotoxic effects of a WEO-polluted soil on *Vigna unguiculata* was significantly ($p > 0.05$) reduced upon amendment with sawdust (Ikhajagbe and Anoliefo, 2010). Atagana (2008) successfully bioremediated contaminated soil containing $>380\ 000\ \text{mgkg}^{-1}$ total petroleum hydrocarbons by composting with sewage sludge. Studies by Laine and Jürgensen (1997) investigated bench-scale composting of chlorophenol contaminated soils using different inoculants: mushroom straw compost, remediated soil and indigenous soil microflora.

Similarly, Joyce et al. (1998) investigated the fate of a mixture of three and four ring PAHs (fluorene, anthracene, phenanthrene, pyrene, benz[a]anthracene) under composting conditions with solid municipal waste. They reported that anthracene, phenanthrene and pyrene were removed effectively. Martens (1982) and Kastner and Mahro (1996) investigated the degradation of naphthalene, anthracene, fluoranthene and pyrene in soil and soil-compost incubations. The study showed that the presence of compost enhanced the removal of the PAHs.

Wischnann and Steinhart (1997) successfully remediated 100% of fluoranthene and pyrene, $>90\%$ of benz[a]anthracene and chrysene and approximately 70% of benzo[a]pyrene removed from the soil mixed with the compost, after 180 days.

Soil amendments such as composts have an enormous potential for bioremediation as they are capable of sustaining diverse populations of microorganisms, such as bacteria including bacilli, pseudomonads, mesophilic and thermophilic actinomycetes and lignin-degrading fungi, all with the potential to degrade a variety of aromatic pollutants. They can act as a soil ameliorant capable of changing pH, moisture content, soil structure and acting as a nutrient source, thereby improving the

contaminated soil environment for indigenous or introduced microbial degradative activity. A soil amendment is mostly effective when thoroughly mixed into the soil. If it is merely buried, its effectiveness is reduced, and it will interfere with water and air movement and root growth. Having an enormous potential for bioremediation, they are capable of sustaining diverse populations of microorganisms, all with the potential to degrade a variety of aromatic pollutants.

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