Annual Review & Research in Biology
2(3): 66-82, 2012

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Changes in Polyaromatic Hydrocarbon Content of a Waste Engine Oil Polluted Soil Exposed to pH Adjustments

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Authors' contributions

This work was carried out in collaboration between all authors. Author BI, with the support of authors NA and GOA, designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors EOO, UAO and NA managed the analyses of the study. Authors BI and NA managed the literature searches. All authors read and approved the final manuscript.

Research Article

Received 23rd October 2012
Accepted 8th December 2012
Published 20th December 2012

ABSTRACT

The present study investigated the role of pH adjustments in the remediation of polyaromatic hydrocarbon (PAH) contents of waste engine oil-polluted soil. Sun-dried top soil (0-10cm) was measured into buckets. Waste engine oil (WEO) was added to soil and mixed thoroughly to obtain similar concentrations of 2.5% w/w oil in soil. The polluted soil was thereafter amended with NPK (15:15:15) fertilizer to enhance microbial activity. The buckets were transferred into a well ventilated screen house with inherent constant room temperature. The entire setup was divided into 5 sets. Each set was irrigated daily with 200ml of different pH solutions (pH 3, 5, 7, and 11) for a period of 3 months. There were reductions in total PAH concentrations. Total polyaromatic hydrocarbon (PAH) of soil was lowest when soil was modified with solutions of pH 5 (78.1 mg/kg) followed by that at pH 9 (90.6 mg/kg), pH 3 (213.5 mg/kg) and pH 11(315.1 mg/kg). Obviously, successful remediation of PAH is pH dependent. Fluorene was totally remediated at pH value from acidity to neutrality; whereas at alkalinity, fluorene content was 0.237 mg/kg and 0.139 mg/kg at pH 7 and 11 respectively.

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Keywords: Natural attenuation; pH; polyaromatic hydrocarbon; probable effect concentration; toxicity equivalent concentration; waste engine oil.

1. INTRODUCTION

Nature and the pressure from anthropogenic sources increasingly expose the environment to changes. This is particularly even so with oil pollution as a major environmental concern in many countries; and this has led to a concerted effort in studying the possibility of using environmental friendly and cost effective methods of remediation. Several components of the oil, e.g. solvents and detergents added during the blending process, aliphatic hydrocarbons and PAHs distilled from crude oil are toxic, and can combine with products of combustion to generate carcinogens and endocrine disrupters (USEPA, 1996).

Polycylic aromatic hydrocarbons (PAHs), such as petroleum and petroleum derivatives, are widespread organic pollutants entering the environment, chiefly, through oil spills and incomplete combustion of fossil fuels. Since most PAHs persist in the environment for a long period of time and bioaccumulate, they cause *Environmental Pollution* and affect biological equilibrium dramatically (Demir and Demirbag, 1999). There have also been elevated concentrations of PAHs at contaminated sites. The rate and extent of distribution and/or accumulation of PAHs in soils are affected by environmental factors such as the organic content, structure and particle size of the soil, characteristics of the microbial population, the presence of contaminants such as metals and cyanides that are toxic to micro-organisms, and the physical and chemical properties of the PAHs (Wilson and Jones, 1993). The effects of these physiochemical properties form the basis for this research. PAH molecule stability and hydrophobicity are two primary factors which contribute to the persistence of PAHs in the environment, particularly the high-molecular weight (HMW) PAHs. Consequently, they have been detected in numerous aquatic and terrestrial ecosystems at concentrations high enough to warrant concern about bioaccumulation. Chrysene is one of the HMW PAHs consisting of four fused benzene rings and among PAHs classified as priority pollutants by the U.S. Environmental Protection Agency (Smith et al., 1989). Microbial biodegradation of HMW PAHs has been found as a possible way to clean up polluted soils and water systems (Alexander, 1999). However, relatively little information is available on microbial metabolism of HMW PAHs (Kanaly and Harayama, 2000). A number of bacterial isolates capable of chrysene metabolism have been described; *Rhodococcus* sp. Strain UW1 (Walter et al., 1991), *Sphingomonas yanoikuyae* which oxidized chrysene (Boyd et al., 1999) while *Pseudomonas fluorescens* utilised chrysene and benz[a]anthracene as sole carbon sources (Caldini et al., 1995). Chrysene oxidation occurs by incorporation of an oxygen molecule in an aromatic ring. This is catalyzed by dioxygenase to a *cis*-dihydrodiol intermediate, which undergoes further metabolism via pyridine nucleotide dependent dehydrogenation reaction to produce catechols. These are substrates for ring cleavage enzymes, which lead to complete mineralization (Hinchee and Olfenbuttel, 1991). There are various remediation technologies, some of which have become controversial, particularly when they involve physical and chemical methods. Physical and chemical methods are most widely used procedures for clean-up, are not simple or favourable to the environment. Environmental friendly approach to remediation therefore becomes imperative. One of such approach relies on the soils inherent ability to remediate contaminants (Ikhajiagbe, 2010; Ikhajiagbe and Anoliefo, 2011; Ikhajiagbe and Anoliefo, 2012a,b,c). The present study therefore aims to investigate if any adjustment in soil pH would significantly impact on the remediation of PAH in petroleum hydrocarbon polluted soil. Soil pH in the present study would not be constantly monitored, as the main objective of the study is to determine how exogenous application of pH solutions can affect bioremediation activities in oil-polluted soils.

2. MATERIALS AND METHODS

Top soil (0-10cm) was collected randomly from an area measuring 50 x 50m on a fallow land situated near the Department of Plant Biology and Biotechnology Screen House, University of Benin, Benin City, Nigeria. Thereafter, 5kg sun-dried soil was placed into large 10-litre bowls with 5 random perforations made with 2 mm diameter nails at the bottom of each bowl. Waste engine oil (WEO) was obtained from an auto-mechanic workshop in Benin City that specializes in repairs of small vehicles. Oil was added to soil in the bowls and mixed thoroughly to obtain similar concentrations of 2.5% w/w oil in soil. The polluted soil was thereafter amended with 4g NPK (15:15:15) fertilizer to enhance microbial activity (Ikhajiagbe and Mgbeze, 2012). The buckets were transferred into a well ventilated screen house with inherent constant room temperature.

The entire setup was divided into 5 sets. The soil pH of each set would be adjusted by daily irrigation of polluted soils with pH solutions. Five different pH solutions were prepared. A 1M NaOH solution was carefully added to distilled water (pH 7) to obtain pH 9 and pH 11 solutions. Orthophosphoric acid was added to distilled water to obtain pH of 3 and 5 respectively. The pH was read on a pH meter. These solutions were prepared and stored in clean jerry cans. As the predetermined water-holding capacity of the soil was 80ml/kg soil, each bucket was irrigated every morning with 200ml pH solutions for a period of 3 months. Care was taken to ensure that the soils in the bucket were properly and equally moistened, while ensuring that pH solutions as well as water soluble fractions of oil in soil did not drain out from the perforations at any time throughout the experimental period. The soils in the bucket were turned and properly mixed once every week to enhance relatively equal surface area of exposure of both pollutant and pH solutions in the soil. The control soil was irrigated with distilled water (pH 7). After three months of soil exposure to pH treatments, soils were taken to the laboratory for analysis.

2.1 Total Organic Matter (TOM) Contents

Half a gram (0.5 g) of each air-dried soil sample was put into a conical flask and 2.5 ml of 1N potassium dichromate solution $K_2Cr_2O_7$ was added and swirled gently to disperse the sample in the solution. Five millilitres (5 ml) of concentrated tetraoxosulphate (VI) acid was added rapidly, into the flask and swirled gently until sample and reagents were mixed and finally swirled vigorously for about a minute. The flask was allowed to stand in a fume cupboard for 30 minutes. Five to ten (5 to 10) drops of the indicator were added and the solution titrated with 0.5N $FeSO_4$ to maroon colour. A blank determination was carried out to standardize the dichromate (Nelson and Sommers, 1982). TOM contents were calculated as follows (Osuji and Nwoye, 2007):

$$\text{TOM (\%)} = \frac{(\text{meq K2Cr2 O7} - \text{meq Fe SO4}) \times 0.003 \times 100 \times 1.3}{\text{Weight of sample (g)}} \times 1.724$$

Where: meq K2 Cr2O7 = 1N X 2.5 ml
meq FeSO4 = 0.5 N X Volume of titrant in ml
0.03 = Milliequivalent weight of carbon
1.30 = Correction factor
Where: 1.724 = Conversion Factor

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

A 10 g sample was extracted with methylene chloride (DCM). The extract was filtered through anhydrous sodium sulphate to remove any trapped water molecule. This was followed by a clean- up/ fractionation of the sample extract into Aliphatic and Aromatic (PAH) components. Finally, the components were concentrated using a rotary evaporator for GC analysis, using FID as detector. Model of GC used was AGILENT 6890.

2.2.1 Extraction Procedure

About 10 g of a well mixed sample was weighed into a dry, clean beaker, previously rinsed with DCM. The weight of the sample taken was recorded. The sample was air- dried previously, ground and sieved to a uniform size. 100 ml of DCM was then added, followed by addition of 1 ml of surrogate spike standard. The beaker was then covered with aluminium foil and allowed to stand overnight. The sample was filtered through Whatman No.41 filter paper packed with 10 g of anhydrous Sodium sulphate, into a round-bottomed flask. The sample extract was concentrated using a rotary evaporator at 60ºC to about 1-2 ml volume, and transferred into a sample vial. The same was done for blank using DCM passed through same procedure. The sample and blank were now ready for clean- up/ fractionation to get the aliphatics and aromatics (PAH).

2.2.2 Fractionation Procedure

2.2.2.1 Preparation of the Fractionation Column

A glass wool fibre was inserted into the base of the column. The column must be a polypropylene type. 10 g of silica gel (60-200 mesh size, Davidson Grade 850 or its equivalent) was weighed, pre-conditioned by baking at 105ºC overnight, and the column was packed with it. The base of the column was tapped to pack the silica gel properly. The column was eluted very well with n-hexane, and care was taken not to allow it to dry out during this period.

2.2.2.2 Fractionation of the Sample Extract

Using a pipette, 1 ml of the sample extract was transferred to the top of the column. It was eluted with 60 ml of n-hexane to the get the aliphatic hydrocarbons and the eluates collected in a conical flask. While the hexane was almost getting dry, 40 ml of DCM was added to elute the PAH components. Also, the eluates were collected in another conical flask. The sample eluates were then concentrated to 1 - 3 ml using the rotary evaporator, before being transferred into sample vials.

2.3 GC Analysis

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

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

Where,
Rf = Response factor = Total Area / Total Concentration, obtained from instrument calibration with standards.
Area is obtained from the chromatogram output.
F.vol is the final volume of the concentrated extract (in ml)
Wt is the initial weight of the homogenized sample (in grams)

2.4 Identification of Soil Microorganisms

Isolation and characterization of bacterial and fungal oil degraders was carried out using the methods of Sabba (1995) and Cheesebrough (1998).

2.5 Computation of Probable Effect Concentration Quotient (PECQ) of PAH Compounds

$$PECQ = \frac{\text{Concentration of PAH component}}{\text{PEC value for that component}}$$

Where mean PECQ > PEC, toxicity is indicated. PECQ predicts the presence or absence of toxicity. PEC value for PAH components are provided from Honeywell (2011).

2.6 Computation of Toxic Equivalency (TEQ) for Polycyclic Aromatic Hydrocarbons (PAH)

Toxic Equivalency factors (TEF) are toxicity potency factors used as a consistent method to evaluate the toxicities of variable mixtures of organic compounds.

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

Where TEQ = Toxic Equivalency, Ti = PAH concentration in soil, and TEF = Toxic Equivalency factor. Compounds having TEF = 0 are not important in calculating TEQ. Benzo[a]pyrene has a TEF value of 1 and it serves as an index PAH for other PAH compounds. Toxicity references calculated above are compared with benchmark values provided by Efroymson et al. (1997).

3. RESULTS AND DISCUSSION

The effects of pH adjustments of the remediation of PAH contents of waste engine oil-polluted soils have thus been studied. The physiochemical properties of the soil used in the present as well as PAH content of waste engine oil used are present on Tables 1 and 2, respectively. Total organic matter (TOM) in the soil at 1 week after pollution (WAP) in a pH 7 medium was 0.61% (Fig. 1). Three months later, within the same pH 7, organic matter was 0.62%, with no significant change. Within the pH ranges, TOM ranged from 0.54 (pH 11) to 0.74 (pH 5).

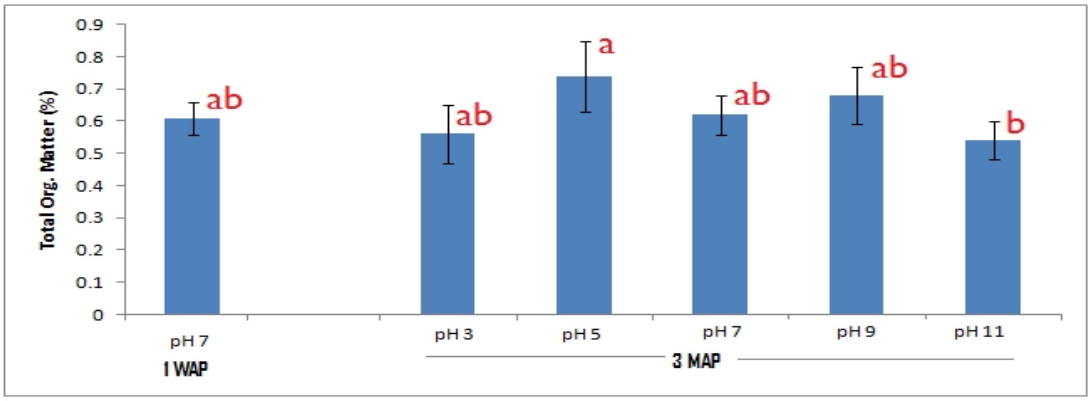

Fig. 1. Impact of pH changes on the total organic matter content of waste engine oil polluted soil. Bars with similar aphabets do bot differ significantly ($p<0.05$) from the other. WAP, weeks after polltion; MAP, months after pollution

Table 1. Physical and chemical properties of soil before waste engine oil contamination

Parameters	Units	Soil
pH	-	6.11
Electrical Conductivity	µs/cm	301.00
Total Org. Matter	%	0.61
Total Nitrogen	%	0.12
Exchangeable Acidity	meq/100 g soil	0.22
Water-holding capacity	ml(water)/kg(soil)	83.00
K	meq/100 g soil	1.43
Ca	meq/100 g soil	15.26
Mg	meq/100 g soil	10.97
P	mg/l	153.00
Clay	%	7.90
Silt	%	13.90
Sand	%	78.20
Fe	mg/kg	998.80
Mn	mg/kg	16.71
Zn	mg/kg	12.12
Cu	mg/kg	4.98
Cr	mg/kg	2.08
Cd	mg/kg	ND
Pb	mg/kg	ND
Ni	mg/kg	3.60
V	mg/kg	0.76
Total Hydrocarbon Content	mg/kg	224.06

ND: Not detected (≤0.0001 mg/kg)

Table 2. PAH 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	**825.8485**

Total PAH at 1 WAP was 923.9 mg/kg compared to 163.7 mg/kg 3 months later at the same pH 7, thus indicating an 82.3% remediation in 3 months. Total PAH of soil was lowest at pH 5 (78.1 mg/kg) followed by that at pH 9 (90.6 mg/kg), pH 3 (213.5 mg/kg) and pH 11(315.1 mg/kg). Obviously, successful remediation of PAH is pH dependent. The PAH component, acenaphthene, was 0.167 mg/kg at 1 WAP (pH 7) compared to 1.872 mg/kg at 3 MAP (pH 7); these values further decreased to 0.336 mg/kg at pH 9 and 0.769 mg/kg at pH 11. Fig. 2 – 6 present chromatographs showing PAH contents of soil 3 months after exposure to waste engine oil pollution and various pH treatments.

At 1 WAP, soil content of fluorene was 0.378 mg/kg. However at 3 MAP fluorene was totally remediated from soils adjusted to pH 3, 5 and 7 respectively (Table 3). Whereas fluorene content was 0.237 mg/kg and 0.139 mg/kg at pH 7 and 11 respectively. Similarly, there was total remediation of phenanthrene at pH 5 and pH 7, though at 3MAP, significant reduction in phenanthrene at pH 3, 9, and 11 (0.355 mg/kg, 0.013 mg/kg and 0.299 mg/kg respectively). There were significant reductions in PAH content of soil at 3 MAP in anthracene, fluoranthene, pyrene, benz(a) anthracene, chrysene, benzo(b) fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,-cd)pyrene, and benzo(g,h,i)perylene in all pH ranges, compared to original values present at 1 WAP. PAHs with lower-molecular-weight (two to four ringed compounds) such as naphthalene, acenaphthylene and fluorene are relatively easy to degrade, in general, the fate of PAHs in the soil depends on the molecular size and topology of the compound (Kanaly and Harayama, 2000). This probably accounts for their rapid degradability compared to other PAH components. Volatilization is the first line of elimination for low molecular weight PAHs. However, as the molecular sizes increase and when exposure to soil particles is prolonged, bioavailability is reduced greatly and, biodegradation rates become slower. In order to enhance the biodegradation processes and making it economically realistic and rapid, it is imperative that the bioavailability of PAHs in soil be increased (Piskonen and Itävaara, 2004). The rates at which PAHs are degraded are also determined by moisture level. The reason is simple, that water is needed for microbial growth and enzymatic/biochemical activities (Leahy and Colwell, 1990). The present study, the buckets got adequate supply of moisture.

Table 3. Polyaromatic hydrocarbon content (mg/kg) as effected by pH adjustments for a period of 3 months after soil exposure to waste engine oil pollution

PAH component	1 WAP	3 MAP				
	pH 7	pH 3	pH 5	pH 7	pH 9	pH 11
Naphthalene	0.5173	0.6801	0.8624	0.5651	1.1689	1.4993
Acenaphthylene	0.1670	0.1645	0.2264	1.8717	0.3362	0.7685
Acenaphthene	0.4560	0.2781	0.3350	0.4213	0.4190	0.7272
Fluorene	0.3578	0	0	0	0.2373	0.1385
Phenanthrene	1.5357	0.3552	0	0	0.0126	0.2996
Anthracene	4.7538	1.2191	0.4834	1.1712	0.5559	2.9376
Fluoranthene	6.5246	2.186	1.9775	3.9038	2.5307	0.1805
Pyrene	40.3273	4.3797	1.7898	6.2468	1.1041	10.45652
Benz(a)anthracene	190.4658	21.4226	12.7000	35.3024	17.7697	44.8713
Chrysene	363.3801	76.9447	28.8077	12.6432	22.5679	123.774
Benzo(b)fluoranthene	72.9912	19.0635	15.7780	5.2771	10.9021	21.7708
Benzo(k)fluoranthene	124.1892	51.8582	9.0027	83.9363	20.7669	62.5217
Benzo(a)pyrene	49.9230	33.6229	4.0727	10.3167	6.3897	20.4295
Indeno(1,2,3-cd)pyrene	29.4184	0.3149	0.8412	1.2631	0.8793	11.2069
Benzo(g,h,i)perylene	38.8968	0.9800	1.2663	0.7963	4.9683	13.4897
TOTAL	**923.9045**	**213.4701**	**78.1437**	**163.7156**	**90.6090**	**315.0731**

WAP Weeks after pollution, MAP months after pollution

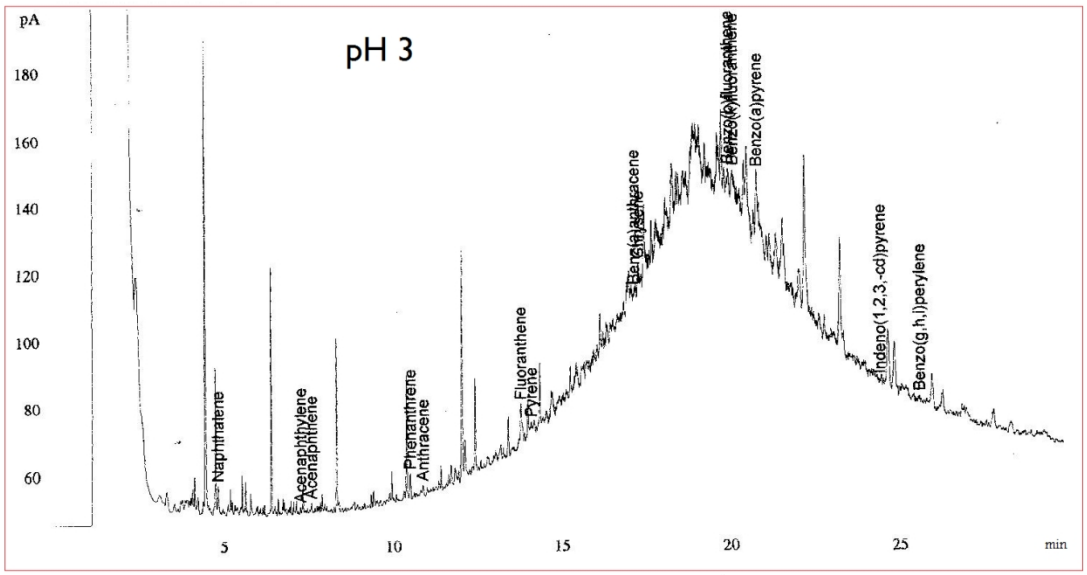

Fig. 2. Chromatograph showing PAH content (mg/kg) of soil 3 months after exposure to waste engine oil pollution and soil pH (3) adjustment

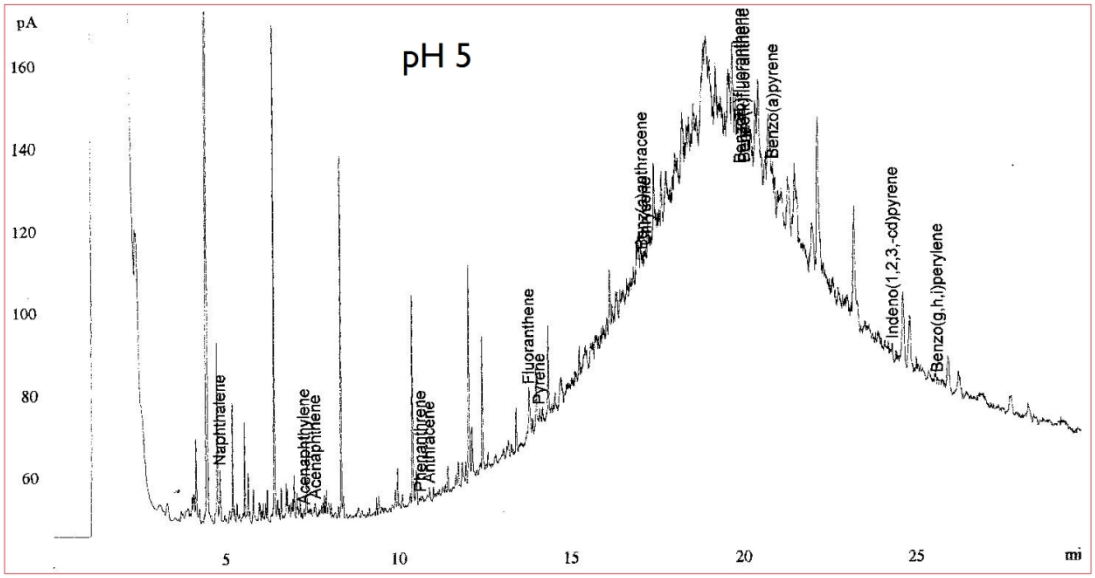

Fig. 3. Chromatograph showing PAH content (mg/kg) of soil 3 months after exposure to waste engine oil pollution and soil pH (5) adjustment

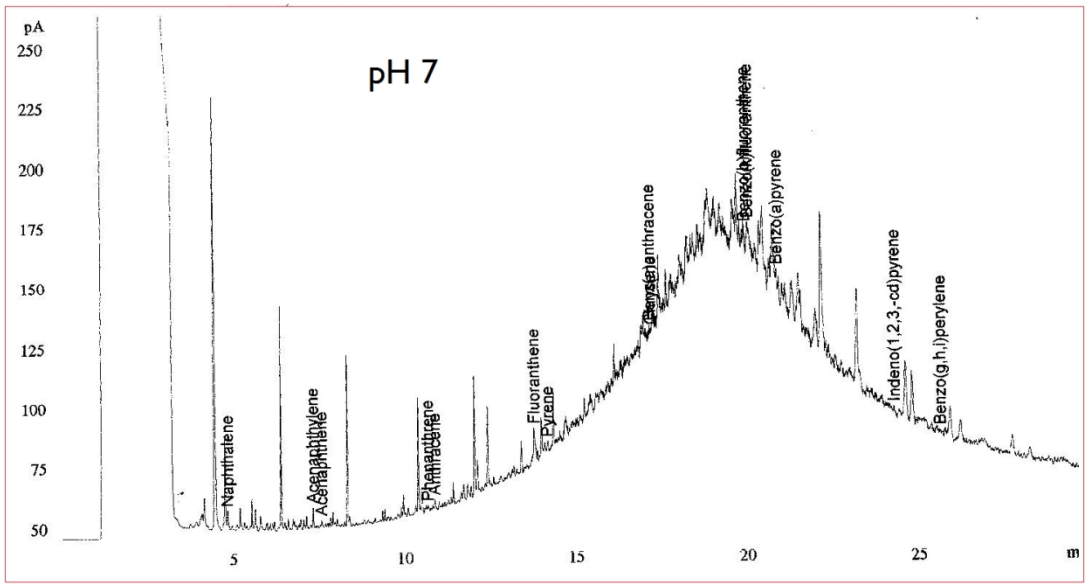

Fig. 4. Chromatograph showing PAH content (mg/kg) of soil 3 months after exposure to waste engine oil pollution and soil pH (7) adjustment

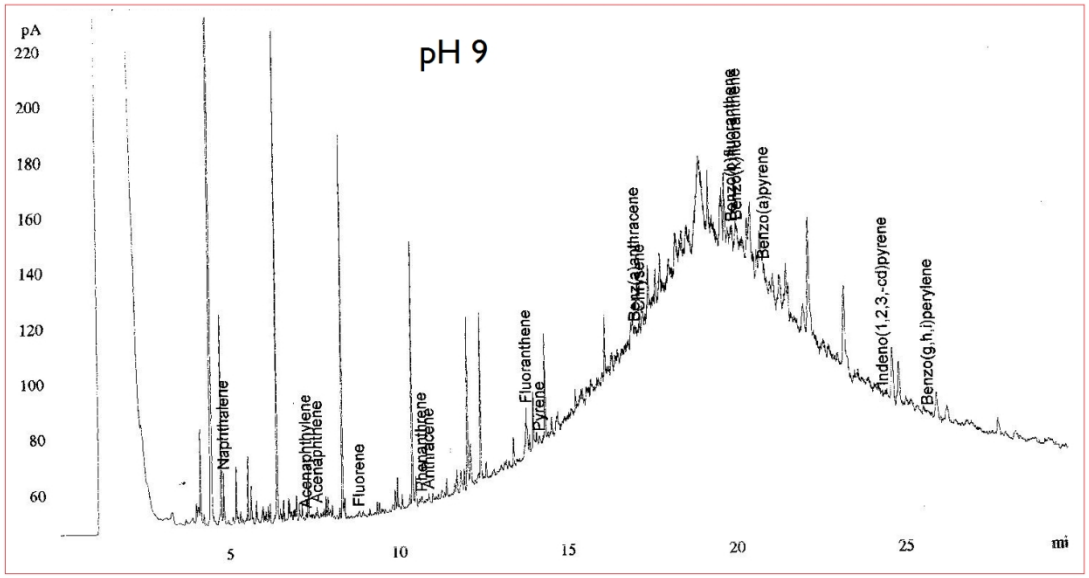

Fig. 5. Chromatograph showing PAH content (mg/kg) of soil 3 months after exposure to waste engine oil pollution and soil pH (9) adjustment

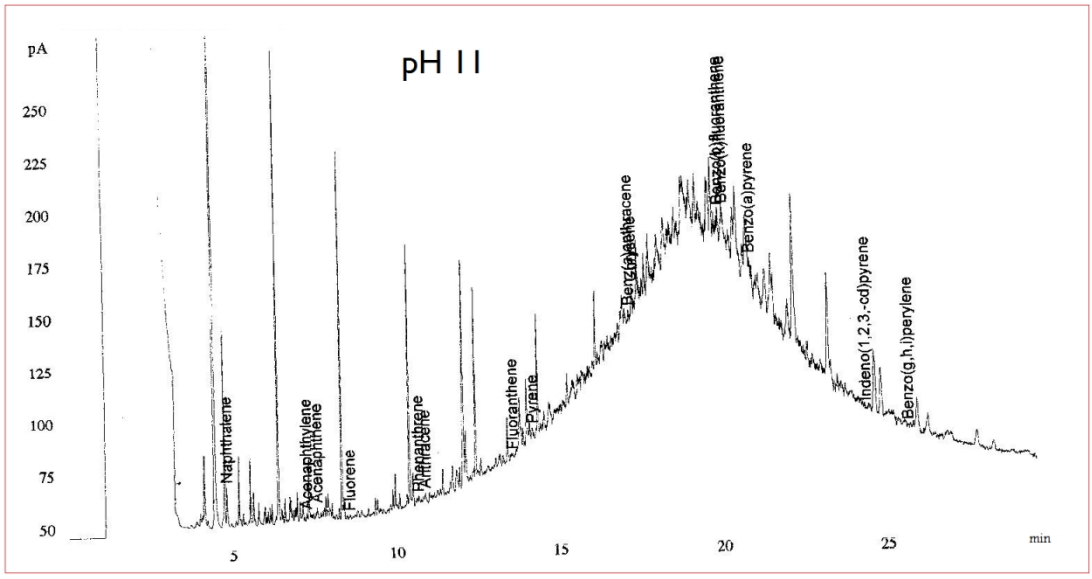

Fig. 6. Chromatograph showing PAH content (mg/kg) of soil 3 months after exposure to waste engine oil pollution and soil pH (9) adjustment

Probable Effect Concentration Quotient (PECQ) of PAH of WEO polluted soil affected by soil pH for a period of three months is presented on (Table 4).

Table 4. Probable Effect Concentration Quotient (PECQ) of PAH content (mg/kg) as effected by pH adjustments for a period of 3 months after soil exposure to waste engine oil pollution

PAH component	1 WAP			3 MAP		
	pH 7	pH 3	pH 5	pH 7	pH 9	pH 11
Naphthalene [0.917]	0.5641	0.74417	0.9405	0.6162	12747	1.6350
Acenaphthylene [1.301]	0.1284	0.1264	0.1740	1.4387	0.2584	0.5907
Acenaphthene [0.861]	0.1296	0.3230	0.3891	0.4893	0.4866	0.8446
Fluorene [0.264]	1.3553	0	0	0	0.8989	0.5242
Phenanthrene [0.543]	2.8282	0.6541	0	0	0.0232	0.5517
Anthracene [0.207]	22.9652	5.8894	2.3353	5.6580	2.6855	14.1913
Fluoranthene [1.436]	4.5436	1.5223	1.3771	2.7185	1.7623	0.1257
Pyrene [0.344]	117.2305	12.7317	5.2029	18.1593	3.2096	30.3968
Benzo(a)anthracene [0.192]	992.0094	111.5760	66.1458	183.8667	92.5505	233.7047
Chrysene [0.253]	1436.2850	304.1292	113.8644	49.9731	89.2012	489.2253
Benzo (b) Fluranthene [0.908]	80.3868	20.9950	17.3767	5.8118	12.0067	23.9767
Benzo(k)Fluranthene [0.203]	611.7695	255.4591	44.3483	413.4793	102.3	307.9887
Benzo(a)pyrene [0.146]	0.3419	230.2938	27.8952	70.6623	43.7651	139.9281
Indeno(1,2,3-cd)pyrene [0.183]	160.7563	1.7208	4.6220	6.9401	4.8049	61.2399
Benzo (g,h,i) perylene [0.708]	54.9400	1.3842	1.7886	1.1247	7.0174	19.0532

Values provided in brackets are PEC values associated with the PAH components (Honeywell, 2011). WAP Weeks after pollution, MAP months after pollution.

When PECQ was greater than PEC value of a particular PAH component of the soil, toxicity was indicated for that particular PAH compound (Ingersoll et al., 2000). At 1 WAP, PEC values for naphthalene, acenaphthylene, acenaphthene, and Benzo(a)pyrene (values range 0.1284 – 0.5641) were less than their corresponding PEC values; an indication that toxicity was not indicated for these components in this treatment. Throughout the entire experiment, PECQ for Acenaphthene was less than corresponding PEC values irrespective of soil pH. However, PECQ for Pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, and benzo (g,h,i) perylene were greater than their corresponding PEC values, an indication for toxicity.

Table 5 shows toxicity equivalent concentration (TEC) of carcinogenic polyaromatic hydrocarbons (c-PAH) of waste engine oil polluted soil under varying pH adjustment. Toxicity equivalent (TEQ) at 1 WAP were given as 19.046 mg/kg (benzo(a)anthracene), 49.923 mg/kg (benzo(a)pyrene), 12.419 mg/kg (benzo(k)fluoranthene, 7.299(benzo(b)fluoranthene), 3.634 mg/kg (chrysene) and 2.942 mg/kg (Indeno(1, 2, 3 – cd)pyrene). These values were higher than their respective toxicity equivalent factors (TEF) which are toxicity potency factors used as a consistent method to evaluate the toxicities of variable mixture of organic compound (USEPA 1997). At pH 5, TEC values of PAH components ranged from 0.0841 – 4.072 mg/kg.

At 1 WAP TEC value of indeno(1,2,3–cd)pyrene was 2.9418 mg/kg compared to its corresponding TEF (0.1 mg/kg). At 3 MAP these values decreases to value lower than the TEF at pH 3, 5, and 9 (0.0314 – 0.0879 mg/kg). Total toxicity equivalent concentration (TTEC) were 43.657 mg/kg at pH 3, 8.1914 mg/kg at pH 5, 23.0085 mg/kg at pH 7, 11.9443 mg/kg at pH 9 and 35.9446 mg/kg at pH 11. Comparatively, TTEC was lowest at pH 5 followed by 9, 7, 11 and 3 respectively. However TTEC values at these pH ranges exceeded the method B clean up level for benzo(a)pyrene (0.137 mg/kg) proposed by Cal.EPA (2005), which are those soil clean up levels that have to be based on unrestricted land use. The implication is that the clean up level for benzo(a)pyrene was not met for these particular soil samples. The pH is not only essential for determining the availability of many soil nutrients but also in determining the fate of many soil pollutants, their breakdown and possible movement through the soil. Therefore, pH in the range of 4.9 – 5.1 might have implications on nutrient availability in the oil-polluted soils. Such pH ranges, for instance, might have affected the solubility of minerals (Feinstein et al., 1986; Marin et al., 2006). It is known that strongly acidic soils (pH 4–5) usually have high concentrations of soluble aluminium and manganese, which are toxic to many plants; nitrogen fixation and decomposition activities are also known to be hindered in strongly acidic soils (Alexander, 1969). These factors pu together significantly impact on microbial activity. The implication of a lowered nutrient composition of soil as well as other factors that are pH-dependent would invariably not favour microbial degradation of organic pollutants in the soil, particularly PAHs.

Table 6 shows total colony counts of bacteria and fungi obtained from waste engine oil polluted soil exposed to 3 months of soil pH adjustment. At 1 WAP, total bacteria count was 3.4×10^5 cfu/g. however, at 3 months later, total bacteria counts was 3.9×10^5 cfu/g. Total bacteria counts at 3 MAP increased to 7.3×10^5 cfu/g at pH 5. Total hydrocarbon degraders increased from 1.5×10^5 cfu/g at 1 WAP (pH 7), to 3.4×10^5 cfu/g at pH 5 during 3 MAP as against 2.8×10^5 cfu/g at pH 3 and 2.9×10^5 cfu/g at pH 9. Total bacteria counts at pH 11 were lowest compared to the control pH 7 (Table 6).

Table 5. Toxicity Equivalent Concentration (TEC) of soil as affected by pH adjustments for a period of 3 months after soil exposure to waste engine oil pollution

PAH (mg/kg)	1 WAP	3 MAP				
	pH 7	pH 3	pH 5	pH 7	pH 9	pH 11
Benzo(a)anthracene [0.1]	19.0466	2.1423	1.2700	3.5302	1.7769	4.4871
Benzo(a)pyrene [1.0]	49.9230	33.6229	4.0727	10.3167	6.3897	20.4295
Benzo(k)fluoranthene [0.1]	12.4189	5.1858	0.9002	8.3936	2.0766	6.2521
Benzo(b)fluoranthene [0.1]	7.2991	1.9063	1.5778	0.5277	1.0902	2.1771
Chrysene [0.01]	3.6338	0.7694	0.2881	0.1264	0.2257	1.2377
Indeno(1,2,3-c,d) pyrene [0.1]	2.9418	0.0314	0.0841	0.1263	0.0879	1.1206
Total TEC (TTEC)	95.2587	43.6574	8.1914	23.0085	11.9443	35.6946

TEF values of c-PAH are given in bracket

Table 6. Total colony counts of bacteria and fungi obtained from waste engine oil polluted soil exposed to 3 months of soil pH adjustments

	Soil pH	Bacteria counts ($x10^5$ cfu/g)	Fungal counts ($x10^5$ cfu/g)	Hydrocarbon bacteria Degraders Counts($x10^5$ cfu/g)	Hydrocarbon Fungal Degraders Counts ($x10^5$ cfu/g)	Percentage hydrocarbon degrading bacteria (%)	Percentage hydrocarbon degrading fungi (%)
1WAP	pH 7	3.4	5.2	1.7	2.8	50.00	53.85
3 MAP	pH 3	6.0	3.6	2.8	1.9	46.67	27.78
	pH 5	7.3	8.9	3.4	4.6	46.58	51.69
	pH 7	3.9	6.0	1.7	2.8	43.59	46.67
	pH 9	5.1	6.8	2.9	3.4	56.86	50.00
	pH 11	4.0	3.0	1.9	1.4	47.50	46.67

Total heterotrophic fungal count was 5.2 x 10^5 cfu/g at 1 WAP (pH 7), and then increased to 6.0 x 10^5 cfu/g 3 months later at the same pH. Total fungal count at pH 5 was 8.9 x 10^5 cfu/g compared to 6.8 x 10^5 cfu/g at pH 9 and 3.6 x 10^5 cfu/g at pH 3. Percentage hydrocarbon degrading fungi at 1 WAP was 53.85%. At 3 MAP, percentage hydrocarbon degrading fungi was 27.78% at pH 3, 51.69% at pH 5, 46.67% at pH 7, 50% at pH 9 and 46.67% at pH 11 (Table 6). A number of microorganisms, including a few of those indentified in the present study have been implicated in the bioremediation of PAHs. *Aspergillus niger* and *A. fumigatus* both metabolize terpenes and PAHs *A. niger* converts the terpene B- myrcene to dihydroxylated derivatives (Yamazaki et al., 1988), 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). Phananthrene is oxidized by *A. niger* to trans-dihydrodiols, phananthrol by *A. niger* to tran-dihydrodiols, phenanthrols and sulfate conjugates. *A. niger* is reported to produce l-methyoxy-phenanthrene (Sack et al., 1997) as well as a ring-cleavage product, protoctaechuate (Yogambal and Karegoudar, 1997).

At 1 WAP, *Micrococcus varians, Bacillus subtilis, Clostridium* sp*.,* and *Pseudomonas* sp*.* were bacteria species present (Table 7). They were all hydrocarbon degraders. Three months later, these bacteria species were also present at pH 5. *Pseudomonas* sp was absent at the pH extremes (pH 3 and pH 11). Ikhajiagbe and Anoliefo (2010) had earlier isolated *Achromobacter* sp, *Clostridium* sp, *Sarcina* sp *and Micrococcus* sp from oil-polluted soil at 5 MAP and 14 MAP.

Fungi species present included *Aspergillus niger, Trichoderma* sp*., Penicillium* sp*., Rhizopus* sp*.* at pH 9 (Table 7). *Aspergillus niger* and *Penicillium* sp were the hydrocarbon degraders present. At pH 5, *Aspergillus niger, Trichoderma* sp*.,* and *Penicillium* sp were present. Prevalent fungi species isolated from 5 – 14 month-old waste engine oil polluted soil were *Aspergillus niger, Penicillium* sp, *Geotrichum* sp, and *Trichoderma* sp*.* (Ikhajiagbe and Anoliefo, 2010).

Table 7. Microbial isolates from waste engine oil polluted soil exposed to 3 months of soil pH adjustments

		1 WAP	3 MAP				
		pH 7	pH 3	pH 5	pH 7	pH 9	pH 11
Bacteria	**Micrococcus varians*	+	+	+	-	+	+
	**Bacillus subtilis*	+	+	+	+	+	+
	Clostridium sp.	+	+	+	+	+	-
	**Pseudomonas* sp.	+	-	+	+	-	-
Fungi	**Aspergillus niger*	+	+	+	+	+	+
	Trichoderma sp.	-	-	+	-	+	-
	**Penicillium* sp	+	+	+	+	+	+
	Rhizopus sp.	-	-	-	+	+	-

**hydrocarbon degraders, + present, - absent*

Soil pH is important because most microbial species can survive only within a certain pH range. Furthermore, soil pH can affect availability of nutrients. Soil nutrient availability has also been previously linked with increased microbial activity (Ikhajiagbe, 2010; Ikhajiagbe and Anoliefo, 2010). Therefore, for enhanced remediation of soil PAH components, a pH range most favourable to enhance nutrient availability, and have concomitant effect on increased soil microbial activity, is key to successful PAH remediation. The present study

thus demonstrated that biodegradation of petroleum hydrocarbons was optimal when soil was irrigated with acidic solution of pH 5.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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