Annual Review & Research in Biology
3(2): 107-123, 2013

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Effects of Watering Regimes on the Intrinsic Qualities of Bioremediated Waste Engine Oil-Polluted Soil

B Ikhajiagbe[1*], G. O. Anoliefo[1], E. O. Oshomoh[1] and Blessing Agbonrienrien[2]

[1]Department of Plant Biol. and Biotechnology, University of Benin, Benin City, Nigeria.
[2]Department of Sci. Lab. Tech, University of Benin, Benin City, Nigeria.

Authors' contributions

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

Research Article

Received 26th January 2013
Accepted 7th March 2013
Published 1st April 2013

ABSTRACT

The present study investigated the effects of soil watering regimes on the intrinsic qualities of bioremediation of a waste engine oil-polluted soil. Five (5) kg of sun-dried top-soil was each placed into large perforated bowls and mixed thoroughly with waste engine oil (WEO) 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. Having previously determined the soil's water holding capacity to be 215 ml/kg soil, the entire setup was divided into 6 sets according to watering regimes. One set was irrigated with 1000 ml distilled water only once a week (1PW); the other sets were irrigated twice in a week (2PW), once a month (1PM), and the other twice a month (2PM). One set was deprived of moisture throughout the duration of the study (NWT), while the control experiment was carefully irrigated daily to saturation (CTRL). The entire experimental set up was left for three months in a well ventilated screen house with inherent room temperature range (28 – 30ºC). Results of the present study showed reductions in heavy metal contents, but in differing degrees. One week after pollution (WAP) concentration of Fe was 1097.34 mg/kg,

**Corresponding author: Email: ikhaj@yahoo.com;*

Mg was 18.4mg/kg, Cu was 5.63 mg/kg and Ni was 2.95 mg/kg. Total hydrocarbon content (THC) was 3425.63mg/kg. However, 3 months later when polluted soil was subjected to varying soil watering regimes, Fe in soil was 875.43mg/kg, Cu was 3.83 mg/kg and THC was 1095.54mg/kg respectively in the control, compared to values for Fe (687.3 mg/kg), Cu (3.83 mg/kg) and THC (445.45 mg/kg) respectively, when soil was wetted once a week. The contamination factor (CF) values presented for Fe, Mg, Cu, and Ni were all less than unity (CF < 1) an indication, that these heavy metals were remediated to levels below when soil was not yet exogenously polluted. There were reductions in polyaromatic hydrocarbon (PAH) contents of the soil. At one WAP total PAH was 923.90mg/kg as against 458.59 mg/kg 3 months later. When soil was wetted once a week, total PAH was 85.98mg/kg, 104.89 mg/kg when irrigated twice a week, 170.74 mg/kg when irrigated twice a month and 302.60mg/kg when soil receive no wetting at all. Comparatively total concentration of PAH was lowest at 1PW. Bacterial isolates of the oil polluted soil subjected to watering treatment once a week were *Bacillus subtilis*, *Pseudomonas* sp., and *Serratia marcescens,* whereas fungal species included *Aspergillus niger, Penicillium* sp. and *Trichoderma* sp.

Keywords: Bioremediation; heavy metals; intrinsic; moisture; natural attenuation; petroleum; polyaromatic hydrocarbons; waste engine oil; watering regimes.

1. INTRODUCTION

Oil exploration activities constitute a major source of environmental pollution. These environmental changes could be drastic and as such affect the ecosystem substantially. However, attention is gradually shifting to oil pollution resulting from activities of artisans including mechanics [1,2]. This has even become more important because soils in these non-oil exploration areas are constantly under threat from oil pollution resulting from the activities of these artisans. They change oil from motor vehicles, generators, and other machines, and dispose of the WEO improperly into gutters, water drains, open vacant plots and farmlands.

The fact that motor mechanic workshops, as well as workshops of other artisans, which use and dispose of WEO, occur in urban and rural areas, in bushes and open plots, means that gardens and farms in such areas are constantly under threat of pollution by WEO. The plots of lands are used temporarily as workshops while awaiting their full development [1]. The pollution resulting from WEO is much more devastating considering the fact that metals present in WEO are not necessarily the same as those present in the unused lubricants. Most heavy metals such as V, Pb, Al, Ni, and Fe, which were below detection in unused engine oil gave high mg/l values in used oil [3]. WEO also contain considerably higher concentrations of PAH [4].

Oil spills not only negatively impact on the ecosystem; they destroy farmlands, with detrimental impact on agricultural crops. Consequently, remediative measures, particularly bioremediation, become imperative; physical and chemical methods are not simple or favourable to the environment. The nature and amount of the pollutant present, the ambient and the seasonal environmental condition, and the constitution of the indigenous microbial community are factors that affect biodegradation of pollutants in the environment. Several methods have also been introduced to increase the rate of hydrocarbon biodegradation in the soil and they include oxygenation by excavation of the soil, microbial seeding [5,6] and

nutrient supplementation [7]. The effect of moisture on soil microbial activity is substantial and subject to intense research. Given the strong influence that water exerts on soil processes including microbial growth, and given the likely changes in precipitation patterns associated with climate change, the question arises as to how changes in soil water availability (water potential) will affect microbial growth efficiencies. More so, changes in soil water content could lead to changes in microbial growth efficiency. Stark and Firestone [8] also observed that a decrease in water availability may cause a change in the physiology of microbes as they adjust to more desiccating conditions. Microbial activity for optimum bioremediation of hydrocarbons requires 30 – 90% soil moisture [9]. Therefore, the aim of the present study was to determine the level of successful bioremediation of an oil-polluted soil when subjected to varying watering regimes.

2. MATERIALS AND METHODS

Top soil (0-10cm), of known physicochemical properties, was collected randomly from an area measuring 50 x 50m on a farmland situated near the Department of Plant Biology and Biotechnology Botanic Garden, University of Benin, Benin City, Nigeria. Thereafter, 5kg sun-dried soil was each placed into large perforated 10-litre bowls with 5 random perforations made with 2 mm diameter nails at the bottom of each bowl. Waste engine oil (WEO) 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. The buckets were transferred into a well ventilated screen house with inherent room temperature (28 – 30ºC). The entire set up was divided into 6 sets according to watering regimes. The soil's predetermined water holding capacity was 215 ml/kg soil; hence soil each bucket was irrigated with 1000 ml distilled water at different times; once a week (1PW), twice in a week (2PW), once a month (1PM), and twice a month (2PM). The fifth set was totally deprived of moisture throughout the duration of the study (NWT), while the control experiment was carefully irrigated daily to saturation (CTRL). The entire experimental set up was left for three months in a well ventilated screen house with inherent room temperature range of 28 – 30ºC; after which physiochemical parameters of soil were determined as well as microbial composition. Treatment units were replicated 5 times and completely randomized.

2.1 Soil Physicochemical Analyses

Soils were dried at ambient temperature (22-25ºC), 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 heavy metal fractions by atomic absorption spectrophotometry [10] as well as polyaromatic hydrocarbon contents (PAH) by Gas Chromatography [2].

2.2 Identification of Soil Microorganisms

The soil samples were air-dried and sieved through a 2 mm mesh to remove undesirable material. The dilution series for the soil sample was done by transferring 1 gram of the soil to nine (9 ml) millimeters of sterile distilled water in sterile glass containers as blank. The glass containers were shaken for 5 minutes and was taken as $l0^{-1}$ dilution factor, 10 ml were then transferred from the 10^{-1} dilution into another 9 ml blank to obtain a 10^{-2} dilution and same process of transfer was repeated twice to obtain a dilution factor of 10^{-4}.

2.3 Heterotrophic Bacterial and Fungal Counts

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

2.4 Isolation of Bacterial and Fungal Oil Degraders

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

2.5 Characterization and Identification of Bacterial Oil-degrading Isolates

The bacterial isolates that were predominantly isolated were identified to their species level using conventional microbiological and biochemical tests as described by Cowan and Steel [11] and Cheesebrough [12].

2.6 Characterization and Identification of Fungal Oil-degrading Isolates

The fungal isolates that were predominantly isolated were identified to their species level by colonial characteristic and microscopic examination of hyphal morphology as well as by structure and nature of the fruiting body.

2.7 Computation of Contamination Factor (CF)

CF expresses the ratio between the eventual concentrations of pollutant against its pre-industrial concentration.

$$CF = \frac{\text{Concentration of pollutant}}{\text{Pre-contamination Concentration}}$$

2.8 Computation of Hazard Quotient (HQ)

HQ expresses the possibility of the contaminant being an ecological risk or a contaminant of potential ecological concern. 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
Screening benchmarks are available at Efroymson *et al.* [13].

2.9 Computation of Probable Effect Concentration (PEC) Quotient of PAH Compounds

PECQ predicts the presence or absence of toxicity

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

PEC value for PAH components were obtained from Honeywell [14]. Where mean PECQ > PEC, toxicity is indicated.

2.10 Computation of Concentration 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 = ETi x TEF
Where TEQ = Toxic Equivalency
Ti = PAH concentration in soi
TEF = Toxic Equivalency factor

Analysis of variance in completely randomized design was done using the SPSS-15 statistical software, and means were separated by using the Least Significant Difference.

3. RESULTS AND DISCUSSIONS

Table 1. shows the physical and chemical properties of soil before waste engine oil contamination. Initial concentration of heavy metal on the soil before pollution, herein known as toxicity reference, show that Fe was 99.8mg/kg Mn was 16.11mg/kg Zn was 12.12mg/kg while total hydrocarbon content (THC) was 224.06 mg/kg. Cr and Cd were undetected or less than 0.001 mg/kg. Metals play an integral role in the life processes of microbes. Some metals, such as Cr, Ca, Mg, Mn, Cu, Na, Ni and Zn are essential as micronutrients for various metabolic activities. The present study thus showed the effects of differing watering regimes on the bioremediation of a waste engine oil-polluted soil. Generally, there were significant decreases in heavy metal contents of polluted soil; differences in the level of reductions were recorded.

At one week after pollution (WAP) concentration of heavy metals showed that Fe was 1097.34 mg/kg, Mg was 18.4mg/kg, Cu was 5.63 mg/kg, and Ni was 2.95 mg/kg. THC was 3425.63mg/kg. However, 3 months later when polluted soil was subjected to varying soil watering regimes, Fe in soil was 875.43mg/kg, Cu was 3.86 mg/kg and THC was 1095.54 mg/kg respectively in the control. However, when soil was wetted once a week, concentration of heavy metal was 687.3mg/kg for Fe, 3.83 mg/kg for Cu and 445.45 mg/kg for THC respectively, compared to 782.3 mg/kg for Fe, 4.85mg/kg of Cu and 987.43 mg/kg as THC when soil was not wetted at all (Table 2). Comparatively, total hydrocarbon content

of polluted soil was lowest while soil was wetted once a week (445.45mg/kg) compared to when soil was wetted daily to its water holding capacity (1095.54mg/kg). THC of soil was comparable for 1 PM (775.54mg/kg) and 2PM (805.9). Reductions in heavy metal contents were more in the treatments that were irrigated than in the water-starved treatments. This may result from metal dissolution in solution and eventual leaching. The presence of heavy metals in soil raises a lot of concerns, especially in their relationships with plant growth. Remediation of these metals and other organic pollutants becomes imperative. However, the control and optimization of bioremediation processes is a complex system involving many factors [9]. These factors include the existence of a microbial population capable of degrading the pollutants; the bioavailability of contaminants to microbial attack; the environmental factors contributing to microbial growth (type of soil, temperature, and soil pH, the presence of oxygen or other electron acceptors, and nutrient content). Bioremediation promotes the microbial metabolism of contaminants by adjusting the water, air and nutrient supply in the soil [15].

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

Parameters	Units	Soil
pH	-	6.11
Electrical Conductivity	µs/cm	301
Total Org. Matter	%	0.61
Total Nitrogen	%	0.12
Exchangeable Acidity	meq/100 g soil	0.22
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	%	7.9
Silt	%	13.9
Sand	%	78.2
Fe	mg/kg	998.8
Mn	mg/kg	16.71
Zn	mg/kg	12.12
Cu	mg/kg	4.98
Cr	mg/kg	2.08
Cd	mg/kg	N.D
Pb	mg/kg	N.D
Ni	mg/kg	3.60
V	mg/kg	0.76
Total Hydrocarbon Content	mg/kg	224.06

ND: Not determined (≤0.001 mg/kg)

Table 2. Heavy metals of soil 3 months after soil exposure to waste engine oil pollution and soil wetting regimes

		Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
		(mg/kg)									
1 WAP	**CTRL**	1097.34^{a}	18.4^{a}	16.4^{a}	5.63^{a}	2.83^{a}	1.42^{a}	1.03^{a}	2.95^{a}	3.55^{a}	3425.63^{a}
3 MAP	**CTRL**	875.43^{b}	10.1^{c}	12.4^{b}	3.86^{bc}	2.04^{b}	0.97^{ab}	0.76^{a}	1.93^{b}	2.72^{b}	1095.54^{b}
	1PW	687.3^{d}	11.5^{c}	11.9^{b}	3.83^{bc}	1.74^{b}	1.01^{ab}	0.81^{a}	1.86^{b}	2.54^{b}	445.45^{d}
	2PW	593.2^{d}	11.7^{bc}	12.3^{b}	3.41^{c}	1.83^{b}	0.87^{b}	0.77^{a}	1.73^{b}	2.46^{b}	702.80^{c}
	1PM	642.3^{d}	10.4^{c}	11.5^{b}	4.33^{bc}	2.16^{b}	0.91^{ab}	0.82^{a}	1.44^{b}	2.85^{b}	775.54^{c}
	2PM	682.1^{d}	12.3^{bc}	12.4^{b}	4.16^{bc}	2.07^{b}	0.77^{b}	0.87^{a}	1.84^{b}	2.63^{b}	805.90^{bc}
	NWT	782.3^{c}	16.1^{ab}	13.2^{b}	4.85^{b}	2.12^{b}	1.06^{ab}	0.92^{a}	1.87^{b}	2.49^{b}	987.43^{b}

Means along the same column with similar alphabetic superscripts do not differ significantly (p>0.05) from the other. WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all

Table 3. Contamination factor (CF) of soil 3 months after soil exposure to waste engine oil pollution and soil wetting regimes

		Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V	THC
Toxicity reference (mg/kg)		998.8	16.71	12.12	4.98	2.08	N.D	N.D	3.60	0.76	224.06
3 MAP	**CTRL**	0.8765	0.6044	1.0231	0.7751	0.9808	$>10^{3}$	$>10^{3}$	0.5361	3.5789	4.8895
	1PW	0.6881	0.6882	0.9818	0.7691	0.8365	$>10^{3}$	$>10^{3}$	0.5167	3.3421	1.9881
	2PW	0.5939	0.7002	1.0149	0.6847	0.8798	$>10^{3}$	$>10^{3}$	0.4806	3.2368	3.1367
	1PM	0.6431	0.6224	0.9488	0.8695	1.0385	$>10^{3}$	$>10^{3}$	0.400	3.7500	3.4613
	2PM	0.6829	0.7361	1.0231	0.8353	0.9952	$>10^{3}$	$>10^{3}$	0.5111	3.4605	3.5968
	NWT	0.7832	0.9635	1.0891	0.9739	1.0192	$>10^{3}$	$>10^{3}$	0.5194	3.2763	4.4070

ND not detected (≤ 0.001 mg/kg). WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all

The contamination factor (CF) in the present study provides that inherent concentration of the relationship between inherent concentration of heavy metal in the soil and the toxicity reference. When CF was less than 1 (CF < 1), the implication was that the inherent concentration of heavy metal due to exogenous concentration of waste engine oil in the present study was significantly ($p<0.05$) lower than when soil was not yet exogenously polluted. The implication being that remediation was significant (Table 3). CF values presented for Fe, Mg, Cu, and Ni were all less than one; an indication that these heavy metals were remediated to levels below the toxicity reference [2]. However, CF was greater than one (CF > 1) in Cd, V, and THC. Soil microorganisms can degrade organic contaminants, while metals need immobilization or physical removal. Although many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of free radicals. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function [16]. One of the ways by which microbial transformations of metals occur is either by reduction-oxidation conversions of inorganic forms or by conversions from inorganic to organic forms and *vice versa* [17]. Dissimilatory metal reduction is one of the ways by which reduction of metals can occur; by this process, microbes utilize metals as terminal electron acceptors for anaerobic respiration [18]. Microbes may possess reduction mechanisms that are not coupled to respiration, but instead are thought to impart metal resistance. Microbial reduction of heavy metals has also been achieved by biomethylation of these metals by a number of different bacterial species including *Pseudomonas* sp., *Escherichia* sp., *Bacillus* sp., and *Clostridium* sp. [19]. The present study thus isolated *Pseudomonas* sp., *Bacillus* sp., and *Clostridium* sp from polluted soils, and these organisms may have been involved in the methylation of Fe, Cr and Mn [20].

Microbial processes can also solubilize metals, thereby increasing their bioavailability and potential toxicity or immobilize them. This reduces the bioavailability of metals. This biotransformation is an important component of biogeochemical cycles of metals, and may be exploited in the bioremediation of metal contaminated soils [21].

Hazard Quotient (HQ) of soil after exposure to watering treatments indicate that the inherent concentration of heavy metals in soil compared to their toxicity benchmark values as presented by Efroymson et al. [13] (Table 3). Values for Fe, Cr, and V were greater than unity; the implication being that toxicity was indicated for these heavy metals, and as such was considered to posses concentrations of ecological concern. However, HQ was less than unity for Mg, Zn, Cu, Cd, Pb, and Ni; an indication that ecological toxicity was not indicated for these heavy metals. The present study also showed reductions in polyaromatic hydrocarbon contents of polluted soil, with the most results recorded for treatments that were irrigated only once a week, compared to the control experiment that received daily water treatment up to soil's water holding capacity (Table 4).

At 1 WAP total PAH was 923.90mg/kg as against 458.59 mg/kg 3 months later (Table 5). When soil was wetted once a week, total PAH was 85.98mg/kg, 104.89 mg/kg when irrigated twice a week, 170.74 mg/kg when irrigated twice a month and 302.60mg/kg when soil receive no wetting at all. There was total remediation of phenanthrene at 2PW, 1PM and 2PM. Comparatively total concentration of PAH was lowest at 1PW and highest when soil received water daily up to water holding capacity. Similarly reduction in PAH was better when soil received no wetting at all than when soil was irrigated daily.

Table 4. Hazard Quotient (HQ) of soil 3 months after soil exposure to waste engine oil pollution and soil wetting regimes

		Fe	Mn	Zn	Cu	Cr	Cd	Pb	Ni	V
Toxicity benchmark (mg/kg)		200.0	500.0	50.0	100.0	1.0	4.0	50.0	30.0	2.0
1 WAP	CTRL	50486	0.0368	0.0328	0.0563	2.83	0.355	0.206	0.0983	1.755
3 MAP	CTRL	4.3772	0.0202	0.248	0.0386	2.04	0.2450	0.0152	0.0643	1.360
	1PW	3.4372	0.0230	0.238	0.0383	1.74	0.0252	0.0162	0.0620	1.270
	2PW	2.966	0.2340	0.246	0.0341	1.83	0.2175	0.0154	0.0577	1.230
	1PM	3.2115	0.0208	0.230	0.0433	2.16	0.2275	0.0164	0.0480	1.425
	2PM	3.4105	0.0246	0.248	0.0416	2.07	0.1295	0.0174	0.0623	1.315
	NWT	3.9115	0.0322	0.264	0.0850	2.12	0.2650	0.0184	0.0623	1.245

WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all

Table 5. Polyaromatic hydrocarbon content (mg/kg) of soil after exposure to waste engine oil pollution and varying soil wetting regimes

PAH component	**1 WAP**	**3 MAP**					
	CTRL	**CTRL**	**1PW**	**2PW**	**1PM**	**2PM**	**NWT**
Naphthalene	0.5173	1.1726	0.9392	1.2441	1.8556	6.0100	2.2769
Acenaphthylene	0.1670	0.6211	0.2713	0.8008	0.4611	2.9079	0.7338
Acenaphthene	0.4560	0.7478	0.2893	0.8619	0.7503	0.3504	0.6249
Fluorene	0.3578	0.8742	0.2206	0.4132	0.4042	0	0.2739
Phenanthrene	1.5357	2.6880	0.6038	0	0	0	1.2979
Anthracene	4.7538	10.9662	0.4320	0.8956	1.2115	1.0061	2.8531
Fluoranthene	6.5246	16.1162	1.7320	3.5919	0.7126	4.4505	1.7805
Pyrene	40.3273	49.5007	0.6627	2.9168	1.9140	1.3192	8.9212
Benz(a)anthracene	190.4658	37.5742	7.9957	7.8765	14.2893	30.3393	81.9616
Chrysene	363.3801	99.7552	11.4059	1.6872	32.9586	21.9468	117.3508
Benzo(b)fluoranthene	72.9912	33.6508	6.5838	15.0921	25.3561	11.1703	11.8153
Benzo(k)fluoranthene	124.1892	133.1705	41.3429	58.6802	55.6353	68.7746	28.2508
Benzo(a)pyrene	49.9230	29.1114	7.6216	7.5029	21.5930	16.1446	20.9390
Indeno(1,2,3-cd)pyrene	29.4184	25.7125	1.2667	2.2075	1.07195	3.4236	3.8242
Benzo(g,h,i)perylene	38.8968	16.9238	4.6165	1.1242	2.2526	2.8947	19.7000
TOTAL	**923.9040**	**458.5852**	**85.9840**	**104.8949**	**160.4662**	**170.738**	**302.6039**

WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all.

Chromatographs showing PAH concentrations of soil are presented in Fig. 1. Aprill and Sims [22] noted that the order of removal among the PAHs correlated with the water solubility of the compound. That is, the most water soluble PAHs showed the greatest extent of degradation in the rhizosphere. According to Vidali [9], condition required for microbial activity is 25-28% of its water holding capacity, whereas optimum value for hydrocarbon degradation is 30 – 90% soil moisture.

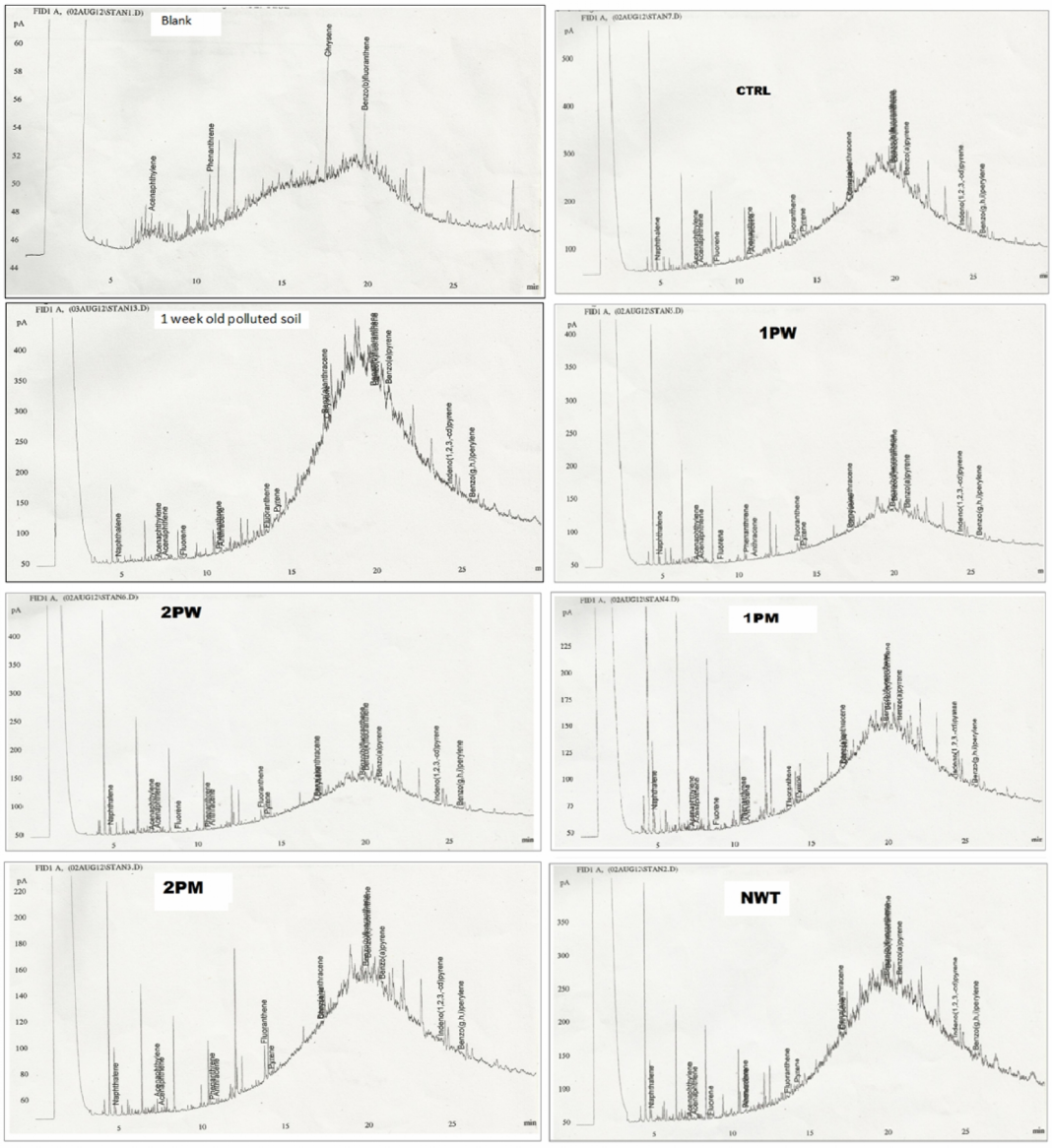

Fig. 1. Chromatograph showing polyaromatic hydrocarbon contents of waste engine oil-polluted soil at one week after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all

Probable Effect Concentration Quotient (PECQ) of PAH content of the soil are presented on Table 6. Result showed that PECQ was greater than 1 (> 1) an indication that poly aromatic content of soil were greater than their respective PECQ values as indicated on Table 6. Generally, the mean PECQ was greater than the respective PECQ of the individual PAH components, the implication being that toxicity was indicated for each of the PAH component. However, for phenanthrene at 2PW, 2PM, and 1PM and fluorene at 2PM, no toxicity was indicated because they were already totally remediated. Total toxicity equivalent concentration was lowest in 1 PW treatment (13.45 mg/kg) compared to the control experiment at 3 MAP (53.12 mg/kg) (Table 7). Many microorganisms grow abundantly in soil-contaminated by petroleum residues [6,23,24]. However, soil moisture influences the rate of contaminant metabolism. This is because it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems. The amount of water in the pore spaces of soil also affects the exchange of oxygen. Under saturated conditions, oxygen can be consumed faster than it is replenished in the soil vapor space and the soil can become anaerobic. This can reduce microbial activity and consequently retard the rate of biodegradation. At very low concentrations hydrocarbons are soluble in water, but most oil spill incidents release petroleum hydrocarbons in concentrations far in excess of the solubility limits [25]. The degree of spreading determines in part the surface area of oil available for microbial colonization by hydrocarbon-degrading microorganisms; in aquatic systems, the oil normally spreads, forming a thin slick [26]. The degree of spreading is reduced at low temperatures because of the viscosity of the oil. In soils, petroleum hydrocarbons are absorbed by plants matter and soil particles, limiting its spreading. Hydrocarbon-degrading microorganisms act mainly at the oil-water interface. These microorganisms can be observed growing over the entire surface of an oil droplet; growth does not appear to occur within oil droplets in the absence of entrained water. Availability of increased surface area should accelerate biodegradation. Not only is the oil made more readily available to microorganisms, but movement of emulsion droplets through a water column makes oxygen and nutrients more readily available to microorganisms.

Aspergillus niger and *A. fumigatus* both metabolize terpenes and PAHs *A. niger* converts the terpene B - myrcene to dihydroxylated derivatives [27], and there is even a report of the ability of *A. niger* to cleave the rings of naphthalene, anthrcene, and Phenanthrene [28]. 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 I-methyoxy - phenanthrene [29] as well as a ring-cleavage product, protoctaechuate [28]. Walker et al.[30] compared the abilities of bacteria and fungi to degrade hydrocarbons. In the present study, there appeared to more bacteria than fungi in similar treatment levels. The ability of certain microorganisms to degrade petroleum seems to be an adaptive process and is governed by environmental conditions. The presence of petroleum may also affect the microbial community through selection of species.

Table 6. Probable Effect Concentration (PEC) of PAH contents (mg/kg) of soil after exposure to waste engine oil pollution and varying soil wetting regimes

PAH component	1 WAP	3 MAP					
	CTRL	CTRL	1PW	2PW	1PM	2PM	NWT
Naphthalene [0.917]	0.5641	1.2788	1.0242	1.3568	2.0236	6.5540	2.4830
Acenaphthylene [1.301]	0.1284	4.7739	2.0853	6.1551	3.5439	2.2351	0.5640
Acenaphthene [0.861]	0.1296	0.8684	0.3369	1.0011	8.7143	0.7259	0. 7259
Fluorene [0.264]	1.3553	3.3115	0.8354	1.5652	1.5309	0	1.0375
Phenanthrene [0.543]	2.8282	4.9503	1.1119	0	0	0	2.3902
Anthracene [0.207]	22.9652	22.9769	2.0869	4.3265	5.826	4.8606	13.7831
Fluoranthene [1.436]	4.5436	11.2229	1.2062	2.5013	0.4962	3.0992	1.2399
Pyrene [0.344]	117.2305	143.8974	1.9263	8.4791	5.5640	3.8349	25.9337
Benzo(a)anthracene [0.192]	992.0094	109.5699	41.6444	41.0232	74.4229	158.0169	402.6884
Chrysene [0.253]	143.6285	304.2894	45.0828	6.6686	130.2709	86.7460	406.3837
Benzo (b) Fluranthene [0.908]	80.3868	37.0603	7.2509	16.6212	27.9252	12.3022	13.0124
Benzo(k)Fluranthene [0.203]	611.7695	656.0123	203.6599	289.0649	274.0656	338.7913	138.3349
Benzo(a)pyrene [0.146]	0.3419	199.3929	52.2027	51.3899	147.8975	110.5794	143.4181
Indeno(1,2,3-cd)pyrene [0.183]	160.7563	31.2158	6.9219	12.631	5.8576	18.7079	20.8969
Benzo (g,h,i) perylene [0.708]	54.9400	23.9036	6.5205	1.5879	3.1816	4.0886	27.8248
Mean PECQ	146.2385	103.6483	24.92641	29.62479	46.08801	50.03613	85.7136

Values provided in brackets are PEC values associated with the PAH components [14]. WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all

Table 7. Toxicity Equivalent Concentration (TEC) of PAH contents (mg/kg) of soil after exposure to waste engine oil pollution and different soil wetting regimes

PAH (mg/kg)	1 WAP	3 MAP					
	CTRL	CTRL	1PW	2PW	1PM	2PM	NWT
Benzo (a) anthracene [0.1]	19.0466	3.7574	0.7995	0.7876	1.4289	3.0339	8.1962
Benzo (a) pyrene [1.0]	49.9230	29.1114	7.6216	7.5029	21.5930	16.1446	20.9390
Benzo (k) fluoranthene [0.1]	12.4189	13.3171	4.1343	5.8680	5.5635	6.8775	2.8251
Benzo (b) fluoranthene [0.1]	7.2991	3.3651	0.6584	1.5092	2.5356	1.1170	1.1815
Chrysene [0.01]	3.6338	0.9976	0.1141	0.0169	0.3296	0.2194	1.1735
Indeno(1,2,3-c,d) pyrene [0.1]	2.9418	2.5712	0.1267	0.2208	0.1072	0.3424	0.3824
Total TEC (TTEC)	95.2587	53.1198	13.4546	14.3962	31.5578	27.7348	34.6977

TEF values of c-PAH are given in bracket [31]. WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control),1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all

Table 8. Microbial isolates from waste engine oil polluted soil exposed to 3 months of soil wetting regimes

		1 WAP	3 MAP					
		CTRL	CTRL	1PW	2PW	1PM	2PM	NWT
Bacteria	**Micrococcus varians*	+	-	-	+	+	+	+
	**Bacillus subtilis*	+	+	+	+	+	+	+
	**Clostridium* sp.	+	+	-	+	-	-	-
	**Pseudomonas* sp.	+	-	+	-	+	+	-
	Pseudomonas aeruginosa	-	+	-	+	-	-	+
	Serratia marcescens	-	-	+	-	+	+	+
Fungi	**Aspergillus niger*	+	-	+	+	-	+	-
	**Aspergillus flavus*	-	+	-	+	+	-	+
	**Penicillium* sp	+	-	+	-	+	+	+
	**Fusarium solani*	-	+	-	+	-	+	-
	Trichoderma sp.	-	+	+	-	-	-	-

**hydrocarbon degraders. WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all*

Table 9. Total colony counts of bacteria and fungi obtained from waste engine oil polluted soil exposed to 3 months of soil wetting regimes

		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 (%)
1 WAP	**CTRL**	3.4	5.2	1.7	2.8	50.00	53.85
3 MAP	**CTRL**	6.3	3.8	1.8	1.2	28.57	31.58
	1PW	7.8	3.1	2.6	1.1	33.33	35.48
	2PW	7.0	2.5	3.2	0.3	45.71	12.00
	1PM	5.8	3.8	2.3	1.3	39.66	34.21
	2PM	5.2	6.2	2.0	1.9	38.46	36.54
	NWT	5.7	3.4	2.1	2.1	30.00	61.76

**hydrocarbon degraders, + present, - absent. WAP Weeks after pollution, MAP months after pollution. CRTL Normal daily wetting (Control), 1PW wetting once in a week, 2PW wetting twice in a week, 1PM wetting once in a month, 2PM wetting twice in a month, NWT no wetting at all*

At 3 months after pollution and treatments application, bacteria isolated from soil irrigated once a week included *Bacillus subtilis, Pseudomonas* sp. and *Serratia marcescens*, where as fungi species included *Aspergilus niger, Penicillum* sp, *Trichoderma* sp (Table 8). Total fungi count in the control was 3.8 x 10^5 cfu/g, compared to 6.2 x 10^5cfu/g when soil was irrigated twice a month (Table 9). It was also observed that of the 5 fungal species isolated from the treated soils, *Penicillium* sp. appeared to be most predominant, occurring nearly in all the treatments. The finding of the presence of higher oil-degrading bacterial populations in contaminated soils corroborates the results of Hubert *et al*. [32] and Michalcewicz [33] that attributed these high microbial populations to the stimulatory effect of additional carbon and energy source in the form of lubricating oil. Oil degraders isolated by Akoachere *et al.* [34] included *Pseudomonas fluorescens, Bacillus mycoides* and *Serratia marcescens*. Nkwelang *et al.* [35] identified *Pseudomonas, Bacillus* and *Acinetobacter* as the major genera of bacteria active in polluted soil.

4. CONCLUSION

Although moisture is important in microbial bioremediation of contaminants, it is however important that water logging of soils may hamper the rate of successful remediation. The metabolism of contaminants is influenced by soil moisture. Therefore, soil moisture of between 25-28% of the water holding capacity is usually required for optimal microbial activity that would eventually amount to successful microbial bioremediation of contaminants [9] is optimal for biodegradation. The present study thus showed that for successful bioremediation of oil-polluted soil, the most successful watering regime is irrigation of soil up to soil's water holding capacity for just once a week.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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