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Remediation of used lubricating oil contaminated soil using organic waste amendments

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ABSTRACT

Contamination of soil by used lubricating oil is more prominent in developing countries. This poses a serious threat to the flora and fauna in the environment. Soil contaminated with 5%, 10% and 15% (w/w) used lubricating oil was amended with organic wastes [banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC)]. The study was conducted under natural conditions for 12 months to determine the effects of each organic waste on biodegradation of used lubricating oil. GC/FID results of the oil extracts at the end of 12 months showed complete degradation below detection limit for C_7 to C_{14} hydrocarbon fractions, in all the organic wastes amended treatments for 5% and 10% oil pollution. C_7 to C_{14} hydrocarbon fractions were not completely degraded in unamended soil and all treatments with 15% oil pollution, except BSG amended soil. BSG amended soil recorded better degradation of C_{29} to C_{36} fractions from 6871 mg/kg to 800 mg/kg, compared to BS and SMC treated soil. Complete degradation of fluorene, phenanthrene, anthracene and pyrene below detection limits were recorded in BSG treated soil after 12 months in 5%, 10% and 15% oil pollution. The results of this study attest to the potential of BSG in enhancing oil biodegradation in soil. Hence, BSG can serve as a good candidate for promoting biodegradation of used lubricating oil in soil environment. © 2012 Trade Science Inc. - INDIA

KEYWORDS

Used lubricating oil;
Organic wastes;
Contamination;
Hydrocarbon;
Biodegradation.

INTRODUCTION

Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. The presence of various kinds of automobiles, vehicles and machinery has caused an increase in the use of motor oil. Oil spillages into the environment have become one of the major problems. Spillage of used motor oils such as diesel or jet fuel contaminates our natural environment with hydrocarbons[1]. Hydrocarbon contamination of the air, soil, freshwater (surface water and groundwater), especially by polyaromatic hydrocarbons (PAHs) has drawn public concern because many PAHs are toxic, mutagenic, and

carcinogenic[2-4].

The illegal dumping of used motor oil is an environmental hazard with global ramifications[5].The release of oil into the environment causes environmental concerns and attracts public attention[6].Used lubricating oil contains several toxic components including up to 30% aromatic hydrocarbons, with as much as 22 ppm benzo (a)pyrene (a PAH). PAHs have a widespread occurrence in various ecosystems that contribute to the persistence of these compounds in the environment[7]. Prolonged exposure to high oil concentration may cause the development of liver or kidney diseases, possible damage to the bone marrow and an increased risk of cancer[8-.9]. Chronic effects of naphthalene, a constituent in used motor oil, include changes in the liver and harmful effects on the kidneys, heart, lungs, and nervous system. Due to their relative persistence and potential for various chronic effects (like carcinogenicity), PAHs (and particularly the alkyl PAHs) can contribute to long term (chronic) hazards.

Remediation of petroleum contaminated systems could be achieved by either physicochemical or biological methods. However, the attendant negative consequences of the physicochemical approach are currently directing greater attention to the exploitation of the biological alternatives[10]. Bioremediation of petroleum hydrocarbon contaminated soils has been recognized as an efficient, economic, versatile, and environmentally sound treatment[11]. Harder[12] estimated that bioremediation accounts for 5 to 10 percent of all pollution treatment and has been used successfully in cleaning up the illegal dumping of used engine oil. Factors such as nutrients may limit the rate of petroleum hydrocarbon degradation. The addition of inorganic or organic nitrogen rich nutrients (biostimulation) is seen as an effective approach to enhance the bioremediation process[13-15].with positive effects of N amendment on microbial activity. Therefore, the objectives of this study are to determine the effects of organic wastes amendments on biodegradation of hydrocarbon fractions (aromatic and aliphatic) in used lubricating oil. It also aims to study the effects of oil concentration on biodegradation of used lubricating oil, within the period of 12 months.

MATERIALS AND METHODS

Collection of samples

The soil samples used for the bioremediation study were collected in a sack from the nursery section of the Asia-Europe Institute, University of Malaya, Kuala Lumpur and was transported to the laboratory for analysis. Used lubricating oil was collected from a Perodua car service centre in Petaling Jaya, while the organic wastes were collected from different locations; banana skin (BS) was collected from the Institute of Postgraduate Studies (IPS) canteen, University of Malaya, brewery spent grains (BSG) was collected from Carlsberg brewery, Shah Alam, Selangor and spent mushroom compost (SMC) was collected from Ganofarm Sdn Bhd, Tanjung Sepat, Selangor.

Soil preparation

The soil was air-dried and passed through a 2 mm sieve to remove stones, root materials and other debris. The air-dried, sieved soil samples were polluted with three different concentrations of used lubricating oil: 5%, 10%, 15% (w/w) and thoroughly mixed. The oil contaminated soils were amended with 10% (w/w) of different organic wastes: banana skin (BS), brewery spent grain (BSG) and spent mushroom compost (SMC). After thorough mixing of the oil contaminated soil with the organic wastes; 1.5 kg each of the soil were packed into polythene plastic bags and set up at the experimentation site, exposed to sunlight and rainfall for the period of twelve months. Two different control experiments were set up. One (control) was oil contaminated soil without organic wastes amendment. The second (control) did not contain organic wastes but the soil used was autoclaved and mixed with 0.5% (w/w) sodium azide to determine loss of oil due to non-biological factor.

Replicate samples were withdrawn from each treatment, every three months, throughout the twelve month period of the experiment, for the analysis of petroleum hydrocarbon loss, polycyclic aromatic hydrocarbon and enumeration of hydrocarbon utilizing bacteria.

Physicochemical analysis of soil and organic wastes

The nitrogen content of soil used for bioremediation

and the organic wastes were determined using the Kjeldahl method, while P and organic C contents were determined using ICP-OES and the furnace method, respectively. pH was determined with a pH meter (HANNA HI 8424) on 1:2.5 (w/v) soil/distilled water after 30 minutes equilibration. Triplicate determinations were made.

Measurement of oil biodegradation

The extent of used lubricating oil biodegradation in soil was determined by suspending 10 g of soil (dried with 10 g of anhydrous sodium sulphate) in 20 ml of n-hexane and dichloromethane (80:20) in a 250 ml capacity Erlenmeyer flask. After shaking for 1 hour on an orbital shaker (model N-Biotek-101), the solvent – oil mixture was filtered using Whatman No. 4 filter paper into a 100 ml Florentine flask. The solvent was removed using a rotary evaporator, followed by analysis of the residual oil. One microlitre of the extracted oil (cleaned with HyperSep SPE) sample was analyzed using gas chromatography with a flame ionization detector (GC/FID). The GC was equipped with cross-linked 5% phenyl methyl siloxane capillary column; HP-5MS. Helium was used as a carrier gas. The oven temperature was started at 50 ^{0}C and raised by 25^{0}C/min until 325 ^{0}C, which was maintained for 11 minutes. The major hydrocarbon fractions were identified and quantified on the basis of their retention time and by comparing them to those of analytical standards.

Enumeration and identification of bacteria

Three replicate samples from each oil polluted soil were withdrawn every 3 months for the enumeration of hydrocarbon utilizing bacteria (HUB). 0.1 ml of serially diluted samples were plated on oil agar prepared from mineral salt medium of Zajic and Supplisson[16].(1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4 \cdot 7H_2O$, 1.2 g KH_2PO_4, 0.01 g $FeSO_4 \cdot 7H_2O$, 0.1 g NaCl, 20 g agar, 1% used lubricating oil in 1000ml distilled water, pH 7·4). Triplicate plates were incubated at 30^{0}C for 5 days before the colonies were counted and randomly picked, pure isolates were obtained by repeated sub-culturing on nutrient agar (Oxoid). The bacterial isolates were characterized using microscopic techniques and biochemical tests and further confirmed by using API 20NE for Gram negative bacteria, and BBL Crystal rapid identification kit for Gram positive bacteria. For Gram positive bacterial identification, colonies of pure culture of bacteria were introduced into the BBL inoculums fluid with the aid of sterile wire loop and vortexed for 10 – 15 seconds. The turbidity was adjusted to the equivalence of McFarland No. 0.5 standard and the entire inoculum was poured into the BBL base that contained different wells. The inoculum was gently rolled with both hands to ensure that all the wells were filled. The wells containing the inoculums were later covered with the BBL lid that contained 29 dehydrated biochemical and enzymatic substrates and a fluorescence control on the tips of plastic prongs. The inoculated panels were incubated for 18 – 24 hours at 35 – 37^{0}C, at the end of the incubation period the wells were examined for colour change or presence of fluorescence that might have resulted from the metabolic activities of the microorganisms. The resulting patterns of the 29 reactions were converted into a ten digit profile number that was used as the basis for identification. The resulting profile number derived from different colour changes and cell morphology were entered into a PC in which the BBL Crystal mind software had been installed to obtain the bacterial identification.

Gram negative bacterial isolates were identified using API 20 NE. Pure culture colonies of bacterial samples were transferred into an ampoule of API NaCl 0.85% medium (2 ml) with the aid of an inoculating wire loop to prepare a suspension with a turbidity equivalent to 0.5 McFarland standard. Tests NO_3 to PNPG in the API panel were inoculated by distributing the saline suspension into the tubes using sterile pipettes. 200 µl of the remaining suspension was added into an ampoule of API AUX medium and homogenized. The cupules tests GLU to PAC were filled with the suspension from API AUX medium followed by an addition of mineral oil to the test cupules labeled GLU, ADH and URE until a convex meniscus was formed. The incubation box was closed and incubated at 29^{0}C ± 2^{0}C. At the end of the incubation period, the results were read based on colour changes and converted into a numerical profile. The identification was performed by using the database (V7.0) with the analytical profile index which had been installed into the PC earlier.

RESULT AND DISCUSSION

Physicochemical properties of soil and organic wastes

The soil sample used for bioremediation had a low N content (0.4%) while brewery spent grain (BSG) recorded an appreciable N content (1.02%) compared to banana skin (BS) (0.4%) and spent mushroom compost (SMC) (0.5%). The pH of the soil was slightly acidic in nature at pH 6.12. The soil used for bioremediation had C:N ratio of 25.7, which was a low C:N ratio for effective biodegradation of oil in the soil; hence, the need for the addition of organic wastes as a source of N and P. BSG had the highest N content among the three organic wastes used. This is one of the most important limiting nutrients for effective bioremediation to take place[10,17].The moisture content of BSG (71.8%) was also higher than those of BS (38.5%) and (62.3%). This might enable the BSG to harbor some important microorganisms that will contribute positively to the biodegradation of oil in the soil. The pH of SMC (5.6) was slightly acidic. The reason for this might be because it was used to grow fungi (mushroom) which grows better in an acidic environment. Therefore, the initial substrate of SMC might have been slightly acidic in nature.

Biodegradation of hydrocarbon fractions

Biodegradation of hydrocarbon fractions present in the used lubricating oil was determined at three months intervals for a period of 12 months, to determine the extent of biodegradation of different hydrocarbon fractions using GC/FID. The hydrocarbon fractions were divided into four fractions which are: $C_7 - C_9$, $C_{10} - C_{14}$, $C_{15} - C_{28}$ and $C_{29} - C_{36}$.

Biodegradation of $C_7 - C_9$ fractions in used lubricating oil

TABLE 1 shows the extent of biodegradation of $C_7 - C_9$ hydrocarbon fractions in soil contaminated with 5%, 10% and 15% used lubricating oil, amended with different organic wastes for a period of 12 months. The results of soil contaminated with 5% of used lubricating oil revealed complete biodegradation (below the detection limit) of the petroleum fractions from the initial concentration of 88 mg/kg within the period of 3 months in soil amended with BS, BSG and SMC. Whereas, it took 6 months before the $C_7 - C_9$ fractions were degraded below the detection limit in unamended and sterile polluted soil. In soil contaminated with 10% used lubricating oil, the petroleum fraction ($C_7 - C_9$) was degraded below the detection limit within the first 6 months in soil amended with BS and SMC, whereas in BSG amended soil the time for degradation below detection limit was within the first 3 months of the study (proving the effectiveness of BSG compared to other organic wastes). The loss of $C_7 - C_9$ below detection limit in unamended and sterile soil polluted with 10% used lubricating oil took 9 months. The results of soil contaminated with 15% used lubricating oil showed complete degradation of $C_7 - C_9$ hydrocarbon fraction in BSG amended soil within the first 6 months whereas degradation below detection level was achieved within 9 months in soil amended with BS, SMC and unamended polluted soil, while those of sterile soil was achieved in the 12th month. The rapid biodegradation of hydrocarbon fractions between $C_7 - C_9$ in all the different treatments to levels below detection limits might be due to the volatility of some of those fractions and the ease of their breakdown (due to their simple molecular structures) by bacteria present in the contaminated soil[18-20].

TABLE 1 : Concentration (mg/kg) of C_7 - C_9 fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

Treatment	Time (months)				
	0	3	6	9	12
Soil+5%oil+BS	88	ND	ND	ND	ND
Soil+5%oil+BSG	88	ND	ND	ND	ND
Soil+5%oil+SMC	88	ND	ND	ND	ND
Soil+5% oil only	88	58	ND	ND	ND
Sterile soil+5%oil	88	67	ND	ND	ND
Soil+10%oil+BS	136	61	ND	ND	ND
Soil+10%oil+BSG	136	ND	ND	ND	ND
Soil+10%oil+SMC	136	52	ND	ND	ND
Soil+10% oil only	136	87	58	ND	ND
Sterile soil+10%oil	136	92	61	ND	ND
Soil+15%oil+BS	206	145	98	ND	ND
Soil+15%oil+BSG	206	63	ND	ND	ND
Soil+15%oil+SMC	206	148	86	ND	ND
Soil+15% oil only	206	174	109	ND	ND
Sterile soil+15%oil	206	185	123	67	ND

ND: Not detected at lowest detection limit of 50 mg/kg

The soil amended with organic wastes, mostly BSG, showed better and faster degradation of the fractions. The reason for this might be due to its positive effects on hydrocarbon degrading bacteria which enhanced their multiplication, thereby increasing the rate of hydrocarbon degradation. This is similar to the study by Ijah and Antai[21] .who reported complete degradation of C_7 to C_{12} fractions within 3 months in soil contaminated with 10% crude oil.

Biodegradation of $C_{10} - C_{14}$ fractions in used lubricating oil

TABLE 2 shows the results of biodegradation of $C_{10} - C_{14}$ hydrocarbon fractions in used lubricating oil-contaminated soil after 12 months. 5% oil-contaminated soil amended with BS, BSG and SMC recorded complete degradation of the hydrocarbon fractions below the detection limit within 6 months compared to those of $C_7 - C_9$ fractions which took only 3 months for the complete degradation in amended soil. There was no complete degradation of the fraction ($C_{10} - C_{14}$) in the sterile polluted soil throughout the 12 month period, while complete degradation was achieved within 9 months in the unamended polluted soil. Soil contaminated with 10% used lubricating oil recorded oil biodegradation below detection limit in soil amended with BSG within the 9th month, whereas that of BS amended soil extended to the 12th month, while complete degradation below the detection limit was not achieved in soil amended with SMC, unamended and sterile polluted soil throughout the 12 month period. The sterile polluted soil at had residual $C_{10} - C_{14}$ fractions of 114 mg/kg in the 12th month.

TABLE 2 : Concentration (mg/kg) of C_{10} - C_{14} fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

Treatment	Time (Months)				
	0	3	6	9	12
Soil+5%oil+BS	139	83	ND	ND	ND
Soil+5%oil+BSG	139	62	ND	ND	ND
Soil+5%oil+SMC	139	91	ND	ND	ND
Soil+5% oil only	139	106	67	ND	ND
Sterile soil+5%oi	139	118	82	64	58
Soil+10%oil+BS	184	139	103	78	ND
Soil+10%oil+BSG	184	117	92	ND	ND
Soil+10%oil+SMC	184	144	112	92	59
Soil+10% oil only	184	156	128	103	64
Sterile soil+10%oil	184	172	154	133	114
Soil+15%oil+BS	242	186	127	76	ND
Soil+15%oil+BSG	242	164	109	61	ND
Soil+15%oil+SMC	242	191	138	92	67
Soil+15% oil only	242	190	156	117	95
Sterile soil+15%oil	242	217	189	153	135

ND: Not detected at lowest detection limit of 50mg/kg

In soil contaminated with 15% used lubricating oil, complete biodegradation below the detection level was only achieved in soil amended with BS and BSG in the 12th month while complete degradation was not achieved in contaminated soil amended with SMC and those of unamended and sterile polluted soil throughout the 12 month study period. The rapid biodegradation of $C_{10} - C_{14}$ fractions has been reported to be among the most rapidly biodegraded components of oil, although they are also susceptible to removal by extensive water washing. Empirically, the first sign of biodegradation is usually n-alkane in the C_{10} to C_{13} range, which probably reflects an optimal carbon number with increasing enthalpy of reaction and decreasing water solubility as the alkane carbon number increases[22].

The results, like those of $C_7 - C_9$ reveal the effectiveness of BSG to effect complete degradation of $C_{10} - C_{14}$ fractions at all the different levels of pollution. This pointed out its ability to stimulate the indigenous bacteria in degrading the hydrocarbon fractions due to its nutrient composition. The results are similar to those reported by Ijah and Antai,[21].who discovered that C_{14} fraction was completely degraded in soil contaminated with 10% crude oil within the period of 12 months. Chang et al.,[23]. also reported substantial degradation of C_{10} to C_{16} hydrocarbon fractions in aged petroleum hydrocarbon-contaminated soil.

Biodegradation of $C_{15} - C_{28}$ fractions in used lubricating oil

The results of biodegradation of $C_{15} - C_{28}$ hydrocarbon fractions in the soil contaminated with 5%, 10% and 15% used lubricating oil and amended with different organic wastes are shown in TABLE 3. The results show that $C_{15} - C_{28}$ hydrocarbon fractions were not degraded below the detection limit in all the treatments. However, the degree of biodegradation varied greatly based on the percentage of oil pollution and organic waste amendments. The reason for incomplete bio-

degradation of these hydrocarbon fractions below detection limit may be due to their complex structural nomenclature, which always poses some significant difficulty to hydrocarbon utilizing bacteria for their complete biodegradation[24].In soil contaminated with 5% oil, BSG amended soil recorded highest biodegradation of $C_{15} - C_{28}$ hydrocarbon fractions from the initial concentration of 3810 mg/kg to 296 mg/kg after 12 months of study. The unamended polluted soil recorded reduction in the hydrocarbon fraction from 3810 mg/kg to 966 mg/kg after 12 months. Studies with soil contaminated with 10% and 15% oil pollution also revealed BSG amended soil, as the best treatment where the oil fractions were reduced from 8150 mg/kg to 676 mg/kg in 10% pollution and from 11341 mg/kg to 1260 mg/kg in 15% oil pollution. The unamended polluted soil and sterile polluted soil recorded very low biodegradation of the $C_{15} - C_{28}$ fractions throughout the 12 month period in soil contaminated with 10 and 15% used lubricating oil. The increase in the biodegradation of $C_{15} - C_{28}$ fractions in soil amended with organic wastes might be due to nutrient composition of the organic wastes, especially BSG. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants, especially N, P and in some cases Fe[10].Depending on the nature of the impacted environment, some of these nutrients could become limiting, hence the addition of nutrients are necessary to enhance the biodegradation of oil pollutants[17,25].

TABLE 3 : Concentration (mg/kg) of $C_{15} - C_{28}$ fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

Treatment	Time (months)				
	0	3	6	9	12
Soil+5%oil+BS	3810	2122	1760	1322	968
Soil+5%oil+BSG	3810	1235	348	321	296
Soil+5%oil+SMC	3810	2231	1750	1428	974
Soil+5% oil only	3810	3601	3510	2161	966
Sterile soil+5%oil	3810	3783	3598	2879	1190
Soil+10%oil+BS	8150	6210	4900	3281	759
Soil+10%oil+BSG	8150	4271	715	691	676
Soil+10%oil+SMC	8150	7012	5100	3301	1630
Soil+10% oil only	8150	7854	7220	7014	6810
Sterile soil+10%oil	8150	8043	7830	7692	7410
Soil+15%oil+BS	11341	10213	7160	6589	5950
Soil+15%oil+BSG	11341	5874	1620	1501	1260
Soil+15%oil+SMC	11341	9531	8534	7840	6670
Soil+15% oil only	11341	11012	10600	10650	9890
Sterile soil+15%oil	11341	10890	9780	10350	10400

Biodegradation of $C_{29} - C_{36}$ fractions in used lubricating oil

Results of biodegradation of $C_{29} - C_{36}$ hydrocarbon fractions in soil contaminated with 5%, 10% and 15% used lubricating oil within the period of 12 months are shown in TABLE 4. The results of the study revealed that these fractions of petroleum hydrocarbons were not properly degraded in all the treatments with the exception of BSG amended soil, where over 90% of the $C_{29} - C_{36}$ hydrocarbon fractions were degraded within the 12 month period. The partial degradation of these hydrocarbon fractions has been reported by different authors that they are not easily degraded by microorganisms in the soil because they are hydrophobic solids at physiological temperatures[19,26].In soil contaminated with 5% used lubricating oil, soil amended with BSG recorded reduction in the concentration of $C_{29} - C_{36}$ from 2643 mg/kg to 221 mg/kg in 12 months. In soil amended with BS and SMC the $C_{29} - C_{36}$ were reduced to 766 mg/kg and 800 mg/kg, respectively,

TABLE 4 : Concentration (mg/kg) of $C_{29} - C_{36}$ fractions in soil contaminated with 5%, 10% and 15% used lubricating oil.

Treatment	Time (Months)				
	0	6	3	9	12
Soil+5%oil+BS	2643	1651	1030	956	766
Soil+5%oil+BSG	2643	1150	278	243	221
Soil+5%oil+SMC	2643	2371	1090	978	800
Soil+5% oil only	2643	2367	2480	1823	1231
Sterile soil+5%oil	2643	2567	2500	2353	1790
Soil+10%oil+BS	5350	4622	3480	1956	647
Soil+10%oil+BSG	5350	2300	520	501	491
Soil+10%oil+SMC	5350	4612	3810	2281	1080
Soil+10% oil only	5350	5002	4390	3813	2762
Sterile soil+10%oil	5350	5191	4719	4225	3891
Soil+15%oil+BS	6871	5814	5140	4756	4520
Soil+15%oil+BSG	6871	3031	919	872	800
Soil+15%oil+SMC	6871	6207	5870	5188	4840
Soil+15% oil only	6871	6752	6350	6213	6130
Sterile soil+15%oil	6871	6692	6310	6241	6160

whereas in unamended soil and sterile contaminated soil, the biodegradation of the hydrocarbon fractions was minimal (reduction from 2643 mg/kg to 1231 mg/kg and 1790 mg/kg, respectively). Soil contaminated with 10% oil recorded reduction in the concentration of these fractions from 5350 mg/kg to 491 mg/kg, 647 mg/kg and 1080 mg/kg in BSG, BS and SMC treated soil, respectively. The low biodegradation of these hydrocarbon fractions might also be attributed to the fact that during biodegradation of hydrocarbon in soil or sediments, low molecular weight fractions are known to be degraded first by microorganisms before degrading the higher molecular weight petroleum fractions[27-29].

Therefore, in this study, possibly, the low molecular weight fractions were first degraded by indigenous microorganisms before the higher molecular weight. This might account for the low biodegradation of the higher molecular fractions in the range of C_{29} to C_{36}.

The results of soil contaminated with 15% used lubricating oil shows rapid degradation of $C_{29} - C_{36}$ fractions in soil amended with BSG from 6871 mg/kg to 800 mg/kg within 12 month. Low biodegradation was recorded in all other treatments at the end of 12 month. The reason for the low degradation of these fractions in all the treatments with 15% oil might be due to the high concentration of oil in the soil. This is known to inhibit the growth of microorganisms with suitable enzyme systems[30].

Biodegradation of PAHs in used lubricating oil

TABLE 5 shows the results of biodegradation of different PAHs within the period of 12 months. The results revealed degradation of fluorene below the detection limit of 0.5 mg/kg in all the treatments and at all pollution levels. Complete degradation of phenanthrene and anthracene was only achieved in soil amended with organic wastes, while the two PAHs were not completely degraded in unamended and sterile polluted soil. In soil contaminated with 15% used lubricating oil, only soil amended with BSG recorded complete degradation of fluoranthene and pyrene below the detection limit. Other treatments did not record complete degradation of fluoranthene and pyrene after the 12 month period. The reason for the complete degradation of PAHs recorded in soil treated with organic wastes might be due to the potential of the

TABLE 5 : PAHs concentration in soil contaminated with 5%, 10% and 15% used lubricating oil after 12 months remediation

Treatment	PAHs concentration (mg/kg)									
	Flu		Phe		Ant		Fth		Pyr	
	0	12	0	12	0	12	0	12	0	12
Soil + 5% oil + BS	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Soil + 5% oil + BSG	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Soil + 5% oil + SMC	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Soil + 5% oil only	6.0	ND	13	ND	6.3	ND	4	ND	5.4	ND
Sterile soil + 5% oil	6.0	ND	13	ND	6.3	ND	4	ND	5.4	1.8
Soil + 10% oil + BS	8.5	ND	16.2	ND	9.6	ND	5.8	ND	6.7	ND
Soil + 10% oil + BSG	8.5	ND	16.2	ND	9.6	ND	5.8	ND	6.7	ND
Soil + 10% oil + SMC	8.5	ND	16.2	ND	9.6	ND	5.8	ND	6.7	1.6
Soil + 10% oil only	8.5	ND	16.2	0.9	9.6	0.8	5.8	0.8	6.7	1.8
Sterile soil + 10% oil	8.5	1.7	16.2	1.5	9.6	1.9	5.8	1.6	6.7	2.1
Soil + 15% oil + BS	10	ND	19.4	ND	11.8	ND	6.9	0.6	9.6	2.1
Soil + 15% oil + BSG	10	ND	19.4	ND	11.8	ND	6.9	ND	9.6	ND
Soil + 15% oil + SMC	10	ND	19.4	ND	11.8	ND	6.9	1.2	9.6	2.2
Soil + 15% oil only	10	ND	19.4	0.9	11.8	0.7	6.9	1.1	9.6	2.3
Sterile soil + 15% oil	10	3.2	19.4	4.1	11.8	1.1	6.9	2.6	9.6	4.3

ND: Not detected at lowest detection limit of 0.5 mg/kg, flu:Fluorene, phe: Phenanthrene, ant: Anthracene, fth: Fluoranthene, pyr: Pyrene

organic wastes to neutralize the toxic effects of the PAHs on the bacteria present in the contaminated soil. This could also have enhanced their abilities to breakdown the PAHs in the contaminated soil. It has been observed that the addition of straw, compost, manure, etc. helps to enhance degradation by improving soil texture, oxygen transfer, and providing energy to the microbial population[31].

Lau, et al.,[32] .observed that the addition of SMC to PAHs contaminated soil reduced toxicity, added enzymes, microorganisms and nutrients for the microorganisms involved in degradation of PAHs. The loss of PAHs recorded in the sterile polluted soil might also have been due to different processes such as volatilization, adsorption, photolysis or chemical degradation, which are known to contribute to PAHs degradation in contaminated soil[31].

Microbial counts

Hydrocarbon utilizing bacteria (HUB) counts in the soil contaminated with 15%, 10% and 5% used lubricating oil are shown in Figures 1 to 3. HUB in soil pol-

luted with 15% used lubricating oil ranged from 1 x 10^5 CFU/g to 216 x 10^5 CFU/g, while HUB counts in 10% oil pollution ranged from 1 x 10^6 CFU/g to 103 x 10^6 CFU/g. The counts in 5% oil pollution ranged from 2 x 10^6 CFU/g to 131 x 10^5 CFU/g at the end of the 12 months study period. Soil amended with BSG recorded the highest counts of HUB in all the oil pollution level compared to all other treatments. The counts of HUB in all the soil amended with organic wastes were appreciably higher compared to that of unamended and poisoned control soil. The reason for higher counts of bacteria in amended soil might be due to the presence of appreciable quantities of N and P in the organic wastes, especially N content in BSG. N is necessary for bacterial biodegradative activities[33-37]. The reason for increased biodegradation of oil in amended soil (as compared to the unamended soil) could also be due to the presence of organic wastes in the soil. This helps to loosen the compactness of the soil, thus providing sufficient aeration for the indigenous bacteria present in the soil, thereby enhancing their metabolic activities in the contaminated soil.

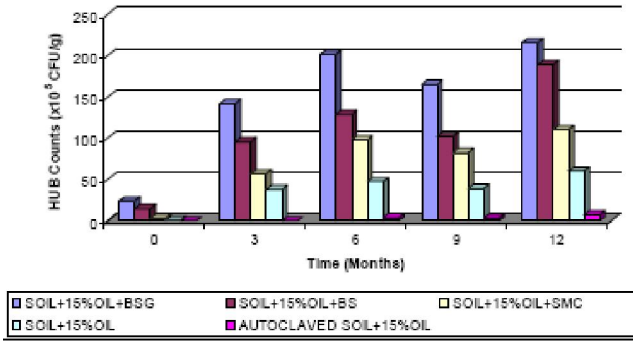

Figure 1 : HUB in soil contaminated with 15% used lubricating oil

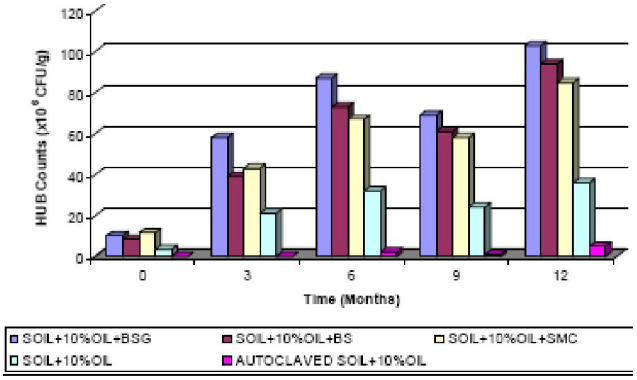

Figure 2 : HUB in soil contaminated with 10% used lubricating oil

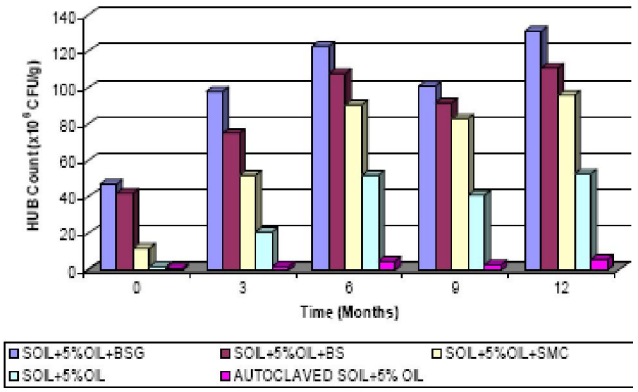

Figure 3 : HUB in soil contaminated with 5% used lubricating oil

The counts of HUB was low in the ninth month in all the treatments, the reason for this might be due to the low level of rainfall characterized with dry season experienced during this period. The counts of HUB in all the treatments correlate positively to the rate of biodegradation of hydrocarbons in the oil contaminated soil, thus suggesting that majority of the oil loss was as a result of microbial degradation. This is similar to the findings of Ijah, and Antai,[34].who reported extensive biodegradation of hydrocarbons in crude oil-contaminated soil by different species of hydrocarbons degrading bacteria in a field study.

The HUB isolated from the used lubricating oil contaminated soil was identified as species of *Acinetobacter, Micrococcus, Pseudomonas aeruginosa, Nocardia, Bacillus megaterium,* and *Corynebacterium.* These bacterial species had been implicated in hydrocarbon degradation by different authors[7,38-40].*Bacillus megaterium* grew more extensively on the oil agar compared to other isolates; this might have been due to the presence of efficient hydrocarbon degradative enzyme systems and the presence of catabolic genes involved in hydrocarbon degradation in the bacterial species[41,42].

CONCLUSION

Biostimulation (with organic wastes) of soil contaminated with 5%, 10% and 15% used lubricating oil was studied for a 12 month period under natural conditions. At the end of 12 months, the oil contaminated soil amended with BSG demonstrated greater potential in enhancing biodegradation of hydrocarbon fractions from C_7 to $C_{36,}$ as well as degradation of PAHs compared to

BS and SMC. The results also pointed out the effects of oil concentration on biodegradation. Partial degradation of hydrocarbon fractions and PAHs were recorded in soil contaminated with 15% and 10% used lubricating oil compared to degradation in soil contaminated with 5%. Therefore, BSG (wastes from brewery) can be utilized to stimulate effective biodegradation of hydrocarbons in soil with minimum oil pollution.

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