Soil Petroleum Hydrocarbon Bioremediation by A Fungi Consortium

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Abstract

Soil pollution is receiving greater and greater attention due to its direct impact on soil, water and public health. Remediation of soil is an important means of restoring soil back to natural condition. The present work was aimed to examining a method of soil decontamination by means of biodegradation which is of crucial ecological significance as its basic mechanisms are based on natural processes. A high percentage of hydrocarbon reduction was obtained during nutrient and bulking agent stimulation. During the first 30 days, the first-order TPH degradation rate constant (k_1) was lower for the treatment without nutrients (natural attenuation) followed by the augmentation, augmentation plus stimulation and stimulation treatments respectively.

Keywords: Bioremediation, Fungi, Petroleum, Hydrocarbon.

تصفیه‌ی بیولوژیکی خاک‌های آلوده به هیدروکربن‌ها با استفاده از قارچ‌ها

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چکیده

آلودگی خاک به خاطر اثر مستقیمش روی خاک، آب و سلامت افراد بیشترو بیشتر مورد توجه قرار می‌گیرد. برگرداندن خاک‌های آلوده به حالت طبیعی از جمله کارهای مهم زیست‌محیطی می‌باشد. هدف پروژه حاضر بررسی تصفیه‌ی بیولوژیکی خاک با استفاده از میکروب‌ها می‌باشد. ازآنجاییکه مکانیسم عمل در این متد بر پایه پروسه‌ای طبیعی استوار می‌باشد از اهمیت اکولوژیکی زیادی برخوردار است. در پروژه حاضر درصد بالایی از کاهش هیدروکربن‌ها با استفاده از نوترینت‌ها و بالکینگ اجنت بدست آمد. در سی روز اول، فاکتورثابت واکنش تغییر هیدروکربن‌ها در تریتمنتی که قارچ‌های فیلامنت دار به آن اضافه شده بود کمتر از تریتمنتی بود که هم قارچ و هم نوترینت اضافه شده بود.

واژه‌های کلیدی: تصفیه ی بیولوژیکی، قارچها، نفت، هیدروکربن.

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Introduction

A changing pattern of anthropogenic activities is linked to inconsistencies in the relationship of ecological systems in the environment. The choice of fossil fuel materials used in energy production is directly responsible for increases in carbon dioxide and other gases resulting in the current trend of global warming.

The increasing use of petroleum has generated various sources of pollution in soil, air and water.

In order to resolve this problem, several techniques have been developed. Among the many techniques employed to remediate polluted sites, environmental friendly technologies of bioremediation are gaining increasing prominence due to their advantages. The advantages of a biological method are that such a process is environmentally friendly in nature and simple to operate. Use of microbial consortia helps multiple metabolic capacities that increase the efficiency of the bioremediation process. Most hydrocarbon degradation studies have been carried out using white rot fungi such as *Phanerochaete chrysosporium*, *Pleurotus ostreatus* and *Trametes versicolor* (Yateem *et al.,* 1998; Mollea *et al.,* 2005). It is important to develop the bioremediation process further using fungi, because of their capacity to incorporate rapidly into the soil matrix and ability to grow in environments with low nutrient concentrations, low humidity and acidic pH (Potin *et al.,* 2004; Mollea *et al.,* 2005).

During the last two decades, a number of researchers have worked on the persistence of hydrocarbons in different natural environments and the possible role of the indigenous microflora in the degradation (Kästner *et al.,* 1994; Balba *et al.,* 1998). There are a few studies on biostimulation using native microorganisms and bioaugmentation of native fungi (Yateem *et al.,* 1998; Ruberto *et al.,* 2003; Straube *et al.,* 2003).

The objective of this study was to determine the effect of bioaugmentation, biostimulation as well as combined biostimulation–bioaugmentation treatments of a soil contaminated with a complex mixture of hydrocarbons.

Material and Methods
Soil Collection

The material selected for this study was from the site used as a motor repairing garages over several years. Polluted soil was selected based on the dark color of the soil. These garages used hydrocarbon-containing materials extensively including diesel, lubricant oils, and other hydrocarbon products.

Oil-contaminated soil was sampled from various sites from Pune city in Maharashtra, India. Samples were collected from multiple sites within an area and mixed to produce composite samples. The soils were collected from the surface to a depth of about 0 to 5 cm with sterile spatulas, using sterilized zip polythene bags and transported to the laboratory and were stored at 4˚C until being processed. Samples contained aged and fresh engine oil.

Processing the soil samples began immediately upon arrival at the laboratory. They were sieved through a (<2mm) mesh screen and thoroughly mixed and air dried. Their pH, conductivity and moisture analysis were analyzed after sieving. Other physicochemical properties were determined thereafter. (Table 1) There was a loss of TPH in sterilized soil, i.e. 0.25 % may be due to heat.

Table 1- Physical and chemical properties of soil composite sample (All values given as percentages %).

Soil condition	Total nitrogen	Total phosphorous	TEM	pH	WHC	TPH	Organic carbon	C:N ratio
NON-ST	0.08	0.02	6.04	5.3	53	6	12.2	610 :4 :1
ST	0.08	0.02	5.8	5.2	50	5.8	11.8	590 :4 :1

Isolation and Identification of Fungal Strains

Fungal strains were isolated by serial dilution from 1×10^{-1} to 1×10^{-7} of 1 g hydrocarbon-polluted soil previously dried and ground. A sample of 200 µL of each dilution was distributed in Petri dishes previously prepared with solid medium of potato dextrose agar (Bioxon). Fungal colonies were selected and plated on the same medium until pure colonies were obtained. The Petri dishes were incubated at 28 ◦C for 5 days. Fungal strains were identified partially by culture preparation and further by scientists from Agarkar Research Institute (ARI) culture collection.

Culturing of the Fungal Inoculums

Soil engine oil extracted was added to a sterile mineral medium (100 ml) in order to obtain a TPH concentration of 10,000 mg L_1. The mineral culture was composed of (NH4)2SO4, 0.5; KH2PO4, 0.5; Kcl, 0.2; MgSO4 _ 7H2O, 0.2 and 0.1 CaCl2 _H2O as per Rennie (1981).

The mineral solution was adjusted to a pH of 6 with phosphoric acid and it was sterilized at 121°C for 18 min. The culture flasks were then inoculated with 10ml of a spore suspension (1.2×10^7 spores mL_1) and incubated at 30°C and 150 rpm until the spore producing germ tube was observed (Cortés *et al.*, 2002). The viability of the spores was monitored by microscopy direct observation. This experiment was carried out in duplicate.

Total Petroleum Hydrocarbon Analysis

The petroleum mixture can be fractionated by silica gel chromatography into a saturate or aliphatic fraction, an aromatic fraction, and a polar fraction (Bergstein and Vestal, 1978). A ten gram sample of polluted soil was mixed with 5 g of NaSO4 anhydrous and was extracted by soxhlet using chloroform as a solvent.

Before starting the extraction, samples were kept in the same solvent for 24 hours, so that all soluble hydrocarbons present in samples could be dissolved. This can also reduce time which is required for soxhlet operation. The extract that contained total oil compounds and some biogenic lipids was estimated by weighing the dry residue after evaporation of the solvent. It was done using rotary evaporator so that pure solvent will be recovered again. The dry extract measured for TPH quantification by difference in weight. The dry extracts were suspended in 60 ml of hexane. The hexane-insoluble fraction containing the polar fraction was determined after filtration of the hexane solution on the solvent-washed Pre-weighed whatman GF/A glass-micro fiber filters. Filters retaining the polar were dried and weighed. The hexane soluble fraction was separated by solid-liquid chromatography on a 15 cm length×1 cm diameter activated (overnight, at 120 ◦C) silica-gel (60-120 mesh) column. Successive elution was performed with 60 ml of hexane (aliphatics), benzene/hexane 1:3 (aromatics). The hydrocarbon fractions eluted were quantified by difference in weight. Analyses of hydrocarbons were carried out in duplicate.

Bioremediation Treatment

The two main limiting factors in hydrocarbon bioremediation are considered as nutrient deficiency as well as hydrocarbon availability. In the present study, the stimulation treatment was carried out through nutrients and bulking agents in order to examine the ability of naturally occurring microbial flora present in the soil for biodegradation. Fungal augmentation was also carried out, in order to examine the role of fungal/ bacteria co-culture in degradation of hydrocarbon.

Sterilized and Non-Sterilized Soil Preparation

The soil was divided into two batches and one batch was autoclaved at 121◦C for 30 min to remove the indigenous microbial population. The other batch was left in natural condition. No crude oil was further added into the soil. As controls, one set of sterile soil and another of the non-sterile soil were left un-inoculated.

Treatments for Each Soil Batch

For each soil batch, three different treatments were considered.

A. Soil without any stimulation but humidified to 70% of its water holding capacity.

B. Soil with nutrient amendment in order to providing optimal C: N ratio of 25: 1 (according to nutrient optimization study).

C. Soil stimulated with nutrient and bulking agent (wheat bran).

Fungal Species Augmentation

For each treatment fungal augmentation was done through three single fungal species selected for this study as well as mix of same three fungi. For each treatment, there was one control in which fungal inoculation was absent. So, for each treatment we considered five options:

1. *Rhizopus* culture
2. *Aspergillus* culture
3. *Penicillium* culture
4. Mixed fungal culture
5. Control without fungal

In order to ensure easy interpretation of the results according to different bioremediation strategies, the controls in treatments A, B and C were named as natural attenuation, nutrient stimulation and nutrient plus bulking agent stimulation respectively. The details of experimental set up are shown in Table 2.

For treatment A (fungal augmentation without stimulation), the humidified soil (50g) was added to a 500 ml sterilized vial and it was inoculated with 10^7 of the corresponding inoculums propagated in liquid culture; total water added to the solid mixture was 70% of its WRC.

For treatment B (fungal augmentation plus nutrient stimulation), in order to have a C: N: P ratio of 250:10:1, the soil was humidified (70%, w/w) with a source of nitrogen $((NH4)2SO4$, and phosphorous $(K2HPO4)$ solution and then inoculation with different fungi was conducted.

Table 2. Different treatments and various fungal species used in the present study.

Treatment	Fungal culture
A (Fungal augmentation without stimulation)	*Rhizopus*
	Aspergillus
	Penicillium
	Mixed fungi
A. Control (natural attenuation)	Control
B (Fungal augmentation plus nutrient stimulation)	*Rhizopus*
	Aspergillus
	Penicillium
	Mixed fungi
B.Control (nutrient stimulation)	Control
C (Fungal augmentation plus nutrient and bulking agent stimulation)	*Rhizopus*
	Aspergillus
	Penicillium
	Mixed fungi
C.Control (nutrient and bulking agent stimulation)	Control

- **A**: Fungal sp. added to the soil batches but nutrient and bulking agent were not added.
- **B**: Fungal sp. plus nutrient were added to the soil batches.
- **C**: Fungal sp. plus nutrient as well as bulking agent were added to the soil batches.
- **A**.control: only water has been added to the soil batches (natural attenuation).
- **B**.control: only nutrient added to the sol batches.
- **C**.control: nutrient as well as bulking agent was added to the soil batches.

For treatment C (fungal augmentation plus nutrient and bulking agent stimulation) about 20 g of barley grain, soaked in water for 48 hours and drained, were placed in each bottle. The bottles were plugged before being sterilized at 121 ∘C for 15 min (15 Ib psi) in an autoclave. Each bottle was aseptically inoculated with one of the selected fungal isolates by slicing the PDA colonized by the organisms into small 5mm

pieces. The bottles were incubated in the dark for three weeks at 25 ◦C to allow complete colonization of the grains. The colonized grains were then inoculated into the contaminated soil at the rate of 5 g of barley to 50 g of soil (fresh weight). This procedure was adapted from Atagana *et al.,* (2005).

Bioaugmentation Treatment

Bioaugmentation treatment was done with 100 g polluted soil and moistened (70% of the water holding capacity) with nutrient solution as described above. Each bottle was inoculated with 10^7 of the corresponding inoculums propagated in liquid culture. The vial content was mixed under sterile conditions to obtain a homogeneous solid mixture.

Jars of all above treatments were covered and incubated at 30°C for 50 days under natural light and passively aerated for 15 min, under sterile conditions, every 2 days. Residual TPH as well as saturate, aromatic and polar fraction were determined as described in determination of hydrocarbon. Abiotic hydrocarbon removal was performed with a sterilized soil without inoculums.

Statistical Analysis

SPSS and Excell packages were used for the statistical work. Correlation between the desired parameters was done based on the Pearson correlation coefficient. Accordingly, the following points were analyzed.

- Each treatment was tested for suitability to remove TPH, saturate and aromatic from polluted soil.
- Each soil batch was tested for suitability to remove TPH, saturate and aromatic through various fungal inoculums.
- All hydrocarbon fraction changes were examined during bioremediation.

TPH Removal by Bioaugmentation

A gradual and homogeneous mycelia colonization of the three identified fungal strains was observed on the soil polluted with 60,400 mg TPHkg_1. Such colonization indicates the capacity of fungal re

adaptation to a highly polluted soil (Canet *et al.,* 2001). Almost all of the treatments after 45 days reached to constant rate of degradation.

Since the objective of the present work was to evaluate the degradation rate of hydrocarbon fractions as well as total petroleum hydrocarbon in different bioremediation treatments, also to evaluate the degradation rate through different indigenous fungal cultures in two soil batches (sterilized and non-sterilized soil). The results were converted the residual hydrocarbon concentration to percentage of reduction through following expression:

% of degradation = [(initial TPH – TPH concentration after treatment)/ Initial TPH] × 100.

Pure fungal culture was of interest because of their ability to synthesize unspecific enzymes that can degrade aromatic structures of high molecular weight (Colombo *et al.,* 1996). Non-sterilized soil augmentation also was of interest in order to evaluate the co-metabolism activity between fungi and bacteria.

Hydrocarbon Degradation Rate

Models are used to show biodegradation of hydrocarbon-contaminated soils by assuming simple first-order kinetics because it is suitable to describe the degradation pattern over time. First-order kinetics imply an exponential decay of substrate concentration with an asymptote to zero. But, in practice, one cannot get zero level by using biological decontamination, even after a prolonged treatment. This is because the remaining hydrocarbons became less available for biodegradation due to their recalcitrance and very limited bioavailability (Châtineau *et al.,* 2003; Demque *et al.,* 1997; Brown *and Donnelly,*1983; Nocentini and Fava, 2000). For these reasons, it is important to avoid using kinetic data from early stages of bioremediation to predict parameters on later hydrocarbon losses, such as the time required to attain a cleanup standard (Brown *et al.,* 1998). When the biodegradation has reached the residual concentration, a further intensive treatment is rarely useful.

Reduction of this residual concentration may often be the critical step to determine the time and limitation for the remediation, and the possibility to attain the regulatory objectives (Nocentini and Fava, 2000).

In this study, an asymptotic concentration was significantly higher than zero, even for the easily biodegradable saturates. To reflect the existence of the remaining or refractory fraction of hydrocarbons, a term representing the refractory fraction was added to a first-order reaction, representing the degradation of hydrocarbon:

$$C_t = C_0 e^{-kt} + b$$

Where C_0 is the initial biodegradable contaminant concentration, C_t the total Concentration of fraction at time t from the start of the test, and b is the estimated refractory fraction determined from the curve fitting.

Results

Treatment Wise Analysis

The following were the major points described based on the type of treatment given to understand biodegradation of hydrocarbons. The results are described as follows.

- Figure 1 shows that natural attenuation with moisture stimulation resulted in a 17% TPH reduction. Moisture plays an important role in supporting growth of soil biota and thus in degradation

The experiment was conducted using augmentation in non-sterilized soil to discover the percentage degradation of aromatic compounds; it showed 24.5% reduction without stimulation and with stimulation was 50.3 % (Figure 2). While aromatic degradation through augmentation plus stimulation in non-sterilized soil was 41.9%.
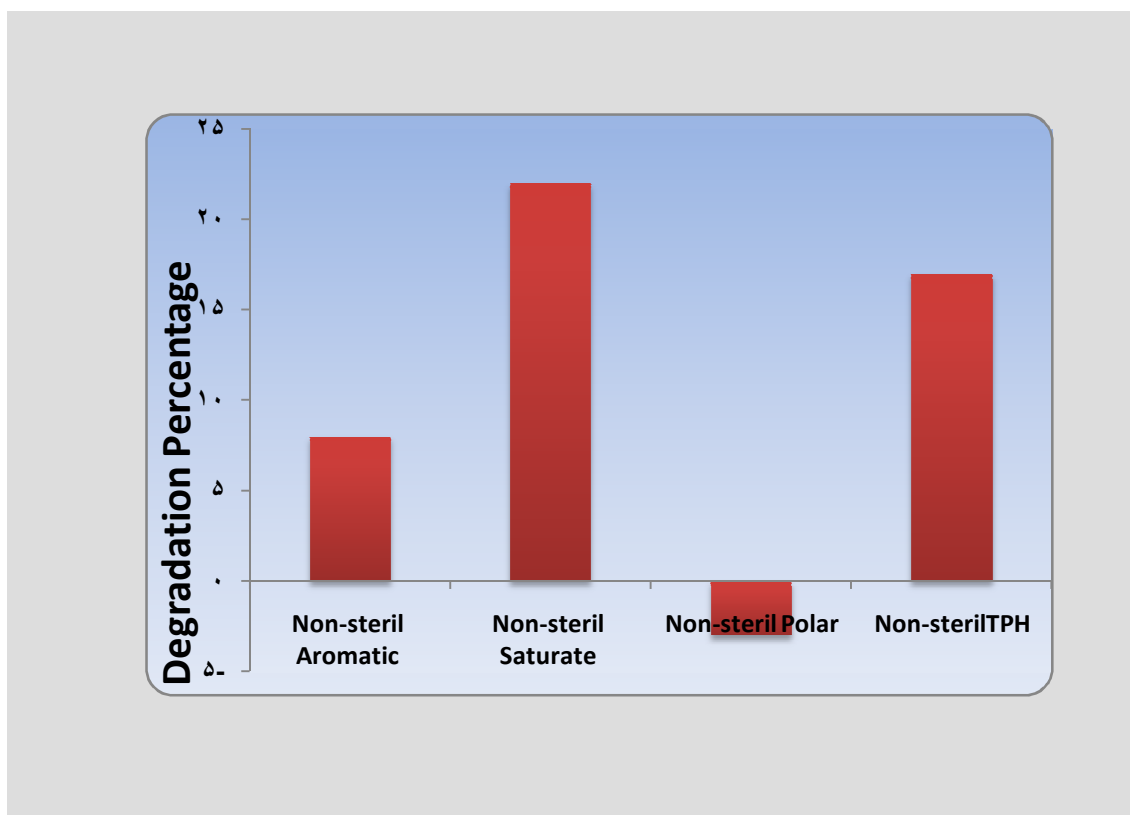

Figure 1. Percentage of hydrocarbon degradation in natural attenuation.
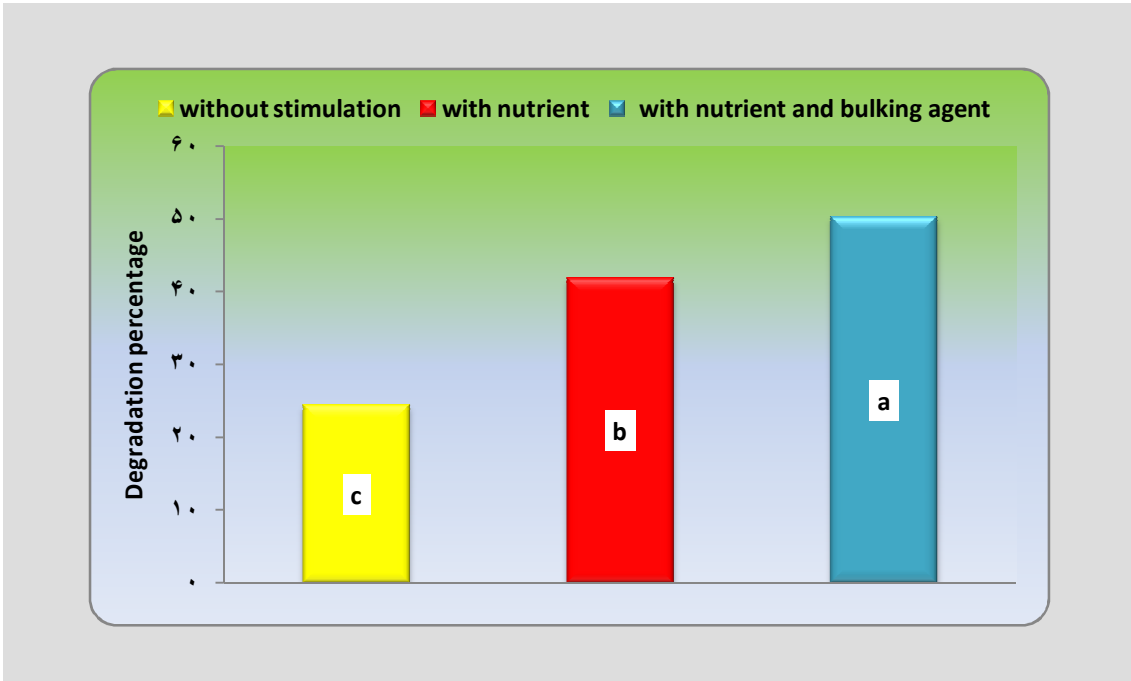
Figure 2. Degradation percentage of aromatic reduction in various non-sterilized soil treatments.

• There was an increase in concentration of polar fraction (Figure 3) in soil bioremediation. This was also similar to that of Juhasz *et al.,* (2005) who reported that through bioremediation of hydrocarbon polluted soil, a sharp decrease in the saturates and aromatics masses and increase in proportional mass of polar were observed.
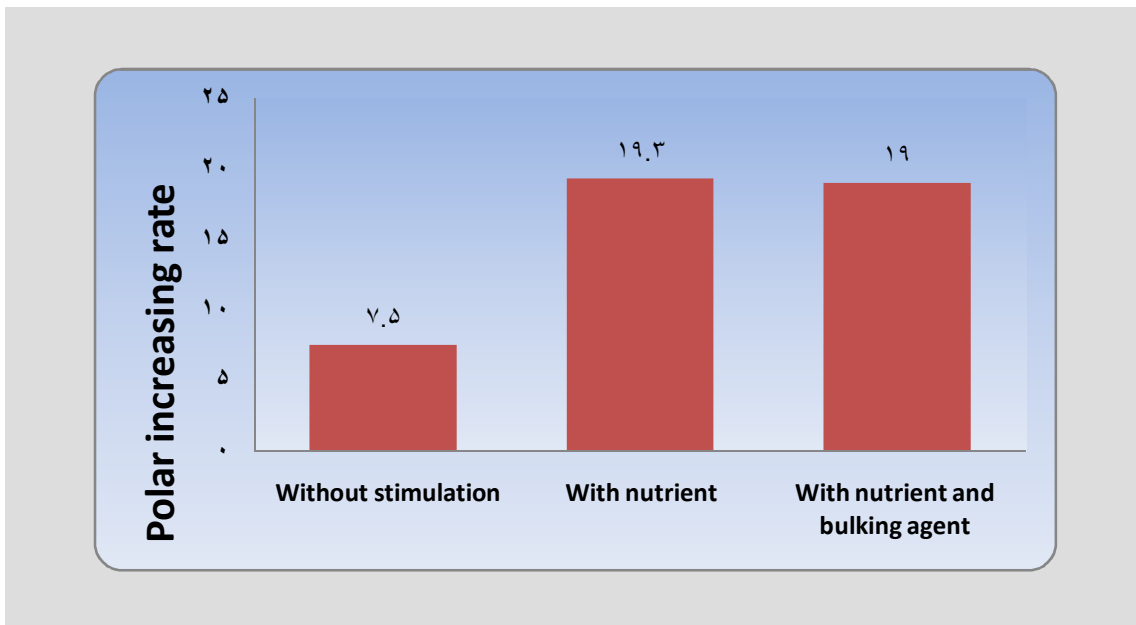

Figure 3. Percentage of polar hydrocarbon changes in various treatments.

- Figure 4 indicates increasing percentage of petroleum hydrocarbon

reduction with nutrients and nutrients plus bulking agent stimulation from 19.33% to 32.37%.

- In the present work aromatic removal with pure fungi augmentation was more than 9%. This was higher than result which Mancera-Lopez *et al.* obtained in filamentous fungal augmentation of soil polluted with hydrocarbon (9%).

- In the present study, 59.67% of total petroleum hydrocarbon was degraded through fungal augmentation plus stimulation treatment.

- Fungal augmentation in sterilized soil resulted in more aromatic hydrocarbon degradation than saturate fraction whereas, in non-sterilized soil, saturate degradation was more than aromatic (Figure 5).

- Figure 6 indicates significant difference (95% confidence) between hydrocarbon degradation percentages in sterilized soil with non-sterilized soil during bioremediation treatment.

Hydrocarbon Degradation Rate Analysis

The pseudo-first order degradation rate constants were calculated in accordance with the related equation and summarized in Table 3.

Biodegradation rates of engine oil calculated showed that, even in optimal nutrient conditions, a significant amount of refractory fractions were remained. This fraction did not degrade even after treatment. For example, 39620, 25130, 28600, 12960 mg kg−1 soil of TPH would remain under natural attenuation, stimulation, augmentation and augmentation plus stimulation treatments, respectively. These refractory or hardly degradable fractions correspond to 82, 52, 59, and 26.82 % of the initial concentration, respectively.
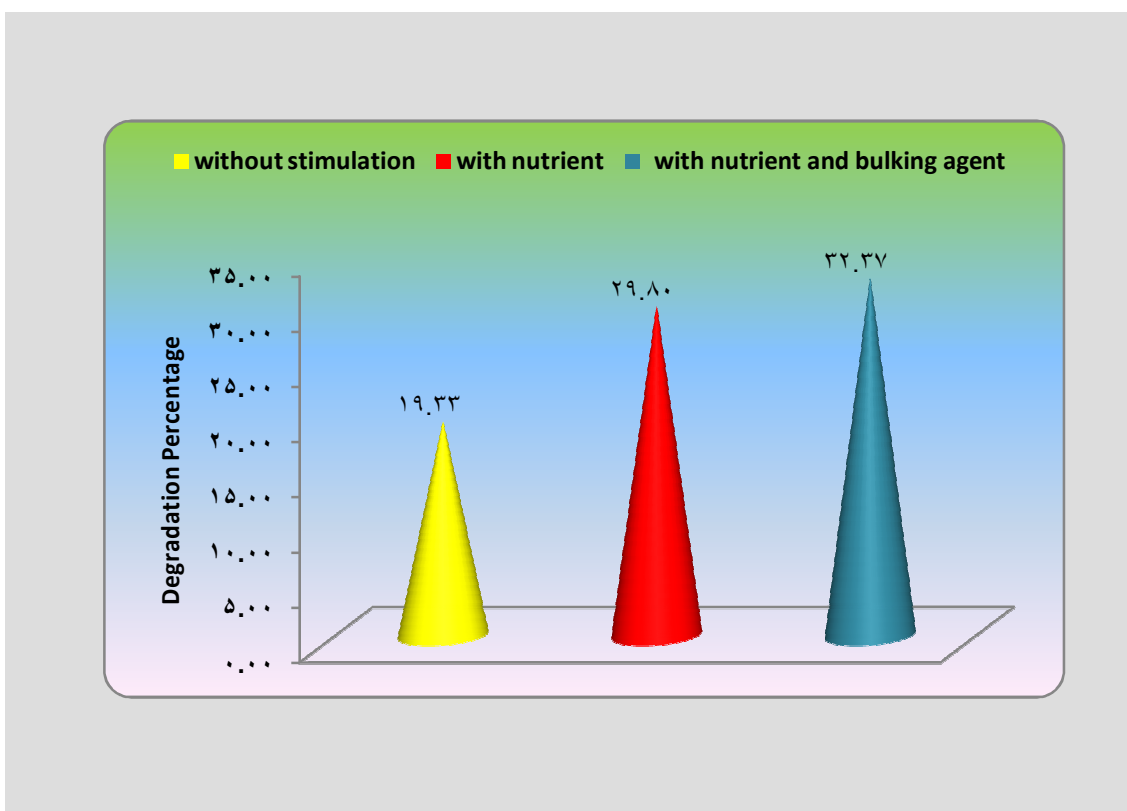

Figure 4. Percentage of total petroleum hydrocarbon reduction in various treatments in both type of soil.
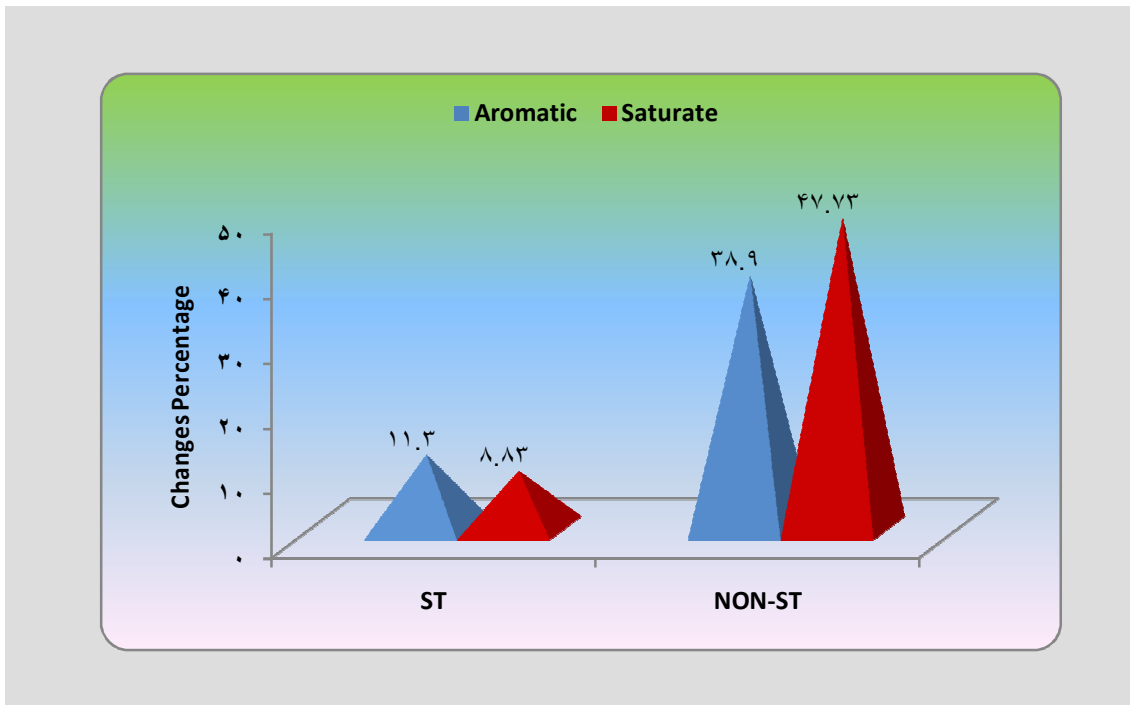
Figure 5. Difference in percentage of hydrocarbon fraction changes in sterilized soil bioremediation with non-sterilized soil bioremediation.
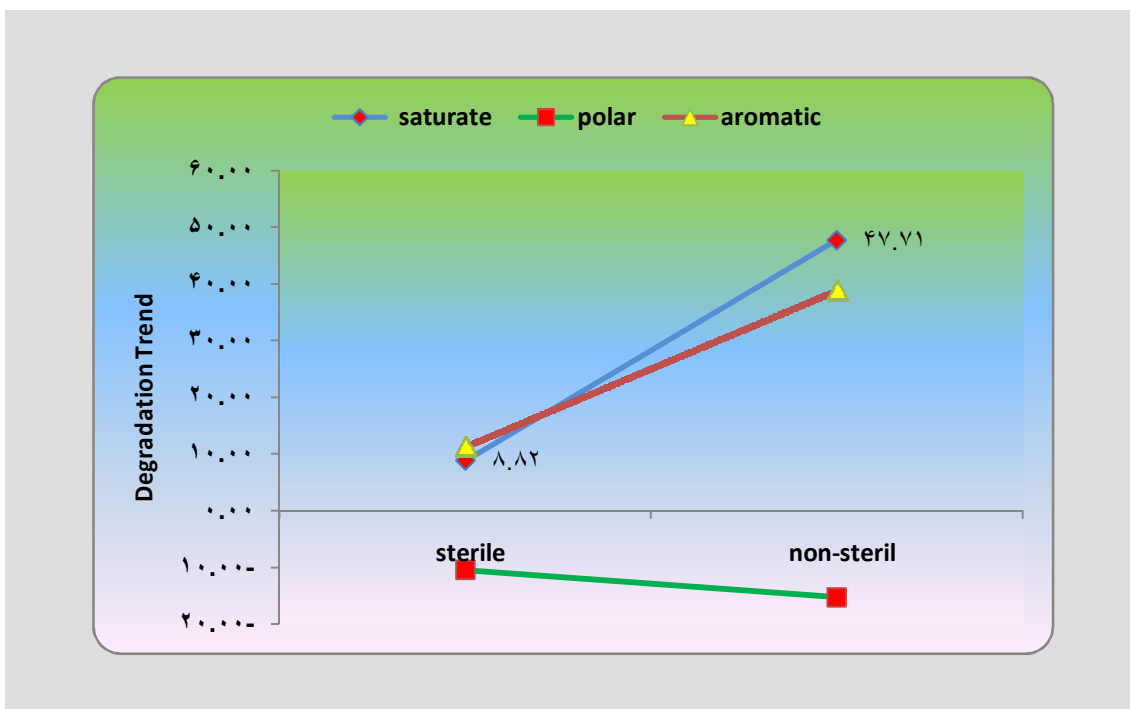

Figure 6. Comparing the degradation trend of different hydrocarbon fraction in sterilized soil with non-sterilized soil.

Table 3. Loss rate constant and hydrocarbon residual concentration during different bioremediation strategy.

Treatment	Total petroleum hydrocarbon			
	Loss rate constant		Residual concentration(g/kg soil)	
	k_1	k_2	Stage 1	Stage 2
Natural Attenuation	0.008	0.009	46.76	43.61
Stimulation	0.03	0.001	36.96	32.58
Augmentation	0.022	0.022	39.93	36.96
Augm+Stim	0.065	0.045	29.95	23.82

During the first 30 days, the first-order TPH degradation rate constant (k_1) was lower for the treatment without nutrients (natural attenuation) followed by augmentation, augmentation plus stimulation and stimulation treatments respectively. In contrast, the rate constant (k_2) for the late stage was higher ($P < 0.05$) in natural attenuation in which nutrient amendment was not present. But in other treatments the rate constant (k_2) for the late stage was lower than first stage. These rate constants are similar to those reported in other studies. First-order kinetics for the removal of petroleum hydrocarbons from soil were previously observed by Taylor and Viraraghavan (1999), who reported a rate constant of 0.01 d–1 during the biodegradation of hydrocarbons in non-amended soil. These values were higher than obtained in the present study by the natural attenuation treatments (0.008-0.009 d–1), indicating the low capacity of soil for biodegradation of petroleum hydrocarbons under natural soil conditions. Also they reported rate constant of 0.03 d–1 during the biodegradation of hydrocarbons in amended soil. It was also the same as the rate constant obtained in first stage of stimulation treatment in the present study. Lee et al., (2006) reported a loss rate constant of 0.006 and 0.02 d–1 for natural attenuation and stimulation of soil contaminated with lubricant oil, which was less than shown in the present study in Table 2. Yerushalmi et al. (2003) reported the original and biostimulated soil rate constant were 0.007 and 0.009 d–1. The present study (0.008, 0.03 d–1 for original and biostimulated soil) indicating the higher capacity of soil under augmentation plus stimulation treatments in compared to the findings of Yerushalmi et al. (2003).

Discussion

1. While 15% engine oil degradation in natural attenuation treatment was reported by Lee et al., (2007), 17% degradation was obtained in the present study. Orchard and Cook (1983) observed a log-linear relationship between water potential and microbial activity. Cho et al. (2000) found that the rate of petroleum hydrocarbon biodegradation was directly related to soil moisture.

2. The highest PAH removal rate reported in the present work could be due to the TPH composition (Davies and Westlake, 1979), as well as to the fact that fungi inocula were previously adapted to the same soil pollution. On the other hand use of bulking agent (wheat bran) could have important role in rapid and higher rate of degradation.

3. In the present study, an increase in polar fraction was observed. Huesemann (1994) also reported that heterogeneous polar fraction could also include biodegradation metabolites that could contribute to an increase in their mass following the biodegradation processes. Zytner (2006) and Genouw et al., (1994) conducted experiments for evaluating fungal ability to degrade aromatic hydrocarbon. Sexstone et al., (1978) found that oil biodegradation in tundra soils was accompanied by the accumulation of polar lipoidal compounds in the soil column. This is due to very low soil biota caused by a low temperature. A similar observation was reported by Jobson et al., (1972) who reported an increase in the polar nitrogen sulfur-oxygen fraction during oil biodegradation in soil.

4. An increasing percentage of petroleum hydrocarbon reduction with nutrients and nutrients plus bulking agent stimulation were seen in the present study.

Several studies have reported positive effects of nutrient supplementation on enhancing oil biodegradation (Roling et al., 2002; Ruberto et al., 2003) and found an increase of 30% of TPH removal, compared to the biostimulation treatment, when bioaugmentation with Acinetobacter. However, these results suggest the importance of supplementation of nutrient to achieve remediation. Autry and Ellis (1992), Margesin and Schinner (1999) and Demque et al., (1997) showed that the addition of microorganisms can accelerate the initial phase of biodegradation and can be advantageous when the contaminants have a toxic effect on the indigenous microorganisms. Whyte et al., (2005) reported a reduced lag time and an increased rate of hydrocarbon mineralization as a result of soil bioaugmentation at temperatures below 10°C.

5. The present work showed degradation was somewhat lower than Yateem et al. (1998), but were similar to the degradation levels attained by Mancera-Lopez et al. (2006). A high percentage of the aromatic compounds are considered toxic for humans (Juhasz and Naidu, 2000). Bossert and Bartha (1986) and Al-Daher et al., (1998) applied different supplements (among them, bioaugmentation with the consortia extracted from the same polluted soil) for the bioremediation of a soil polluted with 32,000– 55,000 mg kg_1 of crude oil. They reported a TPH degradation of 60%. In hydrocarbon removal, the size of the inocula, as well as by environmental conditions plays important role (Cunningham and Philp, 2000).

6. Since in sterilized soil only inoculated filamentous fungi were present whereas, in non-sterilized soil, augmentation of both fungi and bacteria were responsible for degradation. Therefore it may be concluded that fungi were more effective in aromatic degradation than saturate whereas bacteria-fungi co-cultures are more effective in saturate degradation than aromatic (Mancera-López et al., 2007). Thus, it seems that the fungi tested partially degrade, co-metabolically oxidize or mineralize

some PAHs to create less toxic products. (Adekunle and Adebambo, 2007). Cerniglia (1984) and Fan and Krishnamurthy (1995) also reported that fungal metabolism was important for the degradation of hydrocarbons, particularly aromatic compounds, and may be more effective than bacterial metabolism for the experimental conditions tested. Naturally occurring microflora in the soil caused a significant increase in the hydrocarbon degradation percentage.

7. The difference in saturate hydrocarbon reduction between sterilized with non-sterilized soil was more than aromatic. So it can be concluded that fungi-bacteria co-cultures were more effective in saturate degradation than aromatic fraction. Rainwater and Scholze (1991) reported that linear and cyclic saturated long-chain hydrocarbons were easily degraded by a large variety of native soil microorganisms, while branched unsaturated hydrocarbons of long-chain, linear and cyclic structures are commonly degraded in synthophysm with exogenous microorganisms. These findings also agree with the percentage of aromatic hydrocarbon obtained in this work. The difference between polar fractions in non-sterilized soil with sterilized soil was not significant. It may be because of the fact that, in sterilized soil, a single microbial culture can degrade the hydrocarbon partially and as compare to mix microflora in non-sterilized soil.

Conclusion

1. Moisture plays an important role in supporting growth of soil biota and thus in degradation.

2. Petroleum hydrocarbon reduction in the treatments with nutrients and nutrients plus bulking agent stimulation was higher than in other treatments under study.

3. Fungi were more effective in aromatic degradation than saturate whereas bacteria-fungi co-cultures are more effective in saturate degradation than aromatic. Thus, it seems that the fungi tested partially degrade, co-metabolically oxidize or mineralize some PAHs

to less toxic products.

4. There was an increase in polar fraction concentration from both sterilized and non-sterilized soil bioremediation.

5. The highest PAH removal rate reported in the present work could be due to the TPH composition as well as to the fact that fungi inocula were previously adapted to the same soil pollution.

6. Use of a bulking agent (wheat bran) could play an important role in rapid and higher rate of degradation.

7. Polar fraction changes follow the same trend as saturate removal indicate that the saturate degradation percentage was increased as polar production increases.

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