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Bioremediation (natural attenuation) of hydrocarbon contaminated soil

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Abstract

Biodegradation of petroleum hydrocarbons was investigated in contaminated soil surrounding Tehran Oil refining Co. Tehran, Iran. During the experiment which took 10 weeks, nitrogen and phosphorus amounts, total hydrocarbon utilizing bacteria (THUB), total heterotrophic bacteria (THB) and total petroleum hydrocarbons (TPHs) were specified. Also trend in TPHs removal and microbial population were studied. Results indicated that, after 10 weeks TPH level reduced from 25073.3 mg kg^{-1} to 7173.10 mg kg^{-1}. The nitrogen and phosphorus concentrations also decreased during degradation. But the total hydrocarbon utilizing bacteria increased from 4.1×10^4 to 11. 9×10^4 cfu g^{-1} at the end of 6^{th} week and finally reduced to 6.5×10^4 cfu g^{-1} at the end of experiment, while the THB reduced from 2.7×10^8 to 0.001×10^8 cfu g^{-1}. In studied soils TPHs significantly has positive correlation with N and P and THB count and negative correlation with THUB. The low microbial correlation shows that a part of the hydrocarbon loss due to natural attenuation probably had to be attributed to abiotic processes. To conclusion, we demonstrated that a significant reduction in the level of one of the most common pollutants, petroleum waste, can be achieved even under unfavorable conditions.

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Introduction

Physical, biological and chemical processes are employed for remediation. Remediation by enhanced natural attenuation (RENA) is a complete bioremediation technique in contaminated soils, sediments and sludge's discharge to the soil. Soil conditions are often controlled to increase the rate of contaminant degradation. Natural attenuation is a passive remedial approach that relies on natural processes to degrade and dissipate contaminants in soil and groundwater; these processes include physical, chemical and biological transformation (e.g. aerobic/anaerobic biodegradation, dispersion, volatilisation, oxidation, reduction, and adsorption) (Cozzarelli *et al.*, 2001).

Pollution caused by petroleum and its derivatives is the most prevalent problem in the environment. The release of crude oil into the environment by oil spills is receiving worldwide attention (Millioli *et al.*, 2009). Soil contamination with oil derivatives are often found in urban soils around industrial areas and in places where natural gas and oil are obtained (Adam *et al.*, 2002; Clark, 2003). Fuel leaks and oil tanker accidents happen and blow through oil pipelines, resulting in release of crude and refined oil into soil and water in the environment (Ebuehi *et al.*, 2005). Crude oil in soil makes the soil condition unsatisfactory for plant growth due to the reduction in the level of available plant nutrient or a rise in toxic levels of certain elements such as iron and zinc (Omosum *et al.*, 2008). This threatens human health and the creatures that are dependent on soil (Aboribo, 2001; Njoku *et al.*, 2009). Total petroleum hydrocarbons (TPHs) are one of the most common groups of persistent organic contaminants (Huang *et al.*, 2005). Relatively high hydrophobicity of Petroleum hydrocarbons results in considerable increase of their ability to accumulate soil and sediment in comparison to aquatic environments (Karthikeyan and Bhandari., 2001). Additionally, high hydrophobicity of these compounds results in their binding to soil and sediment particles and finally leads to decrease of bioavailability of these contaminants for biological sorption (Luepromchai *et al.*, 2007; Escalante-Espinosa *et al.*, 2005). Therefore, suitable solutions for removal or control of these soil contaminants must be found. Remediation has been defined as the management of a contaminant at a site so as to prevent, reduce or mitigate damage to human health or the environment, which can also lead to quick recovery of the affected lands (Doelman, 1994). Physical, biological and chemical processes are employed for remediation. Remediation by enhanced natural attenuation (RENA) is a complete bioremediation technique in contaminated soils, sediments and sludge's discharge to the soil. Soil conditions are often controlled to increase the rate of contaminant degradation (Odu, 1978, Gradi, 1985). Natural attenuation is a passive remedial approach that relies on natural processes to degrade and dissipate contaminants in soil and groundwater; these processes include physical, chemical and biological transformation (e.g. aerobic/anaerobic biodegradation, dispersion, volatilisation, oxidation, reduction, and adsorption) (Cozzarelli *et al.*, 2001). Bioremediation process enhances the indigenous bacteria via the addition of oxygen and nutrients to degrade petroleum hydrocarbon to carbon dioxide and water. The actual mechanism involved, which is mediated by microbes, is known as biodegradation (Atlas 1981, Colwell and Walker, 1977).

Most studies about aromatic hydrocarbon degradation have been conducted on groundwater (Bockelmann *et al.*, 2001) and aquifers (Griebler *et al.*, 2004) in laboratory and/or field studies; however little research has been carried out into soil samples (Aislabie *et al.*, 2004). Wegwu *et al* (2010) in their study, indicated that attenuation method is one of the best techniques for soil refinement in contaminated soils with crude oil. In other study three methods include; natural attenuation, biostimulation and bioaugmentation introduce as effectives methods for removal of TPHs from soils (Lee *et al.*, 2011).

Studies have been conducted to isolate and characterize hydrocarbon degraders from oil spill sites but little have been done to determine the changes in soil nutrients and TPHs as bioremediation of the spill site progresses. This study was providing information on the effectiveness of RENA techniques on microorganism's population in petroleum hydrocarbon polluted soil. Specifically, the experiment was designed to isolate and characterize hydrocarbon utilizing bacteria and also determine the changes in soil nutrient as function of time. The main objective of the present study was to investigates, using RENA process in 10 weeks timeframe, to verify the TPHs, nitrogen and phosphorus amounts, total hydrocarbon utilizing and total heterotrophic bacteria in a contaminated soil settlement at Tehran Oil refining Co., Iran.

Material and methods

Sample Collection

The sampling site was located at Azim abad, 15 km south of the Tehran state of Iran, named Tehran Oil refining Co. (within longitude 51°25'22"E and latitude 35°32'31"N).

Ten soil samples from surface (0-30 cm depth) were taken from around the waste spillage pond. Control sample was obtained far away from waste spillage pond to determine the background levels of petroleum hydrocarbons in the unaffected soil for comparison with the contaminated site.

The preliminary process of bioremediation took a period of 10 weeks. The bioremediation process comprises field experiment and laboratory simulation, with some physicochemical and microbial analyses. The concentration of TPHs, nitrogen and phosphorus were determined, while the total heterotrophic bacteria and total hydrocarbon utilizing bacteria were committed.

Process description

For remedy the contaminated soils the Remediation by RENA techniques were used that described by Ebuehi *et al.*, (2005).

Field experiments

Spiking of soils

The soils evenly moistened until they were soft and then allowed water penetrates the soil matrix (Ebuehi *et al.*, 2005).

Initial Tilling

A week after wetting in order to mixing the soil and breaking the lumps, soils were tilled. Composite samples were taken for physicochemical and microbial assessment (Ebuehi *et al.*, 2005).

Secondary Tilling

A week after the initial tillage, the soils were tilled and homogenized. The purpose of tillage was uniform distribution the petroleum contaminants and break up the soil lumps to fine particles. As a result of breakage, the surface area of particles increases. The composite samples were collected for analysis (Ebuehi *et al.*, 2005).

Windrow Construction

Windrows/ridges were built after the second plowing. Windrows were created for access to more aeration and air conditioning and optimize the efficiency of the processes, which makes the microorganisms are exposed to oxygen and helps in the biodegradation process of the petroleum hydrocarbon (Ebuehi *et al.*, 2005).

Breaking down Windrows

After 3 to 4 weeks to build windrows of exposure, they were broken (Ebuehi *et al.*, 2005).

Addition of Water

Water was added to soil samples to raise biodegradation process of the petroleum hydrocarbon by microorganisms. The water increase the biodegradation when penetrate into the soil (Ebuehi *et al.*, 2005).

Laboratory experiments

Determination of soil properties

Soil samples were transferred to the laboratory for testing and measuring soil physical and chemical properties.

Soil texture and sand, silt and clay percentage was analyzed using standard methods according to Gee and Bauder (1986). Total nitrogen was measured by Kjeldahl digestion (Bremner, 1996; Betancur-Galvis *et al.*, 2006), Olsen phosphorus was measured in solution extracted by $NaHCO_3$ 0.5 M at pH 8.5 (Olsen and Sommers, 1982), pH and EC was measured in a L/S:1/1 extract (ASTM 2000).

Total organic carbon is an alternative analytical method for measuring petroleum hydrocarbons using the wet oxidation technique as previously reported by Nelson and Sommers (1975).

Total petroleum hydrocarbons analysis

Total petroleum hydrocarbons from soil samples have been investigated by Soxhlet extraction method with same proportion from n-hexan and di-chloromethan (Christopher *et al.*, 1988).

For TPH analysis, gas chromatography (GC) was used (US.EPA, 1984). The gas chromatograph used was a GC-14A (Shimadzu, Japan), supplied with a flame ionization detector (FID) and a DB-5 capillary column. The capillary column was 30 m long.

Microbial enumeration

Hydrocarbon-degrading bacteria's was enumerated using a Most Probable Number (MPN) method and heterotrophic bacteria's was enumerated using colony enumeration on cultivation environment adapted from Wrenn and Venosa (1996).

Bushnell Haas medium (composition: magnesium sulfate 0.2 g L^{-1}, calcium chloride 0.2 g L^{-1}, potassium monophosphat 1 g L^{-1}, ammonium phosphate dibasic 1 g L^{-1}, potassium nitrate 1 g L^{-1} and ferric chloride 0.5 g L^{-1}) supplemented with 2% (w/w) NaCl with pH=7.2 then sterile by autoclave at 120 °C for 20 min.

3g of soil samples was placed in a vial containing 10 ml of Bushnell Haas medium supplemented with 2% (w/w) NaCl and mixed to form slurry for 30 min, then 1 ml of this slurry was placed into a vial containing 9 ml of Bushnell Haas medium supplemented with 2% (w/w) NaCl. A dilution series was prepared from this sample, from 10^{-1} to 10^{-12}, and used to inoculate plates. After diluting risasorin identifier in a rate of 90 µl added to pipes and then sterilized crude oil in a rate of 0.2 ml should be added to each pipe and Plates for enumeration of hydrocarbon-degrading bacteria were incubated at room temperature (26-27°C) for 2 weeks. After of two weeks, while color variation was observed, pipes extracted from incubation, and all plates were read using a MPN chart to determine bacteria, it is necessary to mention that all the needed instruments were sterilized (Gogoi *et al.*, 2003; Chorom *et al.*, 2010).

Results and discussions

The physicochemical characteristics of soil samples influenced by petroleum waste were shown in Table 1. The contaminated soil samples used for the present study contained 101350.7 mg kg^{-1} of average total petroleum hydrocarbon. Average of nitrogen and phosphorus contents was 33.2 and 10.2 mg kg^{-1} respectively.

Hydrocarbons degradation

Hydrocarbons in soil can dissipate/degrade in different ways, namely: (i) by physical processes, including the evaporation of the hydrocarbons according to their characteristics (boiling point, solubility and vapour pressure), soil properties (porosity, density and permeability) and climatic conditions (temperature and rainfall); (ii) by chemical processes, including hydrolysis, oxidation, reduction, photodecomposition and adsorption that depends on the chemical characteristics of the compound (functional groups, chemical structure and polarity), the soil properties (colloidal fraction and moisture); (iii) by microbiological processes, which mainly involve biodegradation by bacterial processes that depend on soil microbiota and environmental conditions (Yong and Mohamed, 1992).

Changes in the residual TPHs of the soil samples are showed in Fig. 1. In all studied samples, the initial TPHs concentrations were decreased. After 70 days, average TPHs was reduced from about 25073.3 mg kg^{-1} to the 7173.1 mg kg^{-1}, which resulted in 71.4% loss in concentration. An obvious degradation was attained during the first 35 days, but a degradation plateau was observed after Day 50 (Fig. 1), where further degradation seemed to be limited. The critical problem for TPHs degradation is their low solubility, which is one of critical limiting factors in the bioremediation process (Christofi and Ivshina, 2002; Toress *et al.*, 2005; Lai *et al.*, 2009).

Table 1. Some physical and chemical characteristics of soil samples.

Contaminated soils	Clay	Sand	silt	Total C	pH	EC	Total N	Olsen P	TPH	PAH
	%					dSm^{-1}	Mg kg^{-1}			
Min	12.0	48.0	40.0	279.2	5.3	7.0	21.4	6.30	101334.0	25321.1
Max	16.0	47.0	37.0	883.6	5.9	7.9	47.6	17.2	101367.1	25876.6
Aver	12.0	48.0	40.0	586.9	5.6	7.4	33.2	10.2	101350.7	25595.4
Control soil	9.0	52.0	39.0	3.2	7.4	8.2	23.2	4.32	2.3	0.002

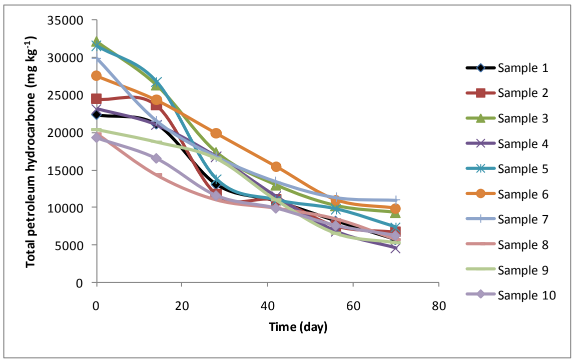

Fig. 1. Degradation of total petroleum hydrocarbons (TPHs) in studied soil samples.

Abiotic loss of TPHs loss has been shown to be less than 10% of total TPHs at 25°C within the first 30 days (Margesin and Schinner, 1997). Based on this result, it can be considered that the reduction of TPHs in these samples was caused by the biological activity of the indigenous microorganisms. Because hydrocarbon degrading microorganisms are known to be ubiquitous, it is possible to degrade TPHs in studied samples without any artificial inoculation (Gallego *et al.*, 2001). Considering TPHs in samples was the only carbon source, the continuous supply of essential nutrients, such as N and P, is necessary to maintain the bioactivity (Pao-Wen *et al.*, 2011). In this study, the drop in rate of TPHs removal was achieved after 35th day in samples may have been caused by the decrease of N and P.

The recommended C/N ratios for soil hydrocarbon bioremediation vary greatly and range at least from 100:1 to 10:1 (Morgan and Watkinson, 1989; Atlas and Bartha, 1992). In studied samples average C/N ratio was 17.5. It is important to add both N and P (Aislabie *et al.*, 1998), although N has been shown to be the major limiting nutrient in arctic soils (Braddock *et al.*, 1997).

Nitrogen and phosphorus changes during degradation

A reduction in P and N were observed during degradation from 9.10 to 1.13, and 30.26 to 0.17 mg kg^{-1} respectively. The N and P concentrations significantly ($p<0.05$) decreased as the remediation process progressed. Their values differed significantly in the first 4 weeks after the primary and secondary soil tillage (Table 2). It shows the hydrocarbon-degrading microorganisms in the first 4 weeks did not produce any significant results.

Table 2. Average TPHs, N and P changes during degradation of hydrocarbon waste in soil.

Before treatment	25073.3	33.2	10.2
After treatment			
2^{nd} week	21424.9	30.26	9.10
4^{th} week	14841.6	25.18	8.23
6^{th} week	11685.9	1.30	4.40
8^{th} week	8733.80	0.62	1.40
10^{th} week	7173.10	0.17	1.13
10^{th} week	7173.10	0.17	1.13

Table 3. Average number of total hydrocarbon utilizating bacteria (THUB) and total heterotrophic bacteria (THB) during degradation of hydrocarbon waste in soil.

	TPHs	Total N	Olsen P	THUB	THB
TPHs	1				
Total N	0.97**	1			
Olsen P	0.91**	0.91**	1		
THUB	-0.58**	-0.58**	-0.69**	1	
THB	0.75**	0.75**	0.79**	-0.90**	1

** Significant at 1% level of significance

However, six weeks after the bioremediation process, concentration of N and P greatly reduced from 30.26 and 9.10 mg kg^{-1} to 0.17 and 1.13 mg kg^{-1} respectively, which indicate a 99.4 and 87.5 % decline in their concentrations respectively. But after 6th week, the rate of decrease was reduced. Presence of hydrocarbon in soil reduces available forms of phosphorus as shown by Dimitrow and Markow(2000), Okolo *et al.*, (2005), Okonokhua *et al.*, (2007).

However, Reduce N and P, thereby reducing TPHs, imply that the N and P content can be used as fertilizer for hydrocarbon-degrading microorganisms. Several studies have reported favorable effects of fertilization on hydrocarbon biodegradation in arctic soils (Whyte *et al.*, 1999) alpine soils (Margesin and Schinner, 1999) and antarctic soils (Wardell, 1995).

Microorganisms

The average number of total hydrocarbon utilizating bacteria (THUB) and total heterotrophic bacteria (THB), in the soils contaminated with petroleum waste at the start of the experiment were 4.1 × 10^4 and 2.7 × 10^8 cfu g^{-1}, respectively.

There was a significant ($p<0.01$) decrease in the populations of THB, after 4 weeks, while those THUB increased. The THB population significantly decreased from 2.7 × 10^8 to 1.8 × 10^8 cfu g^{-1} after 4 weeks, showing a 33.3 % of the THB population, while the THUB increased from 4.1 × 10^4 to 9.2 × 10^4 cfu g^{-1}. This is indicative of the increased biodegradation by the THUB. The THUB continued its upward rise to 11.9 × 10^4 cfu g^{-1} at the end of 6th week. The THUB increased until there was no more contamination before a reduction, showing that the hydrocarbon-utilizing bacteria had now migrated to other soil locations since it feeds on petroleum hydrocarbon (Ebuehi *et al.*, 2005). After 6 weeks of the remediation process, the THUB count reduced. The THUB population finally reduced to 6.5 × 10^4 cfu g^{-1} at the end of 10th week.

Correlations

Correlations between studied parameters in the study are presented in Table 4. In studied soils TPHs significantly and positively correlated with N and P, indicating the relevance of nutrients. Also soil hydrocarbon and nutrient content correlated positively with THB count. This significant correlation represents the importance of biodegradation.

The low microbial correlation (Table 4) shows that a part of the hydrocarbon loss due to natural attenuation probably had to be attributed to abiotic processes. To conclusion, we demonstrated that a significant reduction in the level of one of the most common pollutants, petroleum waste, can be achieved even under unfavorable conditions.

Table 4. Correlation coefficients and significance levels for parameters investigated during the study.

	THUB (cfu g^{-1})	THB (cfu g^{-1})	TPHs (mg kg^{-1})
Before treatment	4.1 × 10^4	2.7 × 10^8	25073.3
After treatment			
2nd week	6.2 × 10^4	2.5 × 10^8	21424.9
4th week	9.2 × 10^4	1.8 × 10^8	14841.6
6th week	11. 9 × 10^4	0.009 × 10^8	11685.9
8th week	8.4 × 10^4	0.001 × 10^8	8733.80
10th week	6.5 × 10^4	0.001 × 10^8	7173.10

Conclusion

Natural attenuation is representative biotechnique that can be used for the remediation of contaminated soil.

In this study, natural attenuation was applied for the remediation of petroleum hydrocarbon contaminated soil and showed the high TPHs degradation efficiency.

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