Iranian J Env Health Sci Eng, Vol.1, No. 1, pp.26-33, 2004

Survey on Physical, Chemical and Microbiological Characteristics of PAH-Contaminated Soils in Iran

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are one of the important groups of organic micro pollutants (Xenobiotics) due to their widespread distribution and low degradability in the environment (atmosphere, water and soil). Some PAHs exhibit carcinogenic and/or mutagenic properties and are listed by the United States Environmental Protection Agency (USEPA) and European Commission (EC) as priority pollutants. In this research three petroleum contaminated sites in Iran were selected in order to separate and classify PAH-degrading microorganisms. Samples were analysed for: soil physico-chemical properties, soil particle size distribution, Ultrasonic extraction of PAH (phenanthrene) and microbial analysis. Ultrasonic extraction method was shown to be a reliable procedure to extract a wide range of PAH concentrations from different soils, e.g. clay, silt, and clay-silt mixtures. Results showed that the extraction rate of phenanthreen in mentioned different soils was in the range of 85 – 100 percent. Results showed that two of three selected sites were contaminated with phenanthrene in the range of 10 – 100 mg/kg of soil, and had a reasonable population of PAH-degrading bacteria, which were enable to adaptate and degradate a concentration range of phenanthrene between 10 and 1000 mg/kg of soil. According to results, it can conclude that, the bioremediation of contaminated soils in Iran may be considered as a feasible practice.

Keywords: *Polycyclic aromatic hydrocarbons (PAHs), Bioremediation, PAH-degrading microorganisms, Ultrasonic extraction.*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) comprise a large and heterogeneous group of organic contaminants which are formed and emitted as a result of the incomplete combustion of organic material (Mulder et al., 2001). Anthropogenic contamination sources, such as road traffic and combustion of fossil fuels predominate, but there are also natural sources, e.g. volcanic eruptions and forest fires (Lee et al., 2001).Polycyclic aromatic hydrocarbons (PAHs) are one of the important groups of organic micropollutants (xenobiotics) due to their widespread distribution in the environment (atmosphere, water and soil). They are produced by incomplete combustion of fossil fuels as well as by diagenetic processes during fossil fuel formation (Khodadoust et al., 2000). In smaller amounts, PAHs are naturally produced by forest fires and possibly microbiological synthesis (Turlough, 1999). PAHs consist of fused benzene rings in linear, angular or clustered arrangements, and contain by definition only carbon and hydrogen atoms. However, nitrogen, sulfur and oxygen atoms

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may readily substitute in the benzene rings to form heterocyclic aromatic compound which are commonly grouped with the PAHs (Feng, and Aldrich, 2000; Lundstedt, 2003; Slavica et al., 2003). Some PAHs exhibit carcinogenic and/or mutagenic properties and are listed by the United States Environmental Protection Agency (USEPA) and European Commission (EC) as priority pollutants (Amellal et al., 2001). Many PAHs have toxic, mutagenic and/or carcinogenic properties.

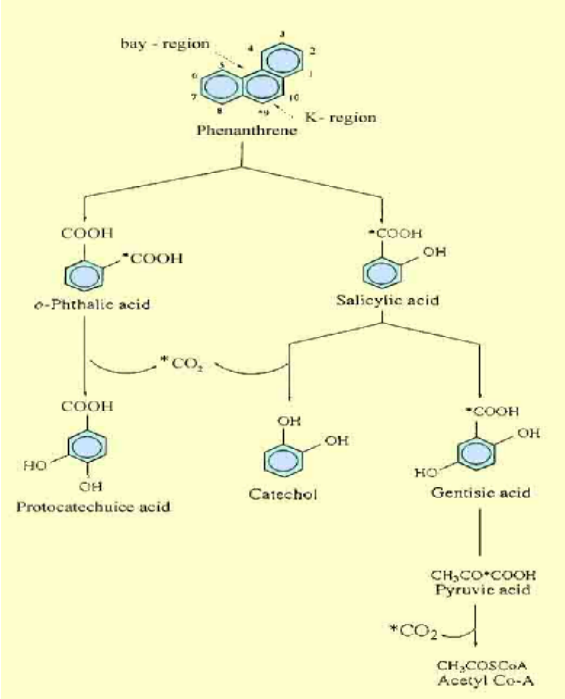
Several microorganisms have been isolated that are capable of degrading PAHs. However, PAHs are relatively persistent and recalcitrant in soils and are more difficult to be deraded than many other organic contaminants under natural conditions (Wilson and lones, 1993).

Since PAHs are hydrophobic compounds with low solubility in water, they have a greater tendency to bind with organic matter or soil, limiting their availability to microorganisms (Lee et al., 2001; Yun et al., 2003).

Consequently, our environment has been become highly polluted with different PAHs. Phenanthrene, a three-ring angular PAH, is known to be a human skin photosensitizer and mild allergen and is mutagenic to bacterial system under specialized conditions (Smanta *et al*., 2002; Yun et al., 2003).

Many PAHs contain a "bay-region" and a "K-region". The bay-and-K-region expoxides, which can be formed metabolically, are highly reactive both chemically and biologically. These epoxides are suspected to be ultimate carcinogens (Turlongh, 1999; Goldman et al., 2001). As phenanthrene contains bay- and K-region (Fig 1), it is also used as a model substrate for studies on the metabolism of bay-region and K-region-containing carcinogenic PAHs such as benzo [a] pyrene, benzo [a] anthracene and chrysene (Cerniglia, 1989; Mastrangela et al., 1997; Falahtpisheh et al., 2001). There are several reports of the degradation of phenanthrene by different bacteria (Bucker et al., 1979; Weis et al., 1998; Stegeman et al., 2001).

Fig.1: Formation of CO_2 from the degradation of



phenanthrene(Cerniglia, 1989)

There are several treatment methods for PAHs contaminated soils including: incineration, fixation, solvent extraction wet oxidation, land-fill and bioremediation. Other than incineration, bioremediation is the only practical considera-tion for complete degradation of organic contaminants (Norris et al., 1994).

Other methods except bioremediation needed to energy, transportation and monitoring.

There is an increasing intrerst in the cleaning of soil contaminated with organic compounds using bioremediation (Wilson and Lones, 1993). Bioremediation has become an intensive area for research and, as result; rapid progress has been made in developing effective micro-bial bioremediation processes (Fig 2) (Smanta et al., 1999). The objectives of this study were as follows:

Separation of PAH degrading micro organisms from contaminated soils in petroleum contami-nated sites in Iran;

Extraction of PAH using ultrasonication method and;

Analysis of extracted samples by HPLC-UVD using a PAH special column.

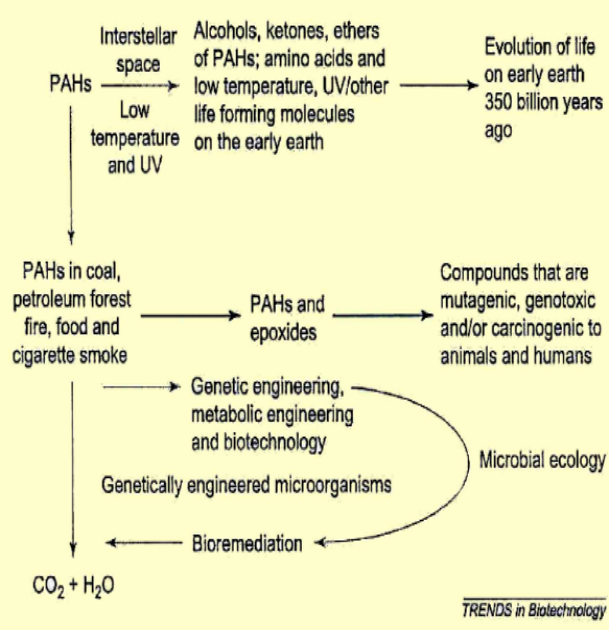


Fig.2: Fate, toxicity and remedittion of polycyclic aromatic hydrocarbons (PAHs) from the environment.

MATERIALS AND METHODS

Initial site investigation

In order to separate PAH-degrading micro-organisms, the soil samples were collected from three different petroleum contaminated sites:

(1) Tehran Oil Refinery site

The oil Refinery of Tehran is located at south part of Tehran, which produces a variety of oil products from crude oil since 19 years.

According to field studies, the site is polluted by oily water effluents and emission by oil combustion stacks. This site was selected as oil refinery site.

(2) Bahregan Oil Zones

Bahregan oil zone is one of the most important rude oil extraction zones of Iran from Persian Gulf.

This site was selected as an oil extraction and transportation from sea to land; the soil samples were collected from oil sludges that are in Bahregan oil zone shore. This site is located between Genaveh and Daylam port, near the Imam Hassan village.

(3) Oil Products Reservoir site

This site is located at northwest of Tehran, where oil reservoirs are provided in order to store the oil products for emergency conditions. This site was selected as oil reservoir site.

Soil sampling

Baseline soil samples for contaminant concentrations, soil chemistry and microbiol-ogy were collected from aged contaminated soils around the above mentioned three sites. The samples were collected in the range of 3-4 kg from the surface and 10 cm deep layer of soil. Prior conducting any analysis, the coarse pieces and stones were separated and the rest were mixed well.

The sub samples were kept cold (3-5) ºC for microorganisms' extraction. Microbial analyses were conducted within 24-48 h after sampling.

Remediation strategy

Soil physico-chemical analyses

Fandamental soil physico-chemical properties were analysed through following standard methods:

- Soli size particle distribution determination based on Unified Soil Classification (ASTM-D-2487);
- Soil moisture content (APHA);
- Organic carbon (ISO, DIS 10694 @11277);
- Polycyclic aromatic hydrocarbon, phenan-threne, (USEPA methods #3550B, #8310 and NIOSH #5506 by PHLC with UV detection (EPA, 2003; NIOSH, 2003).

PAH analysis

Phenanthrene concentration was measured by HPLC, with chromatographic conditions as follows:

- Analytical column: C_{18} Ultra Sep ES PAH QC Speica, 60 × 2 mm ID
- Folwrate (ml/min): 0.5
- Injection rate: 50 µl
- Elute: Acetionitirl/water: 40-100%
- UV detector wavelength: 254 nm

According to pre-test results the optimum elute condition for phenanthrene was determined at 60/40 (actonitril/water) (Herbert, 2003).

PAH extraction methods

There are four important methods for solid phase extraction (SPE) of PAHs from contaminated soil as below:

- *Soxhlet Extraction*
- *Sonication*
- *Supercritical Fluid Extraction using CO*
- *Microwave Assisted Extraction*

In this study, extraction was conducted by sonication method using ultrasonic instrument (*RK31H, Bandelin Electronic,Sonorex, 35 kHz, Germany*), because of fast and easy extraction process compared with other methods.

Certified phenanthrene contaminated soil samples (lg) were dried at air temperature and after sieving (through 50 meshes sieve), were suspended in 10 ml of acetonitril and extracted in ultrasonic bath at 40-45ºC for 45- 60 minutes. Extracts were settled for 10 min, and centrifuged at 5000-6000 rpm for 15 min. Elutes were filtered by 25mm, 2µm pore size, PTFE membrane filter and submitted to HPLC-UVD analysis.

Soil microbial analyses

Microorganisms were extracted from the contaminated soils by mixing of 1g soil with 10 ml of sterile $Na_2P_2O_7$ solution (2.8g/ liter) in 50 ml erlen mayer for 2h on a shaker (250 rpm) (Kastner et al., 1998). The soil particles were allowed to sediment for 30 min. the supernatant was diluted and plated on solid media.

Initial microbial analysis was conducted both for fungi and bacteria. Fungi determination test was carried out on solid media containing cloroamphenicole and cyclohexamide.

Total CFU were determined using plate count agar or HPC (hetrothrophic plate count) media. Also MPN analyses were conducted using lactose broth media with 15 tubes method (APHA, 1998).

Microorganisms were extracted from the contaminated soils, and supernatant after dilution by 10^{-3}- 10^{-5} times was plated on solid media. After separation of bacteria, the stock samples of separated microbes were prepared by adding of 500 µl of polluted sample and 500µl of glycerin together. The stock samples were holed at - 80ºC for future pilot stuty.

RESULTS

Soil physical analyses

Regarding to next studies on effects of soil structure in PAH degradation in contaminated soil, the soil particle size analysis was carried out on different soil samples, which collected from three before mentioned sites.

The soil particle size determination analysis was conducted using the stack of sieves from 4 to 200 meshes opening size. The results for determination of particle size of contaminated soils of oil refinery of Tehran, Bahregan oil zone, and oil products reservoir sites are presented in Tables 1, 2 and 3 respectively.

After determination particle size distribution of soils, to determine the type of soil the percentage of gravel, sand, silt and clay were measured.

Properties of contaminated soils are presented in Table 4.

Table 1: Determination of particle size of contaminated soil from oil refinery of Tehran

Sieve No. (mesh)	Opening (mm.)	Mass retained (grams) Mr	Retained% (Mr/M)×100	Finer (%)
4	4.75	0	0	100
10	2.00	0.9	0.225	99.8
35	0.50	98.93	24.73	75.07
50	0.30	48.39	12.1	62.97
60	0.25	25.26	6.31	56.66
70	0.21	25.1	8.78	47.88
80	0.18	1.76	0.44	47.44
100	0.15	18.76	4.69	42.75
120	0.12	39.19	9.8	32.96
200	0.075	91.7	22.92	10.04
Pan	--	40	10	----
Total weight	--	400 g	100	----

Table 2: Determination of particle size of contaminated soil from Bahregan oil zone

Sieve No. (mesh)	Opening (mm)	Mass retained (grams) Mr	Retained % (Mr/M)×100	Finer (%)
4	4.75	3.73	1.24	98.76
10	2.00	9.28	3.09	95.7
35	0.50	21.90	7.30	88.4
50	0.30	5.65	1.88	86.52
60	0.25	5.24	1.75	84.8
70	0.21	80.07	26.69	58.11
80	0.18	11.61	3.87	54.24
100	0.15	35.16	11.72	42.52
120	0.12	102.6	34.2	8.32
200	0.075	4.11	1.37	6.9
Pan		20	6.90	--
Total weight	----	300 g	100	----

Table 3: Determination of particle size of contaminated soil from oil reservoir site (Northwest part of Tehran)

Sieve No. (mesh)	Opening (mm)	Mass retained (grams) Mr	Retained% (Mr/M)×100	Finer (%)
4	4.75	13.49	4.5	95.5
10	2.00	67.23	22.41	73.09
35	0.50	78.65	26.2	46.89
50	0.30	73.51	24.5	22.39
60	0.25	10.85	3.6	18.79
70	0.21	22.35	7.45	11.35
80	0.18	0	0	11.35
100	0.15	4.47	1.48	8.86
120	0.12	11.95	3.98	4.86
200	0.075	4.5	1.5	4.38
Pan		13	4.38	-
Total weight	-	300 g	100	-

Table 4: Physico – chemical properties of contaminated soils

	Sample Locations		
Item	**Oil refinery of Tehran**	**Behragan Oil Zone**	**Oil Reservoir Site (Tehran)**
Gravel (%)	0	1.24	4.5
Sand (%)	90	91.86	91.12
Silt (%)	--	6.9	--
Clay (%)	10	--	4.38
Moisture (%)	6.4	1	4
pH	6.8	7.1	7
Organic carbon (%)	7.89	7.312	6.72
Bulk density (g/ml)	0.7	0.6	0.75
Uniformity coefficient (Uc)	3.70	1.79	7.8
Coefficient of curvature (Cc)	0.49	0.73	0.61
Effective size(mm)	0.075	0.12	0.16
Type of soil *	SP-SC	SP-SM	SP-SC

* ***Note:***

• SP-SC: Clayey – sands, poorly graded sand – clay mixture.

• SP-SM: Silty – sands, poorly graded sand – silt mixture.

• Soil classification has been done based on Unified Soil Classification for coarse–grained soils.

Soil microbiology

Initial analysis of soils for fungi determination; do not show any growth on solid media. But CFU and MPN analysis results for different three types of contaminated soils are presented in Table 5.

Table 5: Initial Soil Microbiology

Type of Soil	Initial soil PAH Conc. (mg/kg)	Ultrasonic extraction Conc. (mg/kg)	Recovery (%)	No. of samples
CLAY	100	90-96	90-96	20
	500	425-470	85-94	20
	1000	850-950	85-95	20
SILT	100	90-100	90-100	20
	500	450-500	90-100	20
	1000	850-970	85-97	20
CLAY & SILT	100	85-95	85-95	20
	500	425-475	85-95	20
	1000	850-950	85-95	20
SILT	2000	1800-2000	90-100	5
	5000	4500-4750	90 – 95	5
	10000	9000-9500	90 – 95	5
CLAY	2000	1800-1980	90-99	5
	5000	4350-4750	87 – 95	5
	10000	9000-9500	90 – 95	5

PAH analysis

In this study phenanthrene is selected as 3-reings PAHs prior the contaminated soils analysis for phenanthrene, it was necessary the rate of extraction of PAH using ultrasonication method to be measured. In this regards, ultrasonic extraction procedure was carried out with different soil types, e.g. clay, silt and clay and silt mixture and different initial soil phenanthrene concentrations. The results for ultrasonic extraction rate in different soils with different phenantherene concentrations are presented in Table 6. Due to sealing of oil reservoirs and leakage control in oil reservoir site of Tehran, the phenanthrene soil contamination was not detected. However, in two other sites the phenanthrene soil contamination was measured because of high pollution of these sites. The results for phenanthrene measurement in contaminated collected soils are shown in Table 7.

Table 6: Ultrasonic extraction of PAH (phenanthrene) from contaminated soils

Sample location	Microbial populations	
	MPN/g soil	CFU/g soil (heterutrophic populations)
Oil Refinery of Tehran	5×10^6	6.5×10^5
Bagregan Oil Zone	4.5×10^6	6.7×10^5
Oil Reservoir Site (Tehran)	8.5×10^7	7×10^6

Table 7: Ultrasonic extraction of PAH (phenanthrene) from collected soil samples

Sample Locations	Phenanthrene Conc.(mg/kg soil)
Oil refinery of Tehran	60 (approx.)
Behragan Island	12 (approx.)
Oil reservoir site (Tehran)	Zero

DISCUSSION

According to sieve analysis and uniformity coefficient (Uc) and Coefficient of curvature (Cc) based on unified soil classification (Table 4), the type of soils were SP-SC (poorly graded, sand-clay mixture) and SP-SM (poorly graded, sand-silt mixture).

Results of phyisco-chemical, PAH concentration, and microorganism's analysis in three types of petroleum contaminated soils collected from different sites in Iran, showed that:

Two of three selected sites were contaminated with phenanthrene in the range of 10-100 mg/kg of soil. According to soil particle size distribution analysis, the contaminated soils were classified as SP-SC (poorly graded clay-sand mixture) and SP-SM (poorly graded sand-silt mixture). Contaminated soil microbiology,

(CFU analysis), showed that the contaminated soils contains the reasonable populations of bacteria (6.5×10^5 - 7×10^6 CFU). Hence, it can be concluded that the bioremediation of contaminated soils in Iran may be considered as a feasible practice.

Inoculation tests results showed that, the separated bacteria demonstrated the same tolerance both with high and low level phenanthrene contaminated soils.

Ultrasonic extraction method was a reliable procedure for extracting of different PAH concentrations from different soils, e.g. clay, silt, and clay-silt mixture. It should be mentioned that, the Ultrasonic extraction procedure needs much more clean up work after injection of elutes to HPLC.

ACKNOWLEDGEMENTS

Authors present their gratitude to the Center for Environmental Research (CER) of Tehran University of Medical Sciences (TUMS), for its financial support for this research.

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