Arab Gulf Journal of Scientific Research, 22 (2), pp 66-73 (2004)

Original Paper Ref. No 2307

Simple Bioremediation Treatments for the Removal of Polycyclic Aromatic Hydrocarbons (PAHs) from the Polluted Desert Soil of Kuwait

طريقة مبسطة لتحليل المركبات الهيدركربونية الأروماتية عديدة الحلقات الموجودة في التربة الملوثة في صحراء الكويت

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Abstract: A soil microcosm test was designed to evaluate the influence of mixing polluted desert soil with clay soil (which is used as an amendment material and for immobilization of bacterial cells) on the biodegradation of petroleum polycyclic aromatic hydrocarbons (PAHs). Residual PAHs in this type of polluted soil were quantified by using GC analysis. At the beginning of the experiment 16 PAHs were resolved, of which the mutagenic and carcinogenic compounds flouranthene and pyrene were more frequent than the other PAHs (14% and 12.4% respectively). Results of total PAH biodegradation show that mixing this polluted desert soil with clay soil or its water extract stimulated the biodegradation of 85.8%-89.1% of these compounds. This is in contrast to 61.7%-75.5% in the absence of clay soil. Moreover when the mixed bacterial culture was immobilized in this clay soil 94.4% of total PAHs were degraded. On the other hand, the free cells of the mixed culture succeeded to remove only 75.5% of these compounds. In this study the six-ringed PAHs were completely degraded in the presence of clay soil. A particularly notable distinction between the immobilized culture (T3) and the other treatments in this biodegradation study is the greater efficiency of the immobilized culture to degrade the individuals of the 16 PAHs, especially the carcinogenic compounds: flouranthene, pyrene, chrysene, benzo(a) pyrene and dibenzo (a,h)anthracene. These results lead to the conclusion that mixing the polluted desert soil with clay soil and/or its water extract seems to be a simple cost effective bioremediation method.

Keywords: Kuwait, bioremediation, polluted desert soil, polycyclic aromatic hydrocarbon, bacteria

المستخلص: قام الباحثون بتصميم عدد من التجارب، لدراسة أثر خلط التربة الملوثة بالمركبات الهيدركربونية بتربة طينية غير ملوثة، وذلك كمحسن للتربة، ومثبت للخلايا البكتيرية - على التحلل البيولوجي للمركبات الهيدروكربونية الأروماتية عديدة الحلقات. وقد تم التعرّف على 16 مركبًا من المركبات الأروماتية عديدة الحلقات عن طريق الكروماتوجرام الغازي، وكان أكثر هذه المركبات شيوعًا المركب فلورانثين Flouranthene، والمركب بيرين Pyrene، وهذين المركبين يعدان من المركبات المسرطنة. وقد أمكن عند خلط التربة الصحراوية الملوثة بالمركبات الهيدروكربونية، بتربة طينية غير ملوثة أو بالمستخلص المائي لها، أمكن تنشيط التحلل البيولوجي لهذه المركبات الحلقية إلى ما بين 85.8% - 89.1% من مجموع هذه المركبات الأروماتية الحلقية، وعند مقارنة ذلك بنتائج معالجة تربة ملوثة لم تخلط بالتربة الطينية، وجد أن نسبة تحلل هذه المركبات لا يزيد عن 61.7% - 75.5% وعند استخدام مزرعة بكتيرية مكونة من خليط من ثلاثة أنواع من البكتيريا محملة على حبيبات التربة الطينية (بغرض تثبيتها)، فإن نسبة التحلل إرتفعت إلى 94% من مجموع هذه المركبات. وفي جانب آخر فقد وجد أنه عند حقن التربة الملوثة بهذه المزرعة الخليط غير المثبتة على حبيبات التربة الطينية، أن نسبة تحلل هذه المركبات لم يزد عن 75.5%. وفي أثناء الدراسة أمكن ملاحظة أن المركبات الهيدروكربونية المكونة من 6 حلقات، قد اختفت تمامًا عند خلط التربة الصحراوية الملوثة بالتربة الطينية غير الملوثة. وكان من المميزات الهامة للمزرعة الخليط المثبتة أنها تسببت في تحليل نسبة كبيرة من المركبات الحلقية التي أمكن التعرف عليها، وخاصة المركبات المسرطنة مثل: فلورانثين، بيرين، كريزين، بنزو(a) بيرين، وثنائي البنزين (a,h) أنثراسين، ومن هذه النتائج أمكن الاستدلال على أن معالجة التربة الصحراوية الملوثة بالمركبات الهيدروكربونية بخلطها بتربة طينية غير ملوثة أو بالمستخلص المائي لها، يعتبر من الطرق الفعالة البسيطة وغير المكلفة.

كلمات مدخلية: الكويت، التحلل البيولوجي، التربة الصحراوية الملوثة، المركبات الهيدروكربونية عديدة الحلقات، بكتيريا.

Introduction

High concentrations of polycyclic aromatic hydrocarbons (PAHs) in the environment are usually associated with the disposal of the combusted materials or petroleum residues. In the environment they exist as compounds having two to seven condensed rings, and they are considered as hazardous soil contaminants (Chang, *et al.* 2002).

Severe contamination of soil results mostly from accidental spillage, as an example, the Gulf oil spill which was caused by Iraqi forces in 1991 during their invasion of Kuwait. More than 60 million barrels of crude oil were released from the destroyed

oil wells to the desert soil of Kuwait, forming more than 330 oil lakes covering an area of about 49km^2 (Al-Awadhi, *et al.* 1995; Salam, 1995). Most of the oil has been pumped by Kuwait Oil Company (KOC) leaving the lake beds heavily contaminated. Although several treatment methods for remediation of these polluted soils have been considered (e.g. Al-Gounaim, *et al.* 1992; Al-Awadhi, *et al.* 1996; Cho, *et al.* 1997a.b; Al-Gounaim and Diab, 1998a,b; Balba, *et al.* 1998; Yateem, *et al.* 1998; Al-Gounaim and Diab, 2002; Diab and Al-Gounaim, 2003), large quantities of this polluted soil remains unremeditated in the desert of Kuwait.

This contaminated desert soil is of concern since some of the PAHs especially the higher condensed compounds with four or more rings and their metabolites, have a variety of mutagenic and carcinogenic effects and are classified as compounds with significant human health repercussions (Kalf, *et al.* 1997; Knopp, *et al.* 2000). Therefore, removal of such pollutants is of particular concern for environmental protection in the State of Kuwait.

PAHs manifest low aqueous solubility and these molecules become increasingly insoluble as the number of rings in the molecule increases. The rate of transformation of PAHs decreases with an increase in the number of fused benzene rings in the molecule (Smis and Overcash, 1983), and the resulting low bioavailability has been claimed to be the chief factor limiting PAH biodegradation (Gauger, *et al.* 1990; Stucki and Alexander, 1987).

Microbial degradation of PAHs is considered to be the major decomposition process for these contaminants in nature and is of great practical interest for the implementation of bioremediation. Microorganisms have the enzymatic capacity to oxidize PAHs that range from naphthalene to benzo (a) pyrene (Cerniglia, 1984).

For soil contaminated with accidental hydrocarbon spills, simple bio-remediation procedures that optimize microbial degradation activity by pH control, nutrient balance, aeration and mixing appear to be cost effective means of cleanup (Bossert and Bartha, 1984).

There are few reports of PAHs biodegradation under environmental field conditions and very few dealing with soil systems specifically (Robert, 1998).

Al-Awadhi, *et al.* (1996) studied the bioremediation of the polluted desert soil in Kuwait using a land forming technique. After ten months of treatments, 49.1% and 39.1% of the aromatic fractions of the residual oil were degraded in lightly and heavily polluted samples respectively.

In 1997, Cho *et al.*(b) carried out bioremediation experiments using moderately polluted desert soil from Kuwait. They found that the decomposed compounds were mainly alkanes and the aromatic fractions of the oil were not well decomposed. They concluded that the slow decomposition of the aromatics was due to low population of the aromatics decomposing microorganisms in the Kuwait desert soil. They further reported that the problem of bioremediation of the oil-contaminated soil of Kuwait might be concentrated on the biodegradation of the aromatic fractions.

Although hundreds of PAHs exist in the polluted environment, the US Environmental Protection Agency (EPA) has identified sixteen unsubstituted PAHs as priority pollutants. These compounds ranged from the two-ringed naphthalene to the six-ringed benzo (ghi) perylene. Accordingly, the present work is focused on the presence and biodegradation of the sixteen PAHs in the polluted desert soil in Kuwait.

Materials and Methods

Collection of soil samples

Polluted soil samples were collected from different sites at the side of an oil lake at Burgan Fields, from a depth of 5-20 cm. At least 10 samples were collected from different spots in the same area and mixed to form a composite sample.

Extraction of the residual hydrocarbons

Five grams of the air dried soil were mixed with five grams of anhydrous sodium sulphate to remove moisture, and Soxhlet extracted with chloroform for 8 hours. The extract was evaporated in a preweighed dish, and the amount of the residual oil was determined.

The extracted residual oil was suspended in n-hexane and filtered to remove the non-soluble fraction (asphaltene). The hexane soluble fraction was fractionated by liquid-solid chromatography into saturates, aromatics and resins. The amount of each fraction was determined as described by (Chaineau, *et al.* 1995). Aromatics were further resolved using GC analysis.

GC analysis of PAHs

Identification and quantification of the individual polycyclic aromatic hydrocarbons were determined using a Chrompack CP 9001 gas chromatograph equipped with a CP 9050 liquid sampler and configured with FID, using helium (Grade 6) as a carrier gas, with a flow rate of 1ml/min. A CP Sil 19CB column (25m long x 0.32mm diameter x 0.2 µm thickness for the stationary phase) was used. Temperature programming of initial holding at 40°C (2 min.), and then heating with a rate of 10°C/min to 295°C (holding 2 min.) was applied. The total time of analysis was 45 minutes. Injector and detector temperatures were 250°C and 280°C respectively. Injection volume was 1µl for all samples. The quantification of PAHs was based on application of reference standard materials (100 ppm for each), obtained from Supelco Co. Samples were run in duplicates and the mean value was taken.

Preparation of the enriched culture

In previous work in our laboratory, a polluted soil sample from the desert of Kuwait was treated until all n-alkanes and iso-alkanes were degraded, leaving behind the hardly degradable compounds of the residual oil. From this type of soil, five grams were introduced into a 250ml conical flask containing 50ml of the medium described by Chaineau, *et al.* (1996). A known weight of the sterilized aromatic fraction (prepared as described by Venkateswaran, *et al.* 1995) was added to the medium.

The inoculated flasks were incubated at 30°C for a period of 30 days or more on a rotary shaker operated at 100 rpm. At the end of the incubation period 10ml of the culture were taken and introduced to a second flask containing the same culture medium and incubated as described above. This experiment was repeated several times to enrich the hydrocarbon-utilizing microorganisms. The appropriate volume of this enriched culture (108 CFU/g soil) was chosen as inocula for treatment (T7).

Isolation and identification of the hydrocarbon-utilizing bacteria

From the enriched culture (prepared as described above) three different types of colonies were abundant on streaked plates. The colonies were picked up, purified and identified according to the criteria of Keddie, *et al.* (1986), Goodefllow and Lechevalier (1989) and Palleroni (1984). Bergey's Manual of Determinative Bacteriology (Holt, *et al.* 1994) was also consulted.

Preparation and immobilization of the mixed bacterial suspensions

Suspension of each of the three identified bacteria was prepared as described by White, *et al.* (1996). The appropriate volume of each suspension was chosen so as to give 108 CFU/g soil. Mixed culture from the three bacterial suspension (1:1:1) was prepared. 50ml of this culture was used for inoculating the soil of treatment (T2). The actual number of colony forming units (CFU) that was added to the soil was determined by the viable plate counts.

For immobilization of the cells of this mixed suspension, 50ml was mixed with 25 grams of sterilized non-polluted clay soil and left for 24h at room temperature, then introduced into the soil of treatment (T3) and thoroughly mixed.

Soil treatments

A soil microcosm test was designed to include seven treatments (T1-T7) in duplicates, each consisting of a 2l beaker containing 400g of the polluted soil sample.

The composition of the different treatments can be found in Table 1. Treatments (T4) and (T5) were applied to the soil to evaluate the effects of adding clay soil of high biological activity (rich in microorganisms and minerals). On the other hand (T6) was applied to evaluate the effect of clay soil extract on the biodegradation activity as compared to that of adding clay soil. Other treatments were designed to study the effects of mixing the polluted soil with free (T2) and immobilized (T3) cells of the mixed culture. In (T7) the soil were mixed with the enriched culture, while in (T1) NP fertilizers only was added. It must be noted that treatment (T2) was designed to show the effect of sterilized clay particles used for immobilization of the mixed culture (T3), while in T4 and T5 non-sterilized clay soil was added.

The moisture content of each treatment was adjusted at 60% of its water holding capacity. All of the above treatments were incubated at 30°C. Each beaker was covered with thin aluminum foil to reduce evaporation of water. A small glass rod was introduced to each beaker for tilling of the soil. The loss of water due to evaporation in each treatment was determined by weighing each system at the beginning of the experiment and every 3-4 days, and the amount of water lost was added.

From each of the above treatments samples were taken at the beginning of the experiment and after 6 months. Extraction and quantification of hydrocarbons were carried out as described above.

Preparation of soil extract was made by adding 500 gram to 1000ml distilled water and autoclaved at 121°C for 30 min. The autoclaved suspension was filtered and made up to 1l with distilled water. Sterilization of the clay soil was made as described by Kastner *et al* (1998).

Table 1 Composition of treatments (T1-T7) applied to the polluted desert soil of Kuwait

Composition	Treatments						
	1	2	3	4	5	6	7
NH_4No_3 + K_2HPO_4 60mg + 30mg/100g soil	+	+	+	+	+	+	+
Free cells of mixed culture	-	+	-	-	-	-	-
Immobilized cells of mixed culture	-	-	+	-	-	-	-
Clay soil: - 10g/100g soil	-	-	-	+	-	-	-
- 20g/100g soil	-	-	-	-	+	-	-
Clay soil extract 20 ml	-	-	-	-	-	+	-
Enriched culture	-	-	-	-	-	-	+

Results and Discussion

The collected soil samples were heavily polluted with crude oil (7.6-8.0%). They were sandy in nature, poor in phosphorus (0.16 ppm) and nitrogen (0.01%) and with pH 8.2 (Al-Gounaim and Diab 1998b). One gram of this soil contained 1.4 x 10^4 CFU of bacteria, 1.6 x10^3 CFU of fungi and 0.3 x 10^3 CFU of oil-degrading bacteria. (Al-Gounaim and Diab, 2002).

When the residual oil was extracted and fractionated, it was composed of saturates (36.6%), aromatics (40.0%), resins (7.6%) and asphaltenes (15.8%).

Residual polycyclic aromatic hydrocarbons (PAHs) in this polluted desert soil were quantified by GC-FID analysis. 16 PAHs were resolved (Table 2, Fig.1), they are:

Naphthalene	(6.0%)
Acenaphthylene	(6.1%)
Acenaphthene	(6.7%)
Flourene	(5.4%)
Phenanthrene	(5.2%)
Anthracene	(5.5%)
Flouranthene	(14.0%)
Pyrene	(12.4%)
Benzo(a)anthracene	(4.2%)
Chrysene	(4.4%)
Benzo(a)flouranthene	(3.6%)
Benzo(k)flouranthene	(4.6%)
Benzo(a)pyrene	(4.5%)
Dibenzo(a,h)anthracene	(3.5%)
Benzo(ghi)perylene	(8.5%)
Indeno(1,2,3-c,d)pyrene	(5.4%)

As a total the amount of these PAH compounds in this type of soil was 1930.8 mg/kg of this polluted desert soil. Weissenfels, *et al.* (1992) estimated 1815.1 mg of these PAHs compounds per kg of sandy soil collected from former wood impregnation plants, and 1027.5 mg/kg of heterogenous soil material from a former tar oil refinery.

Although hundreds of PAHs exist in the polluted environment, the U.S. Environmental Protection Agency (EPA) has identified the same above 16 PAHs as priority pollutants and they are monitored routinely for regulatory purposes.

In the present results (Table 2) the mutagenic and carcinogenic compounds flouranthene and pyrene were more frequent (15.3.0% and 12.4% respectively) than the other PAH compounds. Flouranthene, the most abundant PAH in environmental samples, has been reported to be cytotoxic, mutagenic and potentially carcinogenic (Rice, *et al.* 1982; West, *et al.* 1986; Boss, *et al.* 1987; Irvin and Martin, 1987; McElroy, *et al.* 1989). This compound is considered more of a potential health hazard by virtue of its abundance than the widely studied but less abundant carcinogen benzo(a) pyrene (Sakia, *et al.* 1985; Babson, *et al.* 1986; Boss, *et al.* 1987 and Mersh-Sundermann, *et al.* 1992).

Table (2) Polycyclic aromatic hydrocarbons (PAHs) of the contaminated desert soil of Kuwait at the beginning of the experiments (0-time) and after six months physical loss(%)

PAHs	No. of rings	0-time		6-month physical loss
		Mg/kg soil	%	
Naphthalene	2	115.0 ± 4.2	6.0 ± 0.2	14.1 ± 1.40
Acenaphthylene	3	118.0 ± 2.8	6.1 ± 0.11	0.5 ± 1.0
Acenaphthene	3	130.0 ± 2.8	6.7 ± 0.21	4.2 ± 0.6
Flourene	3	104.8 ± 3.0	5.4 ± 0.10	7.0 ± 0.3
Phenanthrene	3	100.0 ± 2.8	5.2 ± 0.10	6.3 ± 0.4
Anthracene	3	105.7 ± 2.6	5.5 ± 0.10	6.4 ± 0.3
Flouranthene	4	269.0 ± 12.7	15.3 ± 0.60	9.2 ± 0.6
Pyrene	4	240.1 ± 7.1	12.4 ± 0.40	8.2 ± 0.6
Benzo(a)anthracene	4	80.5 ± 2.8	4.2 ± 0.10	9.9 ± 0.4
Chrysene	4	85.5 ± 2.4	4.4 ± 0.14	9.2 ± 0.3
Benzo(a)flouranthene	5	70.0 ± 1.4	3.6 ± 0.03	5.4 ± 0.3
Benzo(k)flouranthene	5	88.5 ± 2.7	4.6 ± 0.14	5.4 ± 0.1
Benzo(a)pyrene	5	86.5 ± 2.1	4.5 ± 0.30	4.0 ± 0.3
Dibenzo(a,h)anthracene	5	68.3 ± 2.4	3.5 ± 0.10	3.4 ± 0.1
Benzo(ghi)perylene	6	164.0 ± 5.6	8.5 ± 0.20	5.4 ± 0.1
Indeno(1,2,3-c,d)pyrene	6	105.0 ± 7.1	5.4 ± 0.40	6.4 ± 0.3
Total		1930.8		8.2

± = standard deviation

Knopp, *et al.* (2000) stated that the four-ringed PAHs chrysene and dibenzo(a,h)anthracene and the six-ringed PAH indeno(1,2,3-c,d)pyrene are considered by the International Agency for Research on Cancer (IARC) as carcinogenic compounds. The German Drinking Water Act recommends the analytical determination of only six PAHs (flouranthene, benzo(a)flouranthene, benzo(k)flouranthene, benzo(a)pyrene, indeno(1,2,3-c,d)pyrene and benzo(ghi)pyrene).

According to the above results, PAH-polluted desert soil in Kuwait is of concern because some of the resolved PAHs are genotoxic, mutagenic and/or carcinogenic. Therefore, removal of such compounds has become of particular concern for environmental protection in the State of Kuwait.

The three bacteria that were used in preparing the mixed culture were identified as *Arthrobacter* sp, *Nocardia* sp. and *Pseudomonas* sp.

Results of total PAH biodegradation (Table 3 and Fig. 1) show that mixing the sandy polluted soil with non-polluted clay soil (10-20%) or its water extract, accelerated the biodegradation speed to reach 85.8-89.1% (i.e removal of 1656.0-1720.3 out of 1930.8 mg/kg soil). As a comparison, in the absence of clay soil 61.7-75.5% (i.e 1190.6-1458.6 out of 1930.8 mg/kg soil) of total PAHs were degraded. Moreover, when the mixed bacterial culture was immobilized on clay particles (T3), 1821.4 mg/kg (94.4%) of the total PAHs were degraded: this is in contrast to 75.5% due to the free mixed culture cells (T2). The NP fertilizers alone (T1) and the enriched culture (T7) failed to degrade more than 61.9% and 69.5% of PAHs respectively.

It is well known that immobilization may increase the biodegradation activity of microorganisms. Anchorage of the introduced cells in the polluted layer, especially by adsorption on the surface of the soil or ameliorant particles could prevent cell washout through the soil profile and their extrusion by local microflora (Siderov, *et al.* 1998). Crocker, *et al.* (1995) discussed that

contaminants sorbet to clay soil should be largely bioavailable to bacteria, since the adsorption rates for these materials are high and some degraders bacteria have the ability to directly utilize the sorbet contaminant.

Results of the biodegradation of the individuals of the 16 PAHs (Table 3, Fig. 1) show that the six-ringed PAHs benzo(ghi)perylene and indeno (1,2,3,-c,d) pyrene (a carcinogenic compound) were completely degraded due to treatments T3-T6 (treatments including clay soil or its extract). Although some investigators reported that high molecular weight PAHs of 5 and 6 rings resist extensive bacterial degradation. Smith, *et al.* (1999) confirmed the almost complete degradation of the aromatics from sandy soil and extensive disappearance from organic soil when these soils were initially inoculated with bacteria. Al-Awadhi, *et al.* (1996) reported that although most of the PAH reduction during the treatment of the polluted desert soil of Kuwait was due to losses of the three and four ringed compounds, considerable reduction was observed in the concentration of the five ringed compounds.

A particularly notable distinction between the immobilized (T3) and free cells (T2) mixed culture and also between T3 and the other treatments in this biodegradation study was the greater efficiency of the immobilized mixed culture to degrade the individuals of the 16 PAHs, especially the carcinogenic compounds: flouranthene (98.0%), pyrene (96.2%), chrysene (94.3%), benzo(a)pyrene (93.9%), and dibenzo(a,h)anthracene (93.6%). Wiesel, *et al.* (1993), found that the immobilized bacterial cells on granular clay improved the biodegradation of PAHs and the immobilized mixed culture proved to be very stable biological system. The microbial growth on this carrier material led to high densities and finally resulted in macroscopic visible biofilm. These immobilized cells can be stored, so this materials seems to be a good alternative for the utilization of free cells in the biological treatments of polluted soil.

Table 3 Effects of the different biological treatments (T1-T7) on the biodegradation of PAHs in the polluted desert soil of Kuwait

PAHs	0-time	Residual PAHs contents (mg/kg soil)						
	mg/kg	T1	T2	T3	T4	T5	T6	T7
Naphthalene	115.0±4.2	25.0±1.4	7.0±0.4	7.4±0.5	13.5±0.8	14.5±0.9	11.5±0.6	6.8±0.4
Acenaphthylene	118.0±2.8	88.8±2.5	49.0±0.4	-	11.7±0.4	18.0±0.7	25.5±2.2	10.4±0.7
Acenaphthene	130.0±2.8	57.3±3.1	19.5±0.8	12.0±0.7	23.8±1.3	18.0±0.8	19.8±1.8	37.8±2.5
Flourene	104.8±3.0	11.8±0.6	9.0±0.3	10.0±0.6	9.2±0.6	14.5±0.9	24.3±2.3	6.5±0.3
Phenanthrene	100.0±2.8	13.3±0.7	12.5±1.0	6.2±0.3	8.5±0.7	8.0±0.4	18.3±1.2	10.5±0.5
Anthracene	105.7±2.6	26.5±1.6	25.0±2.3	15.5±1.2	22.0±1.4	21.5±1.8	27.3±2.6	65.0±4.6
Flouranthene	269.0±12.7	24.8±2.1	61.0±3.4	5.4±0.4	18.5±1.1	17.5±1.3	18.3±0.8	46.0±3.6
Pyrene	240.1±7.1	62.7±3.4	33.3±2.6	9.4±0.3	21.0±2.0	27.0±2.1	25.5±1.7	37.8±3.1
Benzo(a)anthracene	80.5±2.8	66.3±5.2	38.5±2.4	11.7±0.6	18.5±0.9	15.7±0.7	19. ±1.2	36.5±4.2
Crysene	85.5±2.4	23.5±1.8	16.0±0.7	4.9±0.2	16.0±0.8	15.0±0.8	17.7±1.8	48.8±6.1
Benzo(a)flouranthene	70.0±1.4	40.2±0.6	23.8±1.8	9.2±0.6	16.3±0.8	12.7±0.4	24.2±1.2	47.0±2.2
Benzo(k)flouranthene	88.5±2.7	69.0±2.2	11.5±0.6	8.0±0.4	11.7±0.7	10.5±0.2	18.5±0.9	27.3±1.2
Benzo(a)pyrene	86.5±2.1	61.0±2.9	36.3±2.7	5.3±0.2	11.3±0.6	11.8±0.3	16.2±0.8	51.8±2.5
Dibenzo(a,h)anthracene	68.3±2.4	45.0±2.8	18.3±1.2	4.4±0.1	8.5±0.3	7.6±0.1	8.4±0.2	29.3±1.8
Benzo(ghi)perylene	164.0±5.6	40.0±7.9	50.0±0.6	-	-	-	-	60.0±3.6
Indeno(1,2,3-c,d)pyrene	105.0±7.1	85.0±4.6	62.5±3.8	-	-	-	-	72.0±4.3
Total	1930.8	740.2	472.7	109.4	210.5	212.3	274.8	593.6

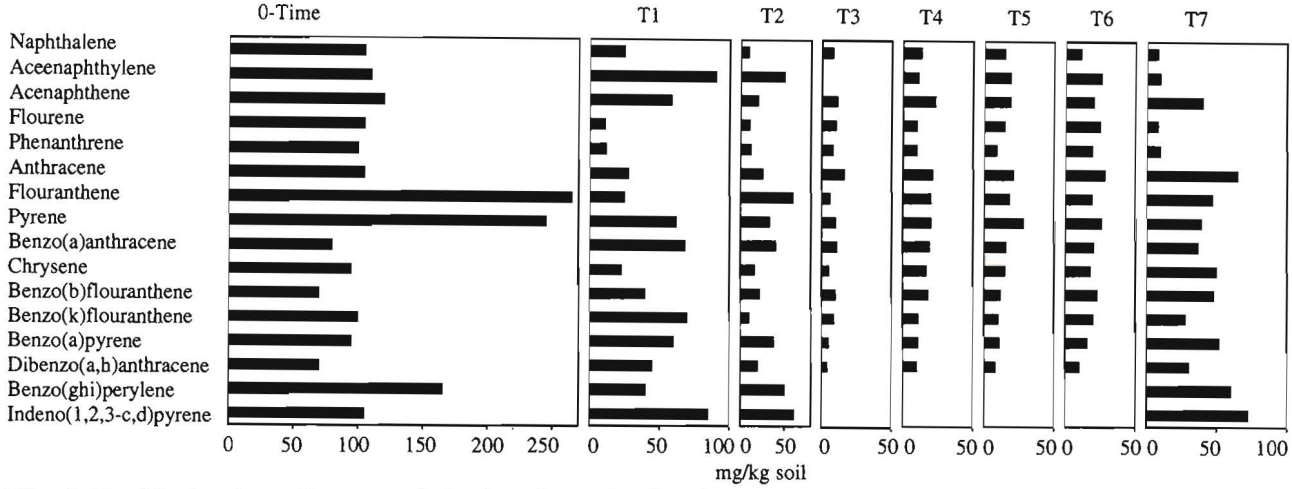

Fig. 1: Residual polycyclic aromatic hydrocrbons (PAHs) in the polluted desert soil of Kuwait by applying treatments T1 - T7

From our biodegradation study (Table 3, Fig. 1) it can be seen that clay soil and its water extract as compared to treatments without clay soil, stimulated the removal of significant amounts of the carcinogenic PAHs: flouranthene (93-93.4%), pyrene (89.0-91.4), chrysene (79.3-82.4%), benzo (a)pyrene (81.3-86.9%), dibenzo(a,h)anthracene (87.6-88.9%), and indeno(1,2,3-c,d)pyrene (100%).

The above results lead to the conclusion that mixing the polluted desert soil with non-polluted clay soil or its water extract seems to be a simple cost-effective bioremediation method. These clay particles could be used as amendment material and at the same time for immobilization of bacterial cells. These immobilized cells (as stated by Wiesel, *et al.* 1993) represent a stable biological system that could be stored without changes in the biodegradation activities of the organisms.

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Received 13th April 2004; in revised form 15th May 2004