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Phytoremediation of Polycyclic Aromatic Hydrocarbons (Pahs) in a Polluted Desert Soil, with Special Reference to the Biodegradation of the Carcinogenic Pahs

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Abstract: The rhizospheres of *Viccia faba, Zea mays* and *Triticum aestivum* were used for the removal of PAH compounds from an oil-polluted desert soil. The results show that, as a total,PAH compounds were reduced by 84.6%, 69.7% and 64.0% in the rhizosphere of *Viccia faba* , *Zea mays* and *Triticum aestivum* respectively. This is in contrast to 51.8% reduction value in the non-rhizosphere soil. Results of GC analysis for the detection of the loss (%) of the 16 PAH individuals show that the roots of the legume plant *Viccia faba* were able to remove 1016.3 (81.2%) out of 1251.7 mg/kg soil of the carcinogenic PAH compounds. This in contrast to 65.6% and 52.1% reduction values in the rhizosphere of the monocot plants *Zea mays* and *Triticum aestivum* respectively. From the non-rhizosphere soil only 29.9% of the carcinogenic PAHs were removed. A particular notable distinction between the rhizosphere of *Viccia faba* and of the other two plants is the greater efficiency of *Viccia faba* roots to degrade the carcinogenic PAH individuals, especially pyrene (91.8%), (90.1%), chrysene (79.4%) and benzo(a)anthracene (76.6%).

Key words: Phytoremediation, Poly aromatic hydrocarbons (PAHs), Contaminated desert soil, Oil pollutants

INTRODUCTION

Petroleum is a complex mixture made of thousands of compounds which can be divided into 4 major fractions: the alkanes, the aromatics, the resins and the asphaltenes. The aromatics especially the recalcitrant polycyclic compounds (PAHs) are of concern owing to their toxicity and tendency to bioaccumulation. PAHs are considered as hazardous because of their mutagenic and carcinogenic activities (Kalf *et al*., 1997).

Significant amount of PAH compounds are found in crude oil. Oil-polluted soil poses a major environmental and human health problem, and the removal of such compounds has become of particular concern for the protection of the environment.

Microorganisms and plants have complementary roles in phytoremediation of the polluted soil. Phytoremediation refers to the use of plants to clean contaminated soil (Joner *et al*., 2004). Frick *et al* (1999) reported the increase of biodegradation of organic contaminants in the rhizosphere soil, the zone of soil directly adjacent to and under the influence of plant roots.

For successful phytoremediation, both plants and microorganisms must survive and grow in crude oil-contaminated soil. Phytoremediation can be applied at moderate contamination levels or after the application of other remediation measures as a polishing step to further degrade residual hydrocarbons and improve soil quality (Schnoor, 1997; Frick *et al*, 1999).

The application of plants for remediation of contaminated soil with PAHs is one of the promising cost and environmental effective approach. Rock and Sayre (1998) estimated phytoremediation clean up coast of $\$162/m^2$ petroleum-contaminated soil compare to $\$810/m^2$ for excavation and incineration.

The objective of the present work is to study the effects of the rhizosphere of a legume plant (*Vicia faba)* and two monocot plants (*Zea mays)* and *(Triticum aestivum)* on the biodegradation of PAH compounds in a polluted desert soil. The advantage of the chosen legume plant is its ability to fix atmospheric nitrogen; this is in addition to the ability of this species to tolerate up to 10% (w/w) crude oil (Radwan *et al*, 2000). On the other hand the advantage of the chosen monocot plants is their extensive branching of the fibrous root system, resulting in a large root surface area per unit volume of surface soil. The fibrous roots would provide a larger surface for colonization by soil microorganisms than a tap root (White *et al*, 2006).

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MATERIAL AND METHODS

Field Experiments:

Were Four plots each of 2x2m^2 were delimited in an area (nursery of the Egyptian Environmental Affairs Agency, Suez Regional branch office) without any history of pollution. The soil in each plot at 0-50 cm depth were ploughed and thoroughly mixed with weathered crude oil so as to give initial concentration of 2.2-2.3% w/w soil. Each plot received the suitable NP concentrations (500 mg ammonium nitrate and 50 mg K_2HPO_4/kg soil).

- Plot No 1 was seeded with 100 viable *Vicia faba* seeds at the beginning of January (the normal growth period of this plant).
- Plot No 2 was seeded by 100 viable grains of *Zea mays* at the beginning of May (the normal growth period of this plant).
- Plot No 3 was seeded by 200 viable wheat *(Triticum aestivum)* viable grains at the beginning of November (the normal growth period of this plant).
- Plot 4 was left without seeding.

Another 4 plots (plots 4-8) received only nutrients (i.e. left unpolluted) to behave as control. The plots were separated by 2m from each other.

The viability of the seeds and grains were tested by soaking in distilled water for 5 minutes. The floated seeds and grains (non viable) were removed. The seeds and grains allowed to germinate and to grow.

After 60 days growth period of each plant, samples were taken from the rhizosphere and non-rhizosphere soil of each plant (both polluted and non-polluted). Samples also were collected from the non-cultivated plots. At the beginning of the experiments soil samples were also collected. Samples were analyzed chemically for the determination of residual hydrocarbons. Each of the developed plant shoot system was carefully removed, dried at 60°C and kept for further studies to detect if PAHs are accumulated in plant tissues or not.

The needed moisture was added (50% of the water holding capacity, (Vecchioli *et al*, 1990) at the beginning of the experiment and periodically to each plot. The soil in each plot was ploughed weekly for aeration.

Determination of the Residual Oil Fractions:

Ten grams of the air-dried soil samples were mixed with 10 grams of anhydrous sodium sulphate to remove moisture. The hydrocarbons were soxhlet extracted with chloroform for 8h. The chloroform extract was evaporated in a preweighed dish.

The extracted residual oil was suspended in n-hexane and filtered through tared filter paper to remove and to determine the insoluble fraction (asphaltene).

The hexane - soluble fraction was fractioned by liquid - solid chromatography into saturates, aromatics and resins. The amount of each fraction was determined (Chaineau *et al*, 1996).

Gas chromatography (GC) analysis of the aromatic fraction for the resolution of PAH compounds.

Although hundreds of PAHs exist in the polluted environment, the US Environmental Protection Agency (EPA) has identified 16 PAH compounds of priority pollutants, and which are monitored routinely for regulatory purposes.

In the present work identification and quantification of the individual 16 PAHs were determined in the aromatic fraction using Varian 3900 gas chromatography equipped with a CP 9050 liquid samples and configured with FID, using helium (Grade G) as a carrier gas, with a flow rate of 1ml/min. A CP Sil 19CB column (25 m long x 0.32 mm diameter x 0.2 mm thickness for the stationary phase 1 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 minutes) was applied. The total time of analysis was 45 min. injector and detector temperatures were 250°C and 280°C respectively injection volume was 1 uL or 2 uL for some samples.

The quantification of PAHs was based on the application of reference standard of the 16 PAHs (100 ppm for each), obtained from Supelco Co. Samples were run in duplicates and the mean values were taken.

RESULTS AND DISCUSSION

The polluted soil sample was sandy in nature, poor in phosphorus (0.19 ppm) and nitrogen (0.02%) with pH (7.6-7.8). one gram of this soil contained 29.0x10^4 CFU/g of total bacteria, 17.3x10^2 CFU/g of fungi and 22.0x10^2 CFU/g of oil-degrading microorganisms (Eman D., 2008, work submitted for publication).

When the residual oil was extracted and fractionated at the beginning of the experiment, it was composed of saturates (35.5%), aromatics (48%), resins (8.4%) and asphaltenes (8.1%).

Residual PAHs in the aromatic fraction quantified by GC-FID analysis. The results (Table 1) show the resolution of 16 different PAH compounds. As a total the amount of PAHs at the beginning of this work was 2854.6 mg/kg soil (Table 1). Weissenfels *et al*., (1992) estimated 1815.1 mg/kg sandy soil collected from former wood impregnation plants, and 1027.5 mg/kg of heterogeneous soil material from a former tar oil refinery.

Table 1: Polycyclic aromatic hydrocarbons (PAHs) in a desert soil polluted with crude petroleum and left for 50 days for physical loss, and used as control (at-0-time):

	PAH_s	No. of rings	mg/kg soil	%
1	Naphthalene	2	16.4 ± 1.1	0.6
2	Acenaphthylene	3	240.8 ± 12.3	8.4
3	Acenaphthene	3	605.5 ± 5.5	21.2
4	Flourene	3	364.2 ± 24.2	12.7
5	Phenanthrene	3	42.5 ± 2.5	1.5
6	Anthracene	3	187.1 ± 4.3	6.6
7	Flouranthene	4	75.3 ± 3.0	2.6
8	Pyrene	4	506.3 ± 16.3	17.7
9	Benzo(a) anthracene	4	142.5 ± 5.0	5.0
10	Chrysene	4	27.2 ± 0.3	1.0
11	Benzo (b) flouranthene	5	35.0 ± 0.0	1.2
12	Benzo (k) flouranthene	5	205.0 ± 5.0	7.2
13	Benzo (a) pyrene	5	37.8 ± 0.3	1.3
14	Dibenzo (ah) anthracene	5	196.3 ± 6.4	6.9
15	Benzo (ghi) perylene	6	111.3 ± 1.2	3.9
16	Indeno (1,2,3-c,d) pyrene	6	61.3 ± 3.8	2.1
Total			2854.6	

From the results (Table 1) it can be observed that acenaphthene and pyrene were more frequent than the other PAHs (21.2% and 17.7% respectively). This was followed by flourene (12.7%), acenaphthylene (8.4%), benzo (K) flouranthene (7.2%), benzo (ah) anthracene (6.9%) and anthracene (6.6%). Other PAHs are of lower frequency, they are in the range of 0.6%-3.9%.

Knopp *et al*. (2000) reported that the four-ringed PAHs chrysene and dibenzo (ah) 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. In addition to the above PAHs, Irwin (1997) reported the carcinogenicity of the following PAHs: Flouranthene, benzo (a) anthracene, benzo (b) flouranthene , benzo (k) flouranthene and benzo (a) pyrene. It was found that some co-carcinogenic activity was noted for both flouranthene and pyrene when combined with mixtures of other PAHs in dermal treatments of mice. PAH compounds usually occur in the presence of other PAHs, i.e. occur in mixtures. These PAH mixtures when present in water, sediments and in the internal tissues of organisms, often tend to be both carcinogenic and phototoxic (Irwin, 1997). Therefore, removal of such compounds has become of particular concern for the environment protection.

In the present study, rhizosphere technology was used for the removal of PAH compounds. Results of the ability of the rhizosphere of *Vicia faba ,Zea mays* and *Triticum aestivum* plants to degrade PAH compounds are found in Table (2) and Fig(1). As a total, PAH compounds were reduced by 84.6%, 69.7%, and 64.0% in the rhizosphere soil of *Vicia faba ,Zea mays and Triticum aestivum* plants respectively. This is in contrast to 51.8% reduction in the non-rhizosphere soil. These results demonstrate successful phytoremediation process as compared to the biostimulation process. Norina *et al* (2004) found positive rhizosphere effects of both maize and oat on microorganisms of the only contaminated soil in comparison with uncontaminated planted soil. Liu *et al*, (2004) reported that when plant roots are stressed e.g. in (PAHs) contaminated soil, the plants can exude certain enzymes to degrade or transform the pollutants. Studies have revealed a range of phenol oxidizing enzyme activities including tyrosinase, catechol oxidase, ascorbate oxidase and laccase.

The above results show that the legume plant *(Vicia faba)* roots stimulated more reduction value of total PAH compounds (84.6%) as compared to the two monocot plants *Zea mays* and *Triticum aestivum*. This may be due to the ability of *Vicia faba* to fix atmospheric nitrogen (Merkl, *et al*., 2005).

Results of GC analysis for the detection and quantification of the loss (%) of the 16 PAH individual in the rhizosphere of the three different plants as compared to the non-rhizosphere soil (Table 2) show that the different plant roots differ in their biodegradation capacity, this may be due to differences in the nature and composition of root exudates which reflect the biodegradation potential of the microbial community in each rhizosphere (Corgie *et al*., 2004). For simplicity the following results can be summarized according to the patterns of the loss (%) of the (PAHs) individuals as follows (Fig. 2):

Table 2: Biodegradation of the 16 PAH compounds in the rhizosphere soil of *Vicia faba (RV), Zea mays (RZ) and Triticum aestivum (RT)* plants as compared to non-rhizosphere soil (*S*).

	PAHs	0-timemg/kg soil	Loss(%) after a period of 50 days			
			S	*RV*	*RZ*	*RT*
1	Naphthalene	16.4 ±1.1	59.3	77.4	51.2	80.5
2	Acenaphthylene	240.8 ± 12.3	76.5	93.1	92.1	77.2
3	Acenaphthene	605.5 ± 5.5	77.1	97.3	78.9	91.2
4	Flourene	364.2 ± 24.2	89.4	88.1	75.6	79.9
5	Phenanthrene	42.5 ± 2.5	37.6	60.0	39.0	9.1
6	Anthracene	187.1 ± 4.3	39.6	64.7	67.0	47.4
7	Flouranthene	75.3 ± 3.0	22.2	66.8	66.1	3.1
8	Pyrene	506.3 ± 16.3	21.6	91.8	81.2	74.9
9	Benzo(a) anthracene	142.5 ± 5.0	32.6	76.6	70.2	50.9
10	Chrysene	27.2 ± 0.3	30.1	79.4	22.8	44.9
11	Benzo (b) flouranthene	35.0 ± 0.0	18.6	90.3	-	-
12	Benzo (k) flouranthene	205.0 ± 5.0	42.9	61.2	42.0	33.9
13	Benzo (a) pyrene	37.8 ± 0.3	6.9	90.1	58.4	43.6
14	Dibenzo (ah) anthracene	196.3 ± 6.4	32.0	90.5	64.8	31.6
15	Benzo (ghi) perylene	111.3 ± 1.2	19.1	59.3	47.9	50.1
16	Indeno (1,2,3-c,d) pyrene	61.3 ± 3.8	66.6	60.2	37.2	72.3
	Total	2854.6	21.8	84.6	69.7	64.0

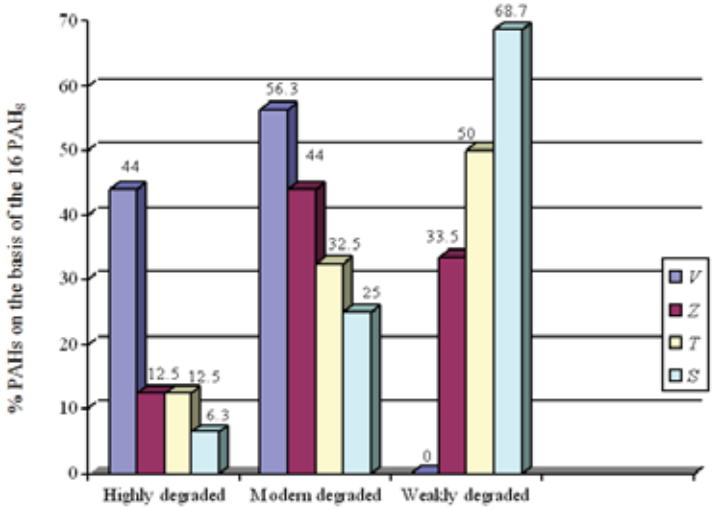

Fig. 1: Patterns of the number of PAHs subjected to phytoremediation by *Viccia faba* (*V*), *Zea mays* (Z), *Triticum aestivum* (T) as compared with the non-rhizosphere soil (*S*)

- **Pattern 1,** it includes the highly degraded PAHs (80-97%). It is represented by 7 (44%), 2 (12.5%) and 2 (12.5%) PAHs from the rhizosphere of *Vicia ,Zea, and Triticum* respectively. From the non-rhizosphere soil only one PAH (6.3%) was observed.

- **Pattern 2,** it includes the moderately degraded PAHs (50-79%). This group is represented by 9 (56.3%), 7 (44%) and 6 (37.5%) PAHs from the rhizosphere of *Vicia, Zea* and *Triticum* respectively. From the non-rhizosphere soil 4 PAHs (25%) were recorded.

- **Pattern 3,** it includes the weakly degraded PAHs (3.1-47.4%). No PAHs of this group was recorded in the rhizosphere of *Vicia.* On the other hand 6 (37.5%) and 8 (50%) PAHs were observed in the rhizosphere of *Zea* and *Triticum* respectively. It is of important to observe that 11 (68.7%) PAHs of this weakly degraded pattern were recorded in the non-rhizosphere soil (Fig. 2).

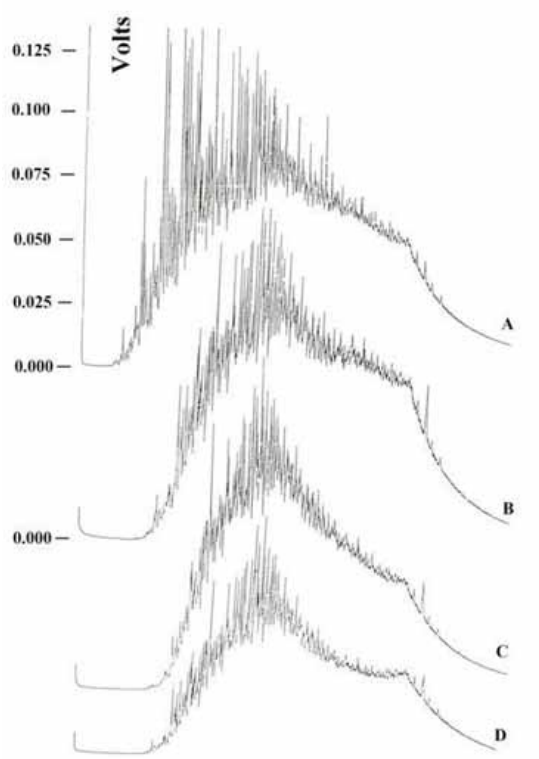

Fig 2: **A :** Gas Chromatograms showing the resolved PAHs at the beginning of the experiment (0-time).
B : Gas Chromatograms showing patterns of the residual PAHs extracted from the rhizosphere of *Triticum aestivum* after 60 days.
C : Gas Chromatograms showing patterns of the residual PAHs extracted from the rhizosphere of *Zea mays* after 60 days.
D : Gas Chromatograms showing patterns of the residual PAHs extracted from the rhizosphere of *Viccia faba* after 60 days.

The results of the biodegradation of the carcinogenic PAHs (Table3) show that *Vicia faba* roots were able to remove as a total 1016.3 (81.2%) out of 1251.7 mg/kg of the carcinogenic PAHs. This is in contrast to 65.6% and 52.7% reduction values in the rhizosphere of *Zea* and *Triticum* respectively. It is of important to observe that only 29.9% of the carcinogenic PAH compounds were removed from the non-rhizosphere soil, i.e. as a result of biostimulation.

Table 3: Biodegradation of the carcinogenic PAHs compounds in the rhizosphere of *Vicia faba(RV), Zea mays (RZ) and Triticum aestivum (RT)* plants as compared with the non-rhizosphere soil (*S*).

Carcinogenic PAHs	0-timemg/kg soil	*S*		*RV*		*RZ*		*RT*	
		Mg/kg soil	% Loss	mg/kg soil	% Loss	mg/kg soil	% Loss	mg/kg soil	%Loss
Flouranthene	75.3 ± 3.0	59.3±1.0	22.2	25.0±1.0	66.8	25.5±1.5	66.1	73.0±3.2	3.1
Pyrene	506.3± 16.3	397.0±9.0	21.6	41.5±1.5	91.8	95.0±5.0	81.2	127.0±3.0	74.9
Benzo(a) anthracene	142.5 ± 5.0	96.5±3.5	32.6	33.3±1.3	76.6	42.5±2.5	70.2	70.0±2.0	50.9
Chrysene	27.2 ± 0.3	19.0±1.0	30.1	5.6±1.1	79.4	21.0±2.0	22.8	15.0±1.0	44.9
Benzo(k) flouranthene	205.0 ± 5.0	117.0±1.0	42.9	79.6±1.1	61.2	119.0±11.6	42.0	135.5±1.9	33.9
Benzo(a) pyrene	37.8 ± 0.3	35.2±0.8	6.9	7.3±0.8	90.1	19.5±0.5	48.4	21.3±1.4	43.6
Benzo(ah) anthacene	196.3 ± 6.4	133.5±3.5	32.0	18.7±1.3	90.5	69.0±1.0	64.8	135.0±5.0	31.6
Indemo (1,2,3-cd) pyrene	61.3 ± 3.8	20.5±0.5	66.6	24.4±0.4	60.2	38.5±1.5	37.2	17.0±1.0	72.3
Total	1251.7	877.7	29.9	235.4	81.2	430.0	56.6	593.8	52.7

A particular notable distinction between the rhizosphere of *Vicia faba* and the rhizosphere of *Zea mays* and *Triticum aestivum* is the greater efficiency of *Vicia* roots to degrade the individuals of the carcinogenic PAHs especially, pyrene (91.8%), benzo(ah) anthracene (90.5%), benzo (a) pyrene (90.1%), chrysene (79.4%) and benzo (a) anthracene (76.6%).

In *Zea mays* rhizosphere, the higher reduction values were 81.2% for pyrene followed by 70.2% for benzo (a) anthracene. The other carcinogenic PAHs were biodegraded in the range of 22.8-66.1%.

In the *Triticum aestivum* rhizosphere, the higher reduction values were 74.9% for pyrene, followed by 72.3% for indeno (1, 2, 3-c,d) spyrene. The other carcinogenic PAHs were biodegraded in the range of 3.1-50.9%.

It must be observed that in the non-rhizosphere soil the higher biodegradation activity was 66.6% for indeno (1, 2, 3-c,d) pyrene. Other values ranged from 6.9-42.9%.

The above results lead to the conclusion that the reduction of PAHs in soil is enhanced in the presence of plants, and out of the three plants used in this phytoremediation process *Vicia faba* demonstrated more successful phytoremediation of the PAHs-polluted soil, this was followed by *Zea mays* and *Triticum aestivum*.

Nichols *et al*. (1997) found that hydrocarbon-degraders specifically were stimulated by the growth of Alfalfa plant *(Medicago sativa L.)*. Norima *et al*. (2004) reported positive rhizosphere effects of maize and oat on microorganisms of the only contaminated soil. The maize has provided a more influence as compared with oat. Lee *et al* (2008) found that the grass plant *Ecinochlora crus-galli* and the legume plant *Astragalusmembranaceus* were suitable candidates for phytoremediation of soils contaminated with PAH pollutants.

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