Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) in the Rhizosphere Soil of *Cyperus conglomeratus*, an Egyptian Wild Desert Plant

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Abstract: Phytoremediation is a promising technology for the clean-up of petroleum hydrocarbon-polluted soil, especially in the developing countries. In the present study, the rhizosphere soil of *Cyperus conglomeratus* (a wild Egyptian desert plan) was collected and studied for the removal of PAH compounds from the polluted desert soil. The rhizosphere soil of this plant was rich in total bacteria and oil-degraders. The rhizosphere soil was able after 180 days to reduce total PAHs from 2329.0 to 576.3 $mgkg^{-1}$ soil (i.e. 75.2% loss), this is in contrast to 45.2% reduction value for the non-rhizosphere soil. The rhizosphere soil significantly enhanced the biodegradation of the 16 PAH individuals (48.4-98.5%) as compared to the non-rhizosphere soil (23.1-94.4). The 2-ringed and the 3-ringed PAHs were highly degraded in the rhizosphere soil (98% and 93.1% respectively) as compared to the other PAH groups. Eight carcinogenic PAHs were resolved among the 16 PAH individuals. The sum of the 8 PAHs decreased in the rhizosphere soil from 1204.5 to 390.9 mgk^{-1} soil, i.e. a reduction of 67.7%, while in the non-rhizosphere soil the reduction value was 41.0%. Collectively, the 5-ringed carcinogenic PAHs were more degraded in the rhizosphere soil (87.8%) than the 4-ringed carcinogenic PAHs (59.2%). Both groups were weakly degraded in the non-rhizosphere soil (34.7% and 30.6% respectively).
A particular notable distinction of the rhizosphere soil of *Cyperus conglomeratus* plant is the greater efficiency to degrade the carcinogenic PAHs especially benzo(a)pyrene, (90.3%), chrysene (86.9%), benzo(a)flouranthene (84.1%) and indeno (1,2,3-c,d) pyrene (82.2%). The present study clearly demonstrates at the first time in Egypt, a successful bioremediation strategy of PAH-contaminated soil by using the rhizosphere effect of the native desert plant *Cyperus conglomeratus*.
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1. Introduction:

Petroleum oil and petrochemicals include significant amount of polycyclic aromatic hydrocarbons (PAHs), which are listed as priority pollutants by the US EPA (Oleszczuk and Baran, 2005). Some of these PAHs are toxic, mutagenic and carcinogenic to human (Kalf *et al*., 1997; Cerniglia, 1993). Accordingly, oil-polluted soils cause a major environmental and human health problem. For the protection of the environment, it is of important to remove such compounds.

Microorganisms and plants have complementary role in phytoremediation of oil-polluted soil (Joner, *et al*., 2004). The success of phytoremediation of hydrocarbon-contaminated soil is dependant on the plant capacity to enhance the microbial activity in the plant rhizosphere, i.e. the main factor for the remediation of contaminated soil is the rhizosphere microflora of the plant adapted to the contaminant (Muratova, *et al*., 2003).

Investigations on the absorption of hydrocarbons by roots and on accumulation and metabolization of these compounds in plant tissues indicate that these processes are not key mechanisms of phytoremediation of hydrocarbon-contaminated soil (Binet *et al*., 2000; Gunther *et al*., 2000).

Phytoremediation is limited by the growth rate of plants. More time may be required to phytoremediate a site, and may need several years. Therefore, for sites that pose acute risks for human and the environment, phytormediation may not the remediation technique of choice (U.S. EPA, 2000). Phytoremediation is also limited to sites with low to moderate contaminant concentrations Collins (2007) demonstrated that if the level of total petroleum hydrocarbons is equal to or less than 3% (w/w), then planting tolerant – species can proceed.

On the other hand, many plant species are sensitive to petroleum hydrocarbons such as polycyclic aromatic hydrocarbons (PAHs), so that,

they grow slowly, and it is time consuming to establish efficient biomass for bioremediation.

To overcome the above limitation, the present study was designed to collect rhizosphere soil from uncontaminated plants and polluting such soil in the laboratory with more than 3% (w/w) petroleum oil (e.g. 4% w/w). As a control non-rhizosphere soil was also collected and contaminated in the laboratory with the same amount of oil. The collected soil samples were then studied for the evaluation of their ability to enhance the biodegradation rate of the pollutant.

Al-Abdulla *et al* (2006) recorded the reappearance of the desert plant *Cyperus conglomeratus* in a polluted area near an oil lake after it was remained absent for a period of time after the great spill.

However, to the best of our knowledge there is no studies on the effect of the rhizosphere soil of the wild plant *Cyperus conglomeratus* on the removal of PAHs from the Egyptian contaminated desert soil. This is in addition to the lack of knowledge regarding the selection and availability of the native wild desert plants suitable for phytoremediation under the Egyptian climatic and ecological conditions.

2. Materials and Methods:

Collection of rhizosphere and non-rhizosphere soils

Samples of studies were collected from non-polluted desert area in the Suez road, in which *Cyperus conglomeratus* plant was dominant. Rhizosphere soil was collected by removing the soil adhering to the plant roots and aseptically introduced to sterile bags. At least 10 *Cyperus* plants in the same area were used for the collection of the rhizosphere soil. All samples were mixed to form one composite sample.

Non-rhizosphere soil samples were collected from 10 location of unvegetated area, and then mixed to form composite sample in sterile bag. Soil samples were collected from 5-25 cm depth.

Physical and chemical analysis of the soil

Physical and chemical analysis of the soil were determined according to the methods described by Jackson (1963-1967).

Soil Treatment

The collected soil samples were air-dried and sieved through 2 mm diameter opening. 100 g of the air-dried of each of the rhizosphere and non-rhizosphere soil was introduced into a 600 ml beaker. For each type of soil two beakers were prepared.

The soil in each beaker was polluted in the laboratory with 4% (w/w) crude petroleum oil and was mixed with NP fertilizer in the form of KNO3 (100 mg) and K2HPO4 (50mg). The moisture content of each treatment was adjusted at 60% of its water holding capacity. Each beaker was covered by a thin aluminum foil to reduce evaporation. For tilling of the soil in each beaker, a small glass rod was used that was an integral part of the treatment. The loss of water due to evaporation in each treatment was determined at the beginning of the experiment and every 4 days, and the amount of water loss was added. All beakers were incubated at 30C^{o} for a period of 180 days.

Microbiological analysis

Total colony forming units per gram air-dried soil (CFU/g soil) of total heterotrophic bacteria, actinomycetes and fungi were counted using the usual dilution plate technique. Nutrient agar (Oxoid) supplemented with 0.4% (w/w) soluble starch was used for the enumeration of total bacteria and actinomycetes. For counting fungi, malt extract-yeast extract agar was used. This medium contained (per litre) 20 g malt extract, 5 g yeast extract and 15 g agar.

Incubation periods at 30 C^{o} were 5-7 days for bacteria and 10-15 days for actinomycetes and fungi. At the end of the incubation periods the developed colonies were counted and expressed as CFU/g air-dried soil.

For counting oil-degrading microorganisms, the three tube Most Probable Number (MPN) method was used as described by Chaineau *et al*., (1996).

Extraction of the residual oil and its fractions

At the beginning of the experiment (O-time) and at the end of 180 days, a known weight (5g) of the air dried soil was mixed with 3g anhydrous sodium sulfate to remove moisture, and then chloroform extracted by using the shaking method described by Chen *et al*., (1996). The extract was pooled and 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. The hexane soluble fraction was fractionated by liquid-solid chromatography into saturates and aromatics (Chaineau, *et al*., 1995). The saturated fraction was discarded, and the aromatic fraction in benzene was reduced to 1 ml under a stream of nitrogen. One µl of this extract was used for GC analysis.

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

Identification and quantification of the individual polycyclic aromatic hydrocarbon (PAHs) were determined using Chromopack CP 9001 gas

chromatogaph equipped with a CP 9050 liquid samples and configured with FID, using helium as a carrier gas, with a flow rate of 1ml/min. CP sil 19CB colum (25 m length x 0.32 mm diameter x 0.2 µm thickness for the stationary phase) was used. Temperature programming of initial holding at 40 C^o (2 min), and then heating with a rate of 10 C^o /min to 250 C^o (holding 2 min) was applied. The total time of analysis was 45 min. Injector and detector temperature were 250 C^o and 280 C^o respectively. Injection volume was 1 µ for all samples. The quantification of PAHs was based on application of reference standard (obtained from Supelco Co.) contained a mixture of the 16 priority PAHs (100 ppm for each individual).

3. Results and Discussion:

The results of mechanical analysis of the soil samples showed that this type of soil contained 5.1% gravel, 43.5% coarse sand, 45.1% fine sand, 4.9% silt and 1.4% clay. Chemical analysis indicated that this soil was characterized by pH 7.8%, poor in P (0.14 ppm), poor in nitrogen (80 ppm) and poor in organic carbon (0.1%).

Results of the total counts (CFU/g dried soil) of microorganisms in the rhizosphere soil of *Cyperus conglomeratus* as compared to non-rizosphere soil at the beginning of the experiment (0-time) and at the end of 180 days incubation period are given in Table (1).

Table 1: Mean CFU counts / g soil of total heterotrophic bacteria, actinomycetes, fungi and oil-degraders in the rhizosphere soil (R) of *Cyperus conglomeratus* plant as compared to the non-rhizosphere soil (S). ±: standard deviation (n=2)

Micgoorganism	O-time			180-days		
	Count / g soil			Count / g soil		
	R	S	R/S	R	S	R/S
Total bacteria $x10^7$	3.2±0.3	0.75±0.07	4.3	34.2±1.7	1.24±0.2	27.5
Actinomycetes $x10^5$	4.7±0.4	0.3±0.03	15.7	1.1±0.14	0.11±0.01	7.8
Fungi $x10^4$	28.4±2.5	1.4±0.3	20.3	18.4±1.1	1.01±0.3	18.2
Oil-degraders $x10^7$	0.5±0.01	0.02±0.001	25.0	18.6±1.1	0.12±0.03	155.0
Oil-degraders (%)	15.6±1.0	2.7±0.2	9.2	54.4±3.4	9.7±0.8	5.6

Generally, counts of the microbial groups were higher in the rhizosphere soil than in the non-rhizosphere soil, this is indicated from the values of R/S (counts in the rhizosphere/counts in the non-rhizosphere) of more than one. The results show that the R/S values of total bacteria and oil-degraders were more pronounced (27.5 and 155 respectively).

Total bacteria ($x10^7 g^{-1}$) in the rhizosphere soil significantly increased from 3.2+0.3 to 34.2+1.7, i.e. with increased factor of 10.69, while in the non-rhizosphere soil no significant increase was detected. Different authors demonstrated that the rhizosphere soil can support microbial communities of several orders of magnitude higher than that in the non-rhizosphere soil (Eman, 2008; Liste and Prutz, 2006; Al-Abdulla *et al*., 2006; Al-Gonaim and Diab, 2002; Nacek *et al*., 2000; Siliciano and Germida, 1998; Curi and Truelova, 1986). Some authors (e.g. Al-Gonaim and Diab, 2002, Schaffner *et al*., 1998) reported that bacterial enumeration is a screening tool which can be used to evaluate the *in situ* response of soil bacteria to petroleum contamination, and the elevation of bacterial densities in the contaminated soil suggested that native soil bacteria are currently biodegrading the oil contaminants.

Results of the count of oil-degraders (Table 1) show that this group of microorganisms was more abundant in the rhizosphere soil of *Cyperus* than in the non-rhizosphere soil.

At the end of 180 days incubation period the counts ($x10^7$ g^{-1}) of the oil-degraders significantly increased from 0.5 to 18.6, i.e. with increasing factor of 37.2 and percentages of 54.4%. On the other hand, no significant increase of the counts in the non-rhizosphere soil was observed. Atlas (1995) reported that hydrocarbon-degrading microorganisms are widely distributed in soils and constitute less than 1% of the total microbial community, but may increase to more than 10% in presence of crude oil. Some authors (e.g. Kim *et al*., 2004; Ling and Gao, 2004; Leigh *et al*., 2002) reported that rhizosphere soil may contain greater numbers of hydrocarbon-utilizing bacteria than bulk soil. Al-Abdullah *et al* (2006) found that oil-polluted non-rhizosphere soil contained very low concentrations of oil-degraders, ranged from 0.1-0.3 $x10^6$ $CFUg^{-1}$ soil, and with percentages

of 5.0-6.6% of the total heterotrophic bacteria. Different authors (e.g. Muratova *et al*., 2003; Wiltse *et al*., 1998; Nichols *et al*., 1997) reported that the efficiency of phytoremediation is connected with high number of degraders microorganisms and their degradative activity.

As for the numbers of actinomycetes and fungi the results (Table 1) show that the count of actinomycetes ($CFUx10^6$ g-1 soil) and of fungi ($CFUx10^4$ g^{-1} soil) in the rhizosphere soil at the end of 180 days decreased from 4.7 (at 0-time) to 1.1 and from 28.4 to 18.4 respectively. In the non-rhizosphere soil the same trend of results was recorded in which actinomycetes and fungal counts decreased from 0.30 to 0.11 and from 1.40 to 1.01 respectively. These results demonstrate negative response of the two groups of microorganisms to the concentration of oil (4%) used in the present study. Muratova *et al* (2003) found a decrease in the number of actinomycetes in contaminated unplanted soil and in the rhizosphere soil of *Phragmitis australis*, and no change was observed in the rhizosphere soil of *Medicago sativa*. Eman (2000) found that the actinomycetes flora in an Egyptian clay soil showed good positive response to oil pollutants at a concentration of 2.5% (w/w) oil, and negative response to the increased concentration of oil. Jensen (1975) found that a number of actinomycetes were able to degrade hydrocarbons, though these organisms do not seem to compete as successfully in a contaminated soil, their lower growth may infer a more dominant role in the later stages of hydrocarbon biodegradation.

In the present study, the negative response of fungi to oil pollutants may find a support in the study Eman (2000) who demonstrated weak response of fungi to the application of 2.5-10% (w/w) oil to an Egyptian clay soil sample. She explained that although the clay soil was rich in fungi, application of 2.5% oil did not stimulate the development of high counts. On the other hand Al-Gounaim *et al* (1995) found that fungi in polluted desert soil in Kuwait exhibited a more positive response to oil pollution.

At the beginning of the experiment the collected non-rhizosphere soil and rhizosphere soil of the wild desert of *Cyperus conglomeratus* were artificially polluted in the laboratory with 4% (w/w) crude petroleum oil. Residual PAH compounds at the starting time of the experiment and at the end of 180 days incubation period were extracted and quantified using GC-FID analysis. The results (Table 2 and Fig. 1) demonstrate the resolution of the 16 US EPA priority PAHs. As a total the sum of the initial 16 compounds was 2329 mg kg-1 soil. Cofield *et al* (2007) estimated initial concentration of 3506 mg kg-1 MGP soil (manufactured gas plants). Al-Gounaim *et al* (2004) found 1930.8 mg kg-1 of the polluted desert soil in Kuwait. On the other hand, Weissenfels *et al* (1992) determined 1815.1 mg kg^{-1} of sandy soil collected from former wood impregnation plants, and 1027.5 mg kg^{-1} in a heterogenous soil material extracted from a former tar oil refinery.

The results (Table 2 and Fig. 1) show that the highest PAH content (mg kg-1 soil) was pyrene (340 mg) followed by flouranthene (270 mg).

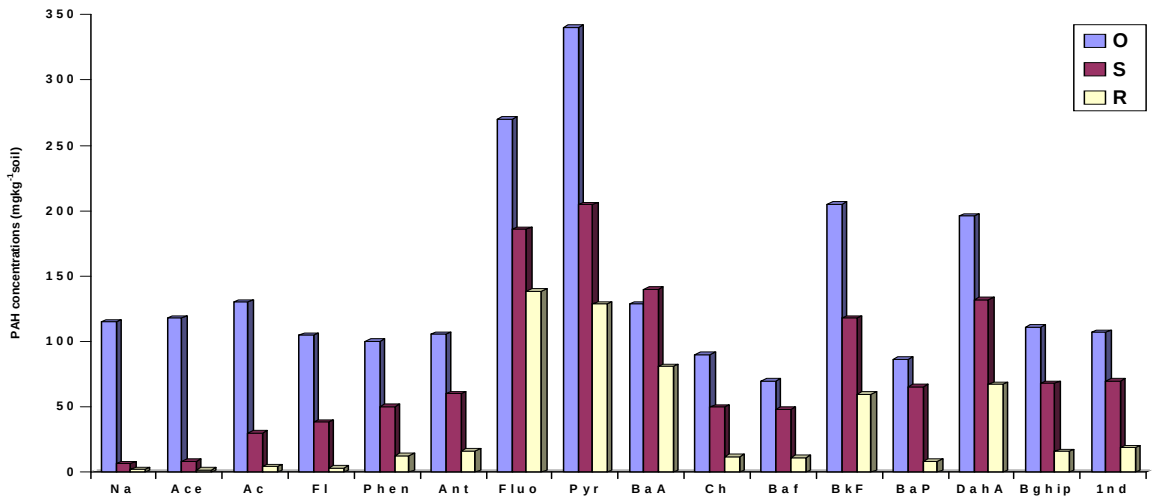


Fig. 1: Residual PAHs (mgkg^{-1} soil) in the rhizosphere soil (R) and in the non-rhizosphere soil (S) of *Cyperus conglomeratus* after 180 days, as compared to O-time.

PAHs

Table (2) Biodegradation of the 16 PAH individuals in the rhizosphere (R) of *Cyperus conglomeratus* as compared to that in the non-rhizsphere soil (S) ± = standard deviation (n-3) NS=Non significant, S=Significant

PAHs	No. of Rings	0-time mg kg^{-1}	180days				P<0.05
			R		S		
			mg kg^{-1}	Loss (%)	mg kg^{-1}	Loss (%)	
1. Naphthalene	2	115.0±7.1	2.3±0.4	98.5	6.4±0.5	94.4	NS
2. Acenaphthylene	3	118.0±2.8	1.8±0.2	98.5	8.3±0.48	93.0	NS
3. Acenaphthene	3	130.0±2.6	4.4±0.3	96.6	30.1±2.6	76.8	S
4. Flourene	3	104.8±5.6	3.4±0.7	96.7	38.4±3.5	63.4	S
5. Phenanthrene	3	100.0±3.8	12.6±2.7	87.4	50.2±6.1	49.8	S
6. Anthracene	3	105.7±5.8	16.3±2.1	84.6	60.2±7.1	43.0	S
Total		558.5	38.5	93.1	187.2	66.5	
7. Flouranthene	4	270.0±18.7	138.3±7.5	48.8	186.1±7.5	31.1	S
8. Pyrene	4	340.0±37.4	128.8±5.7	62.1	204.6±17.7	39.8	S
9. Benzo[a]anthracene	4	182.0±22.4	81.1±6.9	55.4	140.0±8.0	23.1	S
10. Chrysene	4	90.0±12.2	11.8±1.5	86.0	50.1±6.3	44.3	S
Total		882.0	360.0	59.2	580.8	34.2	
11. Benzo[a]flouranthene	5	70.0±3.7	11.1±1.4	84.1	48.2±3.3	31.1	S
12. Benzo[k]flouranthene	5	205.0±18.7	53.8±7.5	73.6	118.4±10.6	42.2	S
13. Benzo[a]pyrene	5	86.5±5.0	8.4±0.5	90.3	65.6±5.4	24.2	S
14. Dibenzo[ah]anthracene	5	196.0±8.9	67.4±5.2	65.6	132.0±4.5	32.7	S
Total		557.5	140.7	74.8	364.2	34.7	
15. Benzo[ghi]perylene	6	111.0±3.7	16.1±103	85.5	66.2±3.5	44.4	S
16. Indeno[1.2,3-c d]pyrene	6	105.0±7.4	18.7±2.2	82.2	70.1±6.7	33.2	S
Total		216.0	34.8	83.9	136.3	36.9	
Total		2329.0	576.3	75.2	1274.9	45.2	

The two compounds represented by 14.6% and 11.6% respectively of all compounds determined. Considerable amounts (mg kg-1 soil) of benzo (k) flouranthene (205 mg), dibenzo(ah)anthracene (196 mg) and benzo(a)anthracene (182 mg) were observed. The concentration of the other PAHs (mg kg-1 soil) were in the range of 70 mg (for benzo (a) flouranthene) to 130 mg (for acenaphthene). Cofield *et al* (2007) found that at the initial of the experiment, the highest PAH content (mg kg-1 soil) was benzo(a)pyrene (750 mg, 21.2%) followed by pyrene (416 mg, 11.9%) and flouranthene (317 mg, 9.0%). Al Gounaim *et al* (2004) found that flouranthene and pyrene were more frequent in the polluted desert soil in Kuwait (15.3% and 12.4% respectively) as compared to the other individuals of the 16 PAHs).

Flouranthene the most abundant PAH in environmental samples, has been reported to be cytotoxic, mutagenic and potentially carcinogenic (McElroy *et al*., 1989; Irvin and Martin, 1987; West *et al*., 1986; Rice *et al*., 1982). This compound is considered more of a potential health hazard by virtue of its abundance than the widely studied but less abundant carcinogenic benzo(a)pyrene (Mersh-Sundermann *et al* 1992; Sakia *et al*., 1985).

In the present study, the sum of the 4-ringed PAHs (Table 2) was of more concentration (882 mg kg^{-1} soil, 37.9%) as compared to the other PAH groups. This was followed by the 3 and 5-ring compounds which were at similar levels (558.7 mg kg-1 soil, 29.9% and 557.5 mg kg-1 soil, 29.0%), on the other hand, the two-ringed and the six-ringed PAHs were relatively low (4.9% and 9.3% respectively). These results find a support in the work of Oleszczuk *et al*. (2007) who found nearly similar results.

Results of the effects of the rhizosphere soil of *Cyperus* plant on the biodegradation of the 16 PAH compounds are found in Table (2) and Fig. (1). These results indicate that the sum of the 16 PAHs recorded after 180 days was 576.3 $mgkg^{-1}$ soil, i.e. with a reduction of 75.2%. This is in contrast to only 45.2% reduction value in the non-rhizosphere control soil. Lee-SangHwan *et al*. (2008) demonstrated that more PAH compounds were dissipated in the rhizosphere soil of grasses and legumes, than in the unplanted soil. Rezek *et al* (2008) estimated 50% reduction of total PAHs in the rizhosphere soil of ryegrass after one year cultivation period. Cofield *et al*. (2007) found that fertilized switchgrass and tall fescue treatment resulted in an overall dissipation of 67%

total PAHs following 12 months treatment. They also found that approximately 28% degradation of total PAHs was observed following 90 days treatment in greenhouse when planted with zucchini.

Results of the biodegradation of the 16 PAHs (Table 2, Fig. 1) show that after 180 days the rhizosphere soil significantly enhanced the dissipation rates of all of the 16 PAH individuals (48.8% - 98.5%) as compared to the non-rhizosphere soil (23.1%-94.4%).

As a total the 2-ringed and the 3-ringed PAHs as compared to the other PAH groups were highly degradable in the rhizosphere soil (98.1% and 93.1% respectively) than in the non-rhizosphere soil (94.4% and 66.5% respectively). The 4-, 5- and 6-ringed PAHs were weakly degraded in the non-rhiosphere soil (34.2% , 34.7% and 36.9% respectively), while more of these PAH groups were degraded in the rhizospehre soil (59.2% , 74.8 and 83.9% respectively). Cofield *et al*. (2007) demonstrated that the 4-ringed and 5-ringed PAHs were more degraded in the planted soil (48.6% and 46.1% respectively) than the 2- 3- and 6-ringed PAHs (3%, 25.8% and 6.1% respectively). Olson *et al*. (2007) reported that the extend of removal of lower molecular weight PAHs was similar for planted and unplanted control soil after 14 month. On the other hand, the total mass of 5- and 6-ring PAHs was significantly greater in planted soil after 7 and 14 months.

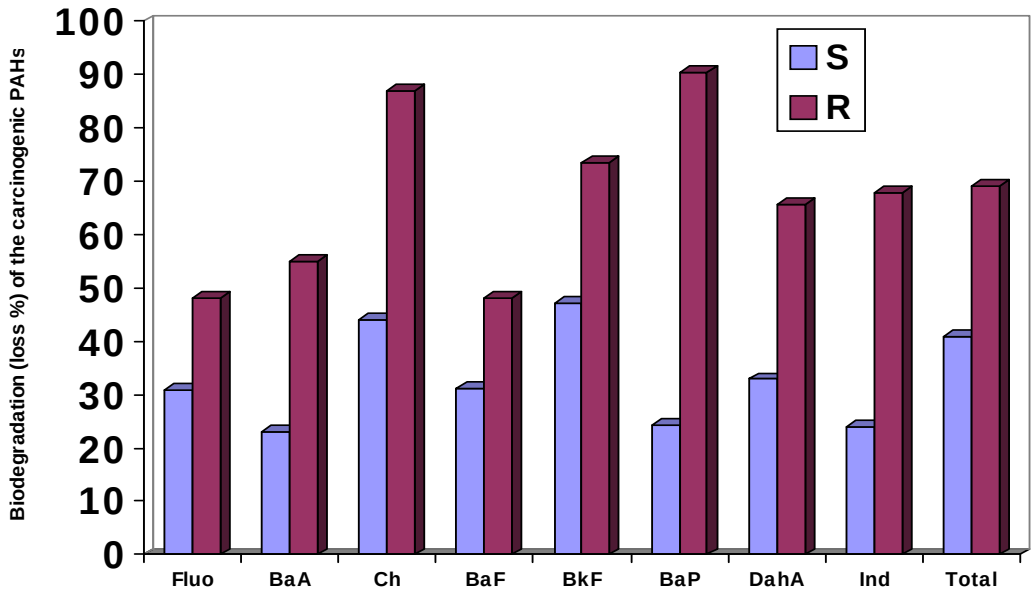
For simplicity, the degradabilities of the 16 PAHs can be categorized into the following groups.

1- Highly degradable PAHs (82.2% - 98.5%). This group includes 13 PAH individuals, of which 11 characterized the rhizosphere soil and only two from the non-rhizosphere soil.

2- Moderately degradable PAHs (55.4- 76.8%). Six PAHs are included in this group, 4 from the rhizosphere soil and two from the non-rhizosphere soil.

Weakly degradable PAHs (23.1- 49.8%). It comprises 13 PAHs, only one of which in the rhizosphere soil, while the remaining 12 PAHs are characteristics of non-rhizosphere soil.

Results of the effect of the rhizosphere as compared to the non-rhizosphere soil on the biodegradation of the carcinogenic PAH compounds are found in Table (3) and Fig. (2). These results show that 8 carcinogenic PAHs were resolved among the 16 PAH individuals. At the beginning of the experiment (0-time) they were represented by a total of 1204.5 $mgkg^{-1}$ soil out of 2329.0 $mgkg^{-1}$ soil (i.e. 51.7%).



Ca

Fig. 2: Biodegradation (loss%) of the different carcinogenic PAH individuals in the rhizosphere soil (R) and in the non-rhizosphere soil (S) of *Cyperus conglomeratus* after 180 days.

Table (3) Biodegradation of the carcinogenic PAH individuals in the rhizosphere soil (R) of *Cyperus conglomeratus* as compared to that in the non-rhizsphere soil (S) ± = standard deviation (n-3)

Carcinogenic PAHs	No. of Rings	0-time mg kg^{-1}	180days			
			R		S	
			mg kg^{-1}	Loss (%)	mg kg^{-1}	Loss (%)
Flouranthene	4	270.0±18.7	138.3±7.5	48.8	186.1±7.5	31.1
Benzo[a]anthracene	4	182.0±22.4	81.1±9.9	55.4	140.0±8.8	23.1
Chrysene	4	90.0±12.2	11.8±1.5	86.9	50.1±9.3	44.3
Total		542.0	231.2	57.3	376.2	30.6
Benzo[a]flouranthene	5	70.0±3.7	11.1±1.4	84.1	48.2±3.3	31.1
Benzo[k]flouranthene	5	205.0±18.7	53.8±7.0	73.6	118.4±10.6	42.2
Benzo[a]pyrene	5	86.5±5.1	8.4±0.5	90.3	65.6±5.4	24.2
Dibenzo[ah]anthracene	5	196.0±8.9	67.4±5.2	65.6	132.0±4.5	32.7
Total		557.5	140.7	74.8	364.2	34.7
Indeno[1,2,3-c,d]pyrene	6	105.0±7.4	18.7±2.2	82.2	70.1±6.7	33.2
Total		1204.5	390.6	67.6	710.5	41.0

At the end of 180 days incubation period the sum of the 8 carcinogenic compounds decreased from 1204.5 mg kg-1 soil to 390.6 $mgkg^{-1}$ soil (i.e. decreased by 67.6%) in the rhizosphere, and to 710.5 $mgkg^{-1}$ soil (i.e. 41.0%) in the non-rhizosphere soil. Collectively, the 5-ring carcinogenic PAHs as compared to the 4-ring PAHs were more degraded (74.8%) in the rhizosphere soil.

According to the degradability propreties of the 8 PAHs, in the rhizosphere soil, they are grouped as follows:

Highly degradable carcinogenic PAHs (80%-90%). They are four compounds namely:

Benzo(a)pyrene (90.3%).
Chrysene (86.9%).
Benzo(a)flouranthene (84.1%).
Indeno(1, 2, 3-c,d) pyrene (82.2%).

Moderately degradable carcinogenic PAHs (55.4-73.6%). This group includes three compounds:

Benzo(k)flouranthene (73.6%).
Dibnezo(ah)anthracene (65.6%).
Benzo(a)anthracene (55.4%).

Weakly degradable PAHs (less than 50%), it is represented only by the compound flouranthene.

As a comparison, all the 8 carcinogenic compounds were weakly degraded in the non-rizosphere soil (23.1%-44.3%).

A particular notable distinction of the rhizosphere soil of *Cypenus conglomeratus* is the greater efficiency to degrade the carcinogenic PAHs especially benzo(a)pyrene (90.3%), chrysene (86.9%), benzo(a)flouranthene (84.1%) and indeno(1,2,3-c,d)pyrene (82.2%).

Knopp *et al* (2000) reported that the four- ringed PAH chrysene and the five- ringed PAH dibenzo(ah)anthracene, and the six-ringed PAH indeno(1,2,3-c,d)pyrene are considered by the International Agency for Research Cancer (IARC) as carcinogenic compounds. Other PAH compounds are known to have carcinogenic activity (Irwin, 1997) such as flouranthene, benzo(a)anthracene, benzo (b) flouranthene, benzo (k) flouranthene and benzo (a) pyrene.

PAH compounds usually occur in mixtures in the environment, and they tend to be more carcinogenic. Irwin (1997) reported that co-carcinogenic activity was noted for both flouranthene and pyrene when combined with mixture of other PAHs in dermal treatment of mice. It is relatively clear that PAH mixtures in water, sediments and organisms internal tissues often tend to be both carcinogenic and phytotoxic.

Rezek *et al* (2008) found that after one year cultivation period of ryegrass (*Lolium perenne*) best degraded PAHs were flouranthene, pyrene and benzo(a)pyrene, while no significant degradation was observed for dibenzo(ah)anthracene and indeno(1,2,3-c,d)pyrene. Oleszczuk and Baran (2007) found that in the rhizosphere soil the content of benzo(a)flouranthene, benzo(a)pyrene and

indeno(1,2,3-c,d)pyrene was less by more than half than the case in the control soil.

The present study clearly demonstrated for the first time in Egypt, a successful bioremediation of PAH compounds by using the rhizosphere soil of the native wild desert plant *Cyperus conglomeratus*. As a result of the lack of knowledge regarding the selection and availability of wild desert plants suitable for phytoremediation under the Egyptian climatic and ecological conditions, a survey must be conducted to determine which plant species will be able to support higher rhizosphere activity, at the same time to accelerate the biodegradation process by applying favorable conditions.

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