Biodegradation of PAH Compounds in the Rhizosphere of *Tamarix nilotica:* A Salt tolerant wild plant

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Abstract: During a scientific visit to a coastal area at Suez, Egypt, it was observed that *Tamarix nilotica* plant naturally dominated on oil polluted site in this area, indicating that this plant is a tolerant of the combined adverse effects of salinity and petroleum pollutants. This observation stimulated a study to investigate the rhizosphere effect of this plant on the degradation and removal of petroleum aromatic hydrocarbons (PAH) compounds from this coastal saline soil. Accordingly, samples were collected from the rhizosphere and from the non-rhizosphere soil and studied. The results show that the rhizosphere soil of *Tamarix nilotica* was rich in total heterotrophic bacteria and oil-degraders. In the rhizosphere soil oil-degraders were of higher percentage (30.7%) compared to the non-rhizosphere soil (4.6%). Residual total petroleum hydrocarbons (TPH) in the non-rizosphere soil was 2.25% (w/w), while in the rhizosphere soil the percentage was 0.9% (w/w). This indicate a reduction of 60% of the TPHs. The saturates fraction in the rhizosphere as compared to the non-rizosphere soil was reduced by 87.5%, while the aromatics were reduced by 60.7%. It is of interest to find that the non-degradable asphaltenes and resins were reduced in the rhizosphere by 1.1% and 2.5% respectively. As a total the amount of PAHs ($mgkg^{-1}$ soil) were 1073.5 and 541.94 in the non-rhizosphere and rhizosphere soil respectively, i.e. with a loss of 49.5% in the rhizosphere. Chrysene and dibenzo(ah)anthracene as compared to the other PAHs were more frequent in the non-rhizosphere soil. These two compounds were reduced by 55.7% and 24.3% respectively in the rhizosphere. As a total the four-ringed PAHs as compared to other PAH groups were highly reduced (60.3%) in the rhizosphere, this was followed by the three-ringed PAH group (52.5%). The five-ringed and the six-ringed groups were weakly reduced (37.8% and 33.8% respectively). The 8 carcinogenic PAH group were collectively reduced in the rhizosphere by 49.1%. A particular notable distinction of the rhizosphere of *Tamarix nilotica* is the greater efficiency to degrade the carcinogenic PAH compounds especially flouranthene (75.4%), benzo(a)anthracene (63.4%) and pyrene (60.2%). Results of Gas Chromatography (GC) analysis for the detection of the accumulated PAHs in the shoot tissue of *Tamarix nilotica* plant growing in the polluted area as compared to that growing in non-polluted area show that the identified peaks in the tissue of both plants were 15 and 14 peaks respectively. The sum of the 15 PAHs was 528 $mgkg^{-1}$ dried tissue, whereas the sum of the 14 PAHs was 769 $mgkg^{-1}$ dried soil. This result indicate an accumulation value of 1.46.

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1. Introduction:

Microorganisms and plants can have complementary roles in phytoremediation of polluted soils. Phytoremediation refers to the use of plants to clean up moderately contaminated soils (Joner *et al*., 2004). When the pollutants are organic compounds, phytremediation may comprise rhizodegradation (microbial degradation in the rhizosphere) and phytodegradation i.e. degradation of compounds absorbed by plants (Flathman and Lenza, 1998).

Rhizodegradation of organic pollutants such as PAHs based on the effects of plant root exudates which constitute different growth factors, enzymes, vitamins, amino acids … etc. (Corgie *et al*., 2004). The application of plants for remediation of soil contaminated with PAHs is one of the promising environmental and cost effective approach. Rock and Sayre (1998) estimated phytoremediation cleanup cost of $\$162/m^2$ petroleum contaminated soil compare to $\$810/m^2$ for excavation and incineration.

To date a great variety of grass species and legumes (Appril and Sims, 1990; Flathman and Lenza, 1998; Pradham *et al*., 1998; Banks *et al*., 1999; Kulakow *et al*., 2000; Davis *et al*., 2004 and Eman, 2008) have been applied for phytoremediation, but the predictive power of these phytoremediation studies is compromised by the brief duration and constant environmental conditions often employed (Muller and Shann, 2006).

Trees have received little attention with regard to the dissipation of PAH compounds, although their perennial life-history and extensive root systems suggest they may be suitable for phytoremediation (Muller and Shann, 2006). Trees such as poplar (Jordahl *et al*., 1997, Newman *et al*., 1999; Tessar *et al*., 2000), alder (Caraman *et al*., 1998), red mulberry

(Olson *et al*., 2001) and pine seedlings (Liste and Alexander, 2000) have been applied for phytoremediation.

Rare investigations have been carried out on the use of salt tolerant plants for phytoremediation of hydrocarbon-polluted soil.

During a scientific visit to a coastal area at Suez, it was observed that *Tamarix nalotica* plant dominated a hydrocarbon-polluted site in this area, indicating that this plant is a tolerant of the combined adverse effects of salinity (2.9% w/w) and petroleum pollutants. As a result of the general lack of knowledge and availability of using *Tamarix nalotica* plant for phytoremediation, the above observation stimulated a study to investigate the rhizosphere effects of *Tamarix nalotica* plant on the degradation and removal of PAH compounds from this coastal saline soil. Accordingly, soil samples were collected from the polluted rhizosphere soil (soil in direct contact with roots) and also from the polluted non-rhizosphere soil (soil 50 cm away from the effects of roots).

2. Material and Methods

Chemical and Physical Analysis of Soil:

Physical and chemical analysis of the soil were determined according to the methods described in Jackson (1967) Soil pH, total salinity, cationic and anionic compositions and total carbonates were determined. Particle size analysis (dry sieving) of tested soil samples was accomplished according to Jackson (1967).

Collection of rhizosphere and non-rhizosphere soil

A polluted coastal area in which *Tamarix nalotica* is dominant was chosen at Suez for the present study. Rhizosphere soil samples (soil in direct contract with roots) and non-rhizosphere soil samples (soil 50 cm away from the roots) were collected from this polluted area .

Counts of total bacteria and fungi

For counting colony forming units (CFU) of bacteria and fungi, the usual dilution plate method was used as described by Al-Gounaim and Diab (1998). Nutrient agar (Oxoid) supplemented with 0.4% solube starch was used for counting bacteria. For counting fungi malt-yeast extract agar was used. Plates for counting bacteria were incubated at 30^{o}C for a period of 5-7 days. Plates for counting fungi were incubated at 25-26^{o}C for 10-12 days. At the end of the incubation periods, the developed colonies were counted and expressed as CFU/g dried soil.

Counting of hydrocarbon-degrading microorganisms

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

The culture medium was dispensed in test tubes, each received 5 ml of the medium. After sterilization, 0.1 ml sterilized oil was added to each tube. One gram of the soil sample was introduced to a test tube containing 10 ml sterilized water, a series of dilution was made. Three tubes of the culture medium were inoculated from each dilution (1 ml for each tube). The inoculated tubes were incubated at 30^{o}C for 21 days. At the end of the incubation period, tubes showing growth were recorded and the MPN values were obtained from the MPN index of the three tubes as found in the Standard Method for the Examination of Water and Waste Water (1989) .

Extraction and determination of the residual oil contents from soil samples and plant shoots

Five grams from each sample of the soil and plant shoot tissue were mixed with 3g anhydrous sodium sulfate to remove moisture and extracted with chloroform by using the shaking method (Chen *et al*, 1996). The extract was pooled and evaporated. The 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 and aromatics (Chaineau *et al*, 1995). The saturated fraction was discarded, and the aromatic fraction in benzene was reduced to one ml. 1µl of this extract was used for GC analysis.

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) had identified 16 PAH compounds as priority pollutants, 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 1 ml/min. A CP Sil 19CB column (25 m long x 0.32 mm diameter x 0.2 µm thickness for the stationary phase 1 was used. Temperature programming of initial holding at 40^{o}C (holding 2 minutes) was applied. The total time of analysis was 45 min., injector and detector temperatures were 250^{o}C and 280^{o}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.

3. Results and Discussion

Physical and chemical analysis (Table 1) of the soil of the area in which *Tamarix nilotica* plant is grown is sandy in nature, with pH 8.1 and total salinity of 2.92%. Nitrogen and phosphorus were of low concentrations (Table 1). The results also show that most of the soluble ions concentrations were of higher values in the rhizosphere soil compared to the non-rhizosphere soil.

The results in Table (2) show that one gram of the rhizosphere soil contained (CFU/g) $30.6x10^6$ of bacteria, $4.2x10^2$ of fungi, and $9.4x10^6$ hydrocarbon-degrading bacteria. This is in contrast to the non-rizosphere soil which contained (CFU/g) $40.2x10^4$ of bacteria, $2.4x10^2$ fungi and $18.3x10^3$ hydrocarbon-degrading bacteria respectively. Oil-degraders in the rhizosphere soil were higher (30.7%) as compared to the non-rhizosphere soil (4.6%).

Residual total petroleum hydrocarbon content of this soil (Table 3) was 2250 mg/100g in non-rhizosphere soil. These results demonstrated that *Tamarix nilotica* plant grown in this habitat is a tolerant to the combined adverse effects of salinity and petroleum hydrocarbon pollutants.

These results indicated the stimulatory effects of the plants roots (especially for HC-degraders), this is confirmed from the values of R/S (Table 2) which ranged from 3 (for the fungi) to 513.7 for HC-degraders. Corgie *et al* (2004) reported that plant roots provide ideal attachment and supply exudates consisting of amino acids, organic acids, sugars, enzymes … etc. Binet *et al* (2000) reported that microbial communities have been documented to be larger and more active in planted versus unplanted soil, and the rhizosphere is often enriched in organisms capable of hydrocarbon degradation. Rugh *et al* (2005) reported the abundance of PAH-degrading bacteria in contaminated soil planted with different native Michigan plant species.

Table 1: Physical and chemical analysis of the rhizosphere soil (R) and non-rhizosphere soil (S) of *Tamarix nilotica*

Particle Diameter (mm)		**%**	**Chemical Analysis**		**%**
2-1 mm	R	15.82	PH	R	7.8
	S	18.82		S	8.1
1-0.5	R	19.40	$CaCO_3$ (ppm)	R	34.6
	S	23.47		S	26.5
0.5-0.25	R	28.77	TS (5)	R	2.92
	S	33.74		S	2.62
0.25-0.125	R	28.77	Ca^{2+} (ppm)	R	13.92
	S	20.56		S	12.60
0.125-0.063	R	14.01	Mg^{+2} (ppm)	R	853.2
	S	3.55		S	269.3
<0.063	R	0.32	No^{+} (ppm)	R	20987.5
	S	0.00		S	14422.5
Texture class	R	Sand	K^{+} (ppm)	R	826.8
	S	Sand		S	644.0
			$11CO_3^{-}$ (ppm)	R	549.0
				S	442.3
			Cl^{-} (ppm)	R	20915.5
				S	18079.5
			SO_4^{2-} (ppm)	R	46915.1
				S	45412.4
			PO_4 (ppm)	R	0.25
				S	0.12
			N_2 (ppm)	R	100
				S	60

Table 2: Mean counts (CFU/g air dried soil) of total heterotrophic bacteria, fungi and oil-degraders in the rhizosphere soil (R) and the non-rhizosphere soil (S) of *Tamarix nilotica* plant.

Microorganisms	CFU / g Soil		R/S Ratio
	S	R	
Total heterotrophic bacteria	$40.2x10^4$	$30.6x10^6$	76.5
Total fungi	$2.4x10^2$	$4.2x10^2$	3.0
Oil-degrader	$18.3x10^3$	$9.4x10^6$	513.7
Percentage oil-degraders	4.6	30.7	

Table 3: Residual oil content and its fractions (mg/100 g soil) in the non-rhizosphere soil (S) and in the rhizosphere soil (R) of *Tamarix nilotica* plant.

Oil and its Fracations	Mg/100 g soil		Reduction (R)
	S	R	
Saturates	800 ± 28.3	100 ± 7.1	87.5
Aromatics	1060 ± 84.5	417 ± 24.0	60.7
Resins	200 ± 14.1	195 ± 7.1	2.5
Asphaltene	190 ± 7.0	188 ± 11.3	1.1
Total	2250	900	60

When the residual hydrocarbons from the rhizosphere soil and non-rhizosphere soil was extracted and fractionated (Table 3), it was found that 2250 mg of TPH/100g soil was extracted from the non-rizosphere soil, this is in contrast to 900 mg/100g of the rhizosphere soil used was measured, i.e. with a reduction of 60%.

As for the different fractions of the TPH extracted (Table 3) it was found that the rhizospehre soil contained less amounts of these fractions (especially the saturates and aromatics) as compared to the non-rhizosphere soil. Saturates fraction was 800 mg/100g soil in the non-rhizosphere soil while in the rhizosphere soil only 100 mg/100 g soil was recorded, i.e. with a reduction of 87.5%. On the other hand, the aromatic fraction was 1060 mg/100 g of the non-rhizosphere soil, this is in contrast to 417 mg/100 g of the rhizosphere soil, i.e. with a reduction of 60%. It is of important to observe that reduction values of 2.5% and 1.1% of the non-degradable resins and asphaltene respectively were recorded in the rhizosphere of this wild salt-tolerant plant (*Tamarix nilotica*). Al-Abdulla *et al* (2006) extracted 706mg/100g soil of the saturated fraction from the rhizosphere soil and 1234.2 mg/100g from the non-rhizosphere soil of the wild desert plant *Salsola imbricate,* i.e. with a reduction of 42.8% in the rhizosphere soil. As for the aromatic fraction, the same trend was observed giving reduction value of 45%, which is slightly higher than the reduction value of the saturates (42.8%). The above authors also calculated the reduction values of the saturates and aromatics fractions in the non-rhizosphere soil and in the rhizosphere soil of the wild desert plant *Cyperus conglomerates.* They recorded loss of 37.1% and 40.2% for the saturates and aromatic fractions in the rhizosphere soil. These authors then recommended the use of these wild desert plants for the phytoremediation of the polluted desert soil.

Polycyclic aromatic hydrocarbons (PAHs) of the residual aromatic fraction in the non-rhizosphere soil and rhizosphere soil of this wild salt-tolerant plant were quantified by GC-FID analysis. The results (Table 4, Fig. 2) show the resolution of 15 different individual PAH compounds in the non-rhizosphere soil, and 13 PAHs in the rhizosphere soil. Naphthalene was absent from both non-rhizosphere and rhizosphere soil, while acenaphthylene and acenaphthene were absent from the rhizosphere soil.

Table 4: Residual PAH contents of the rhizosphere soil (R) of *Tarmarix nilotica* plant as compared to non-rhizosphere soil (S). Loss (%) of PAHs is also given.

PAH Compounds	No. of Rings	S mg/kg Soil	R mg/kg soil	Loss %
1. Naphthalene	2	-	-	-
2. Acenaphthylene	3	8.1 ± 0.50	-	100
3. Acenaphthene	3	4.7 ± 0.10	-	100
4. Flourene	3	15.3 ± 0.40	5.6 ± 0.43	63.4
5. Phenanthrene	3	100.7 ± 3.10	51.10 ± 2.70	49.3
6. Anthracente	3	22.0 ± 1.60	14.87 ± 1.90	52.5
Total		150.80	71.570	52.50
7. Flouranthene	4	68.3 ± 5.40	16.8 ± 1.28	75.4
8. Pyrene	4	57.3 ± 2.47	22.8 ± 0.92	60.2
9. Benzo(a)anthracene	4	72.6 ± 4.77	26.6 ± 2.83	63.4
10. Chrysene	4	277.6±12.60	122.9 ± 5.97	55.7
Total		475.8	189.1	60.3
11. Benzo(a)flourathene	5	15.33 ± 1.25	7.57 ± 1.04	50.6
12. Benzo(k)flouranthene	5	83.0 ± 2.16	44.77 ± 3.24	46.1
13. Benzo(a)pyrene	5	109.0 ± 7.36	55.70 ± 4.14	49.0
14. Dibenzo(ah)anthracene	5	156.0 ± 4.32	118.13±16.19	24.3
Total		363.33	226.17	37.8
15. Benzo(ghi)perylene	6	13.3 ± 0.85	7.3 ± 1.80	45.1
16. Indeno(1,2,3-c,d)pyrene	6	70.0 ± 1.63	47.8 ± 3.81	31.7
Total		83.3	55.10	33.8
Total		**1073.50**	**541.94**	**49.5**

As a total the amount of PAHs (mg/kg soil) were 1073.5 and 541.94 mg/kg oil in the non-rhizosphere and –rhizosphere soil respectively. Weissenfels *et al* (1999) estimated 1815.1 mg/kg sandy soil collected from former wood impregnation plant. Al-Gounaim *et al* (2006) estimated 2256.8 mg/kg of moderately polluted (2-3% oil w/w) desert non-rhizosphere soil, and 1264.9 mg/kg of the rhizosphere soil of the wild desert plant *Cyperus conglomeratus,* i.e. with a reduction value of 40.0%. On the other hand, the same authors found 1643.9 mg/kg of the non-rhizosphere soil, and 532.6 mg/kg of the rhizosphere soil of the wild desert plant *Salsola imbricate.* This demonstrated 67.6% loss in the rhizosphere of this plant. Davis *et al* (2004) reported that phytoremediation is emerging as potentially cost effective technology; plants have often been shown to reduce the level of PAHs in soil. Rugh *et al* (2005) reported that root-micobe interaction is considered to be the primary process of PAH phytoremediation, since bacterial degradation has been shown to be the most dominant pathway for environmental PAH dissipation. However, the precise mechanism driving PAH rhizostimulation symbiotic largely unsolved.

Most studies on PAH phytoremediation have utilized herbaceous annuals and grasses. When compared to these plant types, Moeller *et al* (2006) suggested that tree roots are structurally and functionally differed, particularly with regard to their perennial and woody nature and relationships with ectomycorrhizal fungi. Unique root dynamics associated with these trials including exudation and root turnover, could lead to relatively more complex rhizosphere dynamics and important effects on PAHs degradation in the rhizosphere.

Rugh *et al* (2005) found that most of the tested plant spieces stimulated biodegradation of a broad range of PAH compounds related to the unplanted soil. Spriggs *et al* (2005) found that the rate of dissipation of PAHs was greater for willow followed by poplar, and the unvegetated soil controls. These data support the hypothesis that trees can enhance the degradation of target PAHs in soil. The above authors reported that it is important to note that the degradation rates were measured under greenhouse conditions. Rate of dissipation under field conditions could vary significantly from these results. Hwan *et al* (2008) found that more PAHs were dissipated in the rhizosphere soil than in unplanted soil. This enhanced dissipation of PAHs in planted soil might be derived from increased microbial activity and plant release enzymes.

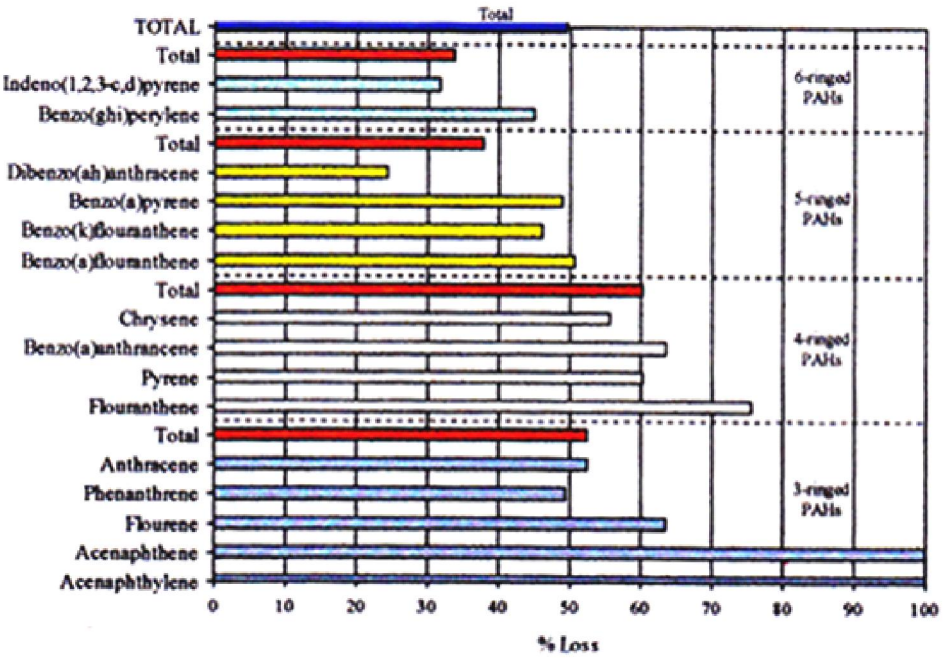

Fig. 2: Reduction values (%) of PAH compounds in the rhizosphere of *Tamarix nilotica* plant (calculated on the basis of PAH content of the non-rhizosphere soil).

In the present work, it can be observed that (Table 4) Chrysene and dibenzo(ah)anthracene as compared to the other PAHs were more frequent in the non-rhizosphere soil (277.6 and 156.0 mg/kg soil respectively). However, this indicates reduction values of the two PAHs in the rhizosphere soil of 55.7% and 43.4% respectively. Spriggs *et al* (2005) reported that the decrease in concentration of chrysene is noticeably smaller in the presence of poplar and willow vegetation.

As a total the four-rings PAHs as compared to the other PAH groups were more reduced in the rhizospehre soil (60.3%). This was followed by the three-rings PAHs (52.5%). The five-ring and the sex-ring PAHs were weakly reduced (37.8% and 33.8% respectively). Spriggs *et al* (2005) reported that the reduction trends of four-ring and five-ring PAHs were noted during 18-monthes study period of phytoremediation, although none were at significant levels when compared to unvegetative controls. Although PAH compouns with increasing number of rings may be microbially degraded, limitation exist which may hinder the bioremediation efficiency.

For simplicity the results in the rhizosphere soil can be summarized in the following point:

PAHs with high reduction values (75.4-100%) were three compounds namely: acenaphthylene (100%), acenaphthene (100%) and flouranthene (75%).

PAHs with reduction values of 50.6-63.4% are six compounds namely: flourene(63.4%), benzo(a)anthracene (63.4%), pyrene (60.2%), chrysene (55.7%), anthracene (52.5%) and benzo(a)flouranthene (50.6%).

The other PAH compounds had reduction values of 24.3% (for dibenzo(ah)anthracene to 49.0% for benzo(a)pyrene.

Spriggs *et al* (2005) found that acenaphthene, anthracene, flouranthene, naphthalene and phenanthrene decreased significantly in green ash *(Fraximus pennsylvanica)* and hybrid poplar *(Populus deltoids)* phytoremediation treatment when compared to the unplanted soil controls.

Rentz *et al* (2004) reported that studies of trees including hybrid poplar, red mulberry and willow indicated that root exudates and/or extracts can actually inhibit the phenanthrene degradation. Muller *et al* (2006) found that pyrene mineralization was not affected by planting. They also found that phenanthrene was consistently lost more rapidly than either anthracene or pyrene.

Results of the reduction values of the carcinogenic PAH compounds (Table 5, Fig. 3) show that as a total the eight carcinogenic PAHs resolved were reduced in the rhizospher soil by 49.1%, and it is important to observe that all of the four-rings PAHs recorded were carcinogenic compounds. This is in contrast to three of the five-rings and one of the six-ring PAHs also are of carcinogenic effects. 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 Aency 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.
*Benzo(a)pyrene.

Table 5: Residual carcinogenic PAH compounds in the rhizosphere soil (R) of *Tamarix* plant as compared to those in the non-rhizosphere soil(s)

Carcinogenic PAHs	No. of Rings	S mg/kg Soil	R Mg/kg soil	Loss %
1. Flouranthene	4	68.3 ± 5.40	16.8 ± 1.28	75.4
2. Pyrene	4	57.3 ± 2.47	22.8 ± 0.92	60.2
3. Benzo(a)anthracene	4	72.6 ± 9.77	26.6 ± 2.83	63.4
4. Chrysene	4	277.6±12.60	122.9 ± 5.97	55.7
Total		475.8	189.60	60.2
5. Benzo(k)flouranthene	5	83.0 ± 2.16	44.77 ± 3.24	46.1
6. Benzo(a)pyrene	5	109.3 ± 7.36	55.70 ± 4.14	49.0
7. Didenzo(ah)anthracene	5	156.0 ± 4.32	118.13±24.30	24.3
Total		348.3	218.60	37.2
8. Indeno (1, 2,3-c,d) pyrene	6	70.0 ± 1.63	47.80 ± 3.8	31.7
Total		894.1	455.5	49.1

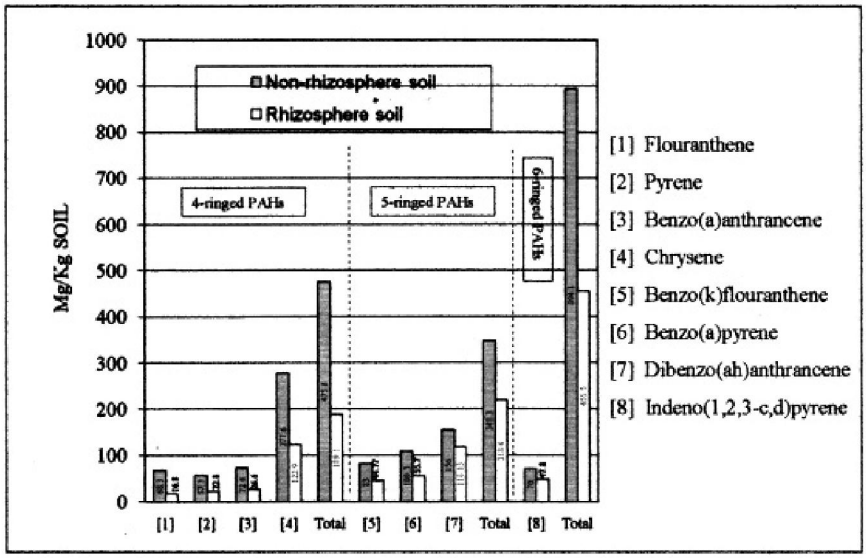

Fig. 3: Carcinogenic PAH compounds (mg/kg soil) in the rhizosphere and non-rhizosphere soils of *Tamarix nilotica*.

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 mixtures of other PAHs in dermal treatment of mice. Collectively, the carcinogenic four-ringed PAHs were highly reduced (60.3%) in the rhizosphere of this plant.

Maximum reduction value in the individual PAHs was 75.4% (for flouranthene) followed by 63.4% (for benzo(a)anthracene) and 60.2% (for pyrene). On the other hand, minimum reduction values were 24.3% (for dibenzo(ah)anthracene) List and Alexander (2000) reported that accelerated dissipation from pyrene-spiked soil has been shown with pine seedling.

Jordahl *et al* (1997) found that population of benzene, toluene and xylene degrading microbes were five times more abundant in the rhizosphere of poplar trees than in the surrounding soil.

A particular notable distinction of *Tamarix nilotica* rhizosphere is the greater efficiency to degrade the carcinogenic PAH compounds especially flouranthene, benzo(a)anthracene and pyrene.

The present results revealed that the roots of the wild salt-tolerant plant *(Tamarix nilotica)* growing in the polluted seashore sediments were densely associated by oil-degraders and other bacterial groups, which reflected the disappearance of higher amounts of TPH and polycyclic aromatic hydrocarbons (PAHs). Accordingly this plant demonstrated successful phytoremediation of this polluted sediments.

To improve and to accelerate this process in a short period of time, Haung *et al* (2005) developed a multi-process phytoremediation system (MPS) that is composed of : land farming (aeration and exposure to light), contaminant-degrading bacteria, plant growth promoting rhizobacteria and growth of the tolerant plants.

Results of the GC analysis for the detection of the accumulated PAH compounds in the shoot tissues of *Tamarix nilotica* plant growing in the polluted sediments as compared to its growth in the non-polluted area (Fig. 3) show that the total number of peaks resolved for PAH compounds were 201 in the tissues of the plant growing in the unpolluted area, on the other hand , 222 peaks recorded for plants tissues of that growing in the polluted sediments, i.e. with increase of 21 peaks. The identified peaks in the tissues of both plants were 15 and 14 peaks of identified PAH compounds for plant in the unpolluted area and plant in the polluted sediment respectively. The sum of the 15 PAHs was 528 mg/kg dried tissue whereas the sum of the 14 PAHs was 769 mg/kg dried tissues. These results indicate accumulation value of 1.46 for total PAHs. The results in Fig. (3) show that the more accumulated PAHs was indeno (1,2,3-c,d) pyrene.

It is of important to note that rare (if none) investigations on the accumulation of PAH compounds in the tissues of salt-tolerant plants. Most studies have utilized the annual and herbaceous non-salt tolerant plants. Kipopulou *et al* (1999) found PAH median values of 161 and 42 Mg/g in lettuce and cabbages respectively, grown in the industrial area of Thessaloniki, Greec. Bakker *et al* (2000) reported that the sum of PAHs in samples of leaves of *Plantago major* collected at 50 m from an oil refinery was 8000 µg/g whereas PAH concentration in grass samples of the same site was 2000 µg/g.

Grova *et al* (2000) reported a concentration of 51.8 µg/g for 8 PAHs in rural grass sample. Nadal *et al* (2004) found that in chard samples the highest value (sum of 16 PAHs) was observed in the residential area, followed by the industrial and the unpolluted zones with concentrations of 179.50 and 28 µg/g dry weight respectively. The same authors reported that the concentrations in vegetation under the influence of petrochemical industries were notable higher.

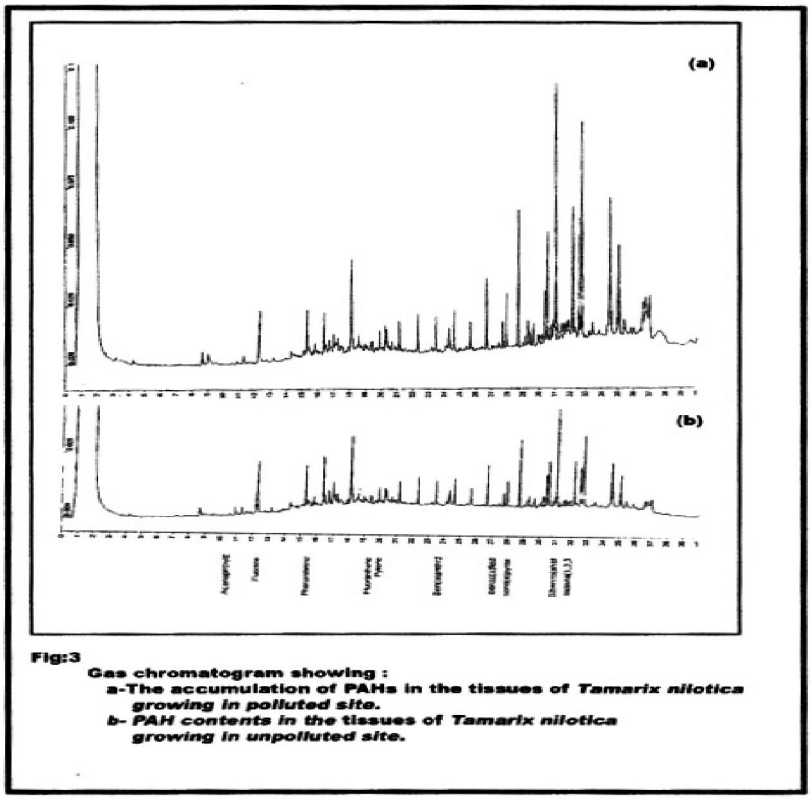

Fig:3
Gas chromatogram showing :
a-The accumulation of PAHs in the tissues of *Tamarix nilotica* growing in polluted site.
b- *PAH contents in the* tissues of *Tamarix nilotica* growing in unpolluted site.

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