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Phytoremediation of Oil Contaminated Desert Soil Using the Rhizosphere Effects

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Abstract: Phytoremediation is a promising technology for the clean up of petroleum contaminated soil.In the present work the rhizosphere of *Vicia faba, Zea mays* and *Triticum aestivuml* plants were tested for their abilities to stimulate the microbial degradation of soil pollutants in desert soil contaminated with 2.2-2.3% crude petroleum oil. The results showed that the roots of the three different plants were density associated with total bacteria, fungi and oil-degrading microorganisms, this is confirmed from the (R+/S+) ratios which ranged from 55.2-250.8 (for total bacteria), 20-131.3 (for fungi) and 95.7-296.1 (for oil-degraders). Percentages of oil-degraders were higher in the rhizosphere soil of *Vicia faba* (62.4%) as compared to the rhizosphere soil of *Zea mays* and *Triticum aestivuml* plants (19.9% and 17.6% respectively). The results of the biodegradation of oil and its fractions showed that great reduction (30%) of total petroleum hydrocarbons (TPHs) was observed in the rhizosphere soil of *Vicia faba* as compared to 16.8% and 13.7% reduction in rhizosphere soil of Zea mays and *Triticum aestivuml* respectively. It was observed also that in the polluted non-cultivated soil the TPHs were reduced by 8.2-10.5% as a result of biostimulation process only (addition of nutrients).The results also showed that *Vicia faba* rhizosphere was able to reduce more of the saturated (47.0%) and more of the aromatics (26.2%) fractions, compared to (37.4% and 8.2%) for *Zea mays* and (33.2% and 3.9%) for *Triticum aestivuml* rhizospheres. It is of interest to find that 5.3% of the hardly degradable fraction resins was degraded in rhizosphere soil of *Vicia faba*. The present results clearly demonstrated that the legume plant *Vicia faba* provided successful phytoremediation process of a contaminated desert soil as compared to the other two plants.

Key words:

INTRODUCTION

Major causes of crude oil contaminated soil include leakage storage tanks and pipelines, land disposal of petroleum wastes and accidental spills. Severe contamination of soil results mostly from accidental spillage, as an example the Gulf oil spill which caused by Iraqi forces in 1991 during their invasion to Kuwait. More than 60 million barrels of crude oil were released to the desert to cover 49 km^2 [1].

Contaminated soils pose a major environmental and human health problem. Microorganisms and plants can have complementary roles in phytoremediation of the polluted soil. Phytoremediation refers to the use of plants to clean contaminated soil [2]. Increased biodegradation of organic contaminants occurs in the rhizosphere, the zone of soil directly adjacent to and under the influence of plant roots [3].

The application of plants for remediation of soil contaminated with petroleum hydrocarbons is one of the promising cost and environmental effective approach. Rock and Sayre [4] estimated phytoremediation clean up costs of \$ 162/m^3 compare to \$ 810/m^3 for excavation and incineration.

For successful phytoremediation both plants and microorganisme must survive and grow in crude oil contaminated soil. Phytoremediation involves growing or encouraging the growth of plants in the contaminated soil either artificially constructed (using cultivated plants) or naturally (using the already existing plants) for a required growth period, to remove contaminants from the site. The plants can be subsequently harvested processed and disposed.

In petroleum contaminated sites, phytoremediation can be applied at moderate contamination levels or after the application of other remediation measures as a

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polishing step to further degrade residual hydrocarbons and to improve soil quality [3,5].

Yateem *et al.* [6] investigated the degradation of total petroleum hydrocarbons (TPH) in the rhizosphere and non-rhizosphere soil of three domestic plants namely, alfalfa (*Medicago sativa*), broad bean (*Vicia faba*) and rayegrass (*Lolium perenne*). Although the three domestic plants exhibited normal growth in the presence of 1% TPH, the degradation was more profound in the case of leguminous plants. They found that the soil cultivated with broad bean and alfalfa was 36.6% and 35.8% respectively, compared with 24% degradation in case of rayegrass. Adams and Duncan [7] found that the legume plant (*Vicia sativ*a) was able to grow in soil contaminated with diesel fuel and the total numbers of nodules were significantly reduced in contaminated plants as compared to control plants, but nodules on contaminated plants were more developed than corresponding nodules on control plants. These authors found that the amount of diesel fuel remaining after 4 months in the legume plant *Vicia sativa* was slightly less than in the rayegrass planted soil.

Rosado and Pichtel [8] studied the decomposition of used motor oil in soil as influenced by plant treatment. Soil contaminated with used motor oil (1.5% w/w) was seeded with soybean (Glycine max), green bean (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), Indian mustard (*Brassica juncea*), mixed grasses / maize (*Zea mays*) and mixed clover (*Trifoleum partense, Trifoleum repense*). After 150 days in the clover treatment the added oil was no longer detected. A total of 67% of the oil was removed in sunflower / mustard and with addition of NPK fertilizer, the oil was completely removed. The grass/ maize treatment resulted in a 38% oil reduction, which increased to 67% with fertilizer application. Based on oil residue and biomass results, the clover and sunflower / mustard treatments are considered superior to other plant treatments in terms of overall phytodegradation of used oil hydrocarbons.

Merkl *et al.* [9] tested three legume plants and three grasses for their ability to stimulate microbial degradation in a sandy soil contaminated with 5% (w/w) crude oil. They showed that the overall advantage of the chosen grass species is their extensive, widely branched root system providing a large root surface for the growth of microbial population. Legumes are considered to be specially promising because of their ability to fix atmospheric nitrogen. Their experiment evaluates the ability of selected species to grow in oil-contaminated soil and enhance oil degradation. Radwan *et al.* [10] reported that *Vicia faba* plant can tolerate up to 10% (w/w) crude oil in sandy desert soil, therefore their potential was assessed for cleaning oily desert soil using rhizosphere technology. They found that the amount of hydrocarbons recovered from oily desert samples supporting *Vicia faba* were lower than in uncultivated oily sand samples.

The objective of the present research is to study the effects of a legume plant (*Vicia faba*) and two monocot plants (*Triticum aestivuml*) and (*Zea mays*) on the changes of the rhizosphere microflora and its degradation potential in response to hydrocarbon-contamination of soil. The advantage of the chosen legume plant is its ability to fix atmospheric nitrogen [9], this is in addition to the ability of this plant species to tolerate up to 10% (w/w) crude oil [10]. On the other hand the advantage of the chosen monocot plants is their extensive widely branched fibrous root system, resulting in 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 [11].

MATERIALS AND METHODS

Field Experiments: Four plots each of $2\times2m^2$ were delimited in an area(nursery of the Egyptian Environmental Affairs Agency, Suez Regional branch office. without no 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 nitrogen and phosphorus (NP) concentrations (500 mg ammonium nitrate and 50 mg K_2HPO_4/kg soil).

- Plot No. 1 was seeded with 100 viable *vicia faba* seed at the beginning of January.
- Plot No. 2 was seeded by 100 viable grains of *Zea mays* at the beginning of May.
- Plot No. 3 was seeded by 200 viable (*Triticum aestivuml*) viable grains at the beginning of November.
- Plot No. 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 were 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 analysed microbiologically and 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 hydrocarbons are accumulated in plant tissues or not.

The needed moisture was added (50% of the water holding capacity, as described by Vecchioli *et al*. [12] 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 and its 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 8 h. The chloroform extract was evaporated in a preweighed dish and the amount of total petroleum hydrocarbons (TPHs) was determined and the loss (%) of TPH was then calculated.

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 fractionated by liquid-solid chromatography into saturates, aromatics and resins. The amount of each fraction was determined according to Chaineau *et al*. [13].

Microbiological Analysis: For counting colony forming units (CFU) of bacteria and fungi, the usual dilution plate method was used. Nutrient agar (Oxoid) medium supplemented with 0.4% (w/w) soluble starch was used for counting bacteria. For counting fungi malt-yeast extract agar was used. The colonies appeared on the different plates were counted and expressed as CFU/g soil. Plates for counting bacteria were incubation 5-7 days at 30°C and for fungi the incubated temperature was 25°C for a period of 10-12 days.

For counting hydrocarbon-degrading microorganisms the three tubes mean probable number (MPN) method was used as described by Chaineau *et al.* [13].

RESULTS AND DISCUSSION

The soil sample used in the present study are sandy soil, with PH 7.6-7.8. This soil was poor in phosphorus (0.19 ppm) and nitrogen (0.02%) contents.

Results of the microbial contents of the polluted and non-polluted plots of *Vicia faba, Zea maize* and wheat (*Triticum aestivuml*) plants are found in Table 1-3 and illustrated in Fig. 1-2.

The results show that the CFU/g of total bacteria, fungi and oil-degraders are higher in rhizosphere soil (both polluted and non-polluted) than in the non-rhizosphere soil of the above three plants. These results reflect the positive rhizosphere effects of the three plants on the microbial communities as indicated from the results of (*R/S*) ratios (Table 1-3) (counts in the rhizosphere/counts in the non-rhizosphere) of more than one. The (R+ / S+) values were more pronounced in the

Table 1: Microbial contents of rhizosphere soil (*R*) and non-rhizosphere soil (*S*) of *Vicia faba* plant after 60 days growth period

		60 days growth period					
	0-time						
Microorganisms	CFU/g soil	*R*+ CFU/g soil	*S*+ CFU/g soil	R+ / S+	*R*-CFU/g soil	*S*-CFU/g soil	R- / S-
Fungi	17.3×10^{2}	10×10^{4}	5×10^{3}	20.0	$6.1\pm0.3\times10^{3}$	$4.5\pm0.5\times10^{2}$	13.6
Total bacteria	29.0×10^{4}	$170.9\pm7.0\times10^{8}$	$29.6\pm1.5\times10^{7}$	57.7	$36.2\pm2.2\times10^{6}$	$1.9\pm0.2\times10^{6}$	19.1
Oil-degraders	22.0×10^{2}	$106.6\pm9.3\times10^{8}$	$3.6\pm0.14\times10^{7}$	296.1	$30.9\pm2.7\times10^{4}$	$5.5\pm0.4\times10^{4}$	5.6
Oil-degraders (%)	0.77	62.4	12.2		0.9	2.9	

R+ = polluted rhizosphere soil, *S*+ = polluted non-rhizosphere soil, *R*-= non-polluted rhizosphere soil, *S*-= non-polluted non-rhizosphere soil.

Table 2: Microbial contents of rhizosphere soil (*R*) and non-rhizosphere soil (*S*) of *Zea mays* plant after 60 days growth period.

		60 days growth period					
	0-time						
Microorganisms	CFU/g soil	*R*+ CFU/g soil	*S*+ CFU/g soil	R+ / S+	*R*-CFU/g soil	*S*-CFU/g soil	R- / S-
Fungi	$17.3\pm0.6\times10^{2}$	$19.7\pm2.1\times10^{5}$	$15.0\pm0.8\times10^{3}$	131.3	$63.0\pm5.7\times10^{4}$	$7.0\pm0.8\times10^{3}$	90.0
Total bacteria	$29.0\pm1.2\times10^{4}$	$742.4\pm21.4\times10^{8}$	$29.6\pm1.5\times10^{7}$	250.8	$173.1\pm5.7\times10^{6}$	$1.9\pm0.2\times10^{6}$	91.1
Oil-degraders	$22.0\pm0.8\times10^{2}$	$147.6\pm6.1\times10^{8}$	$2.6\pm0.10\times10^{7}$	132.2	$27.4\pm3.0\times10^{4}$	$5.5\pm0.4\times10^{4}$	5.0
Oil-degraders (%)	0.77	19.9	8.8		0.20	2.9	

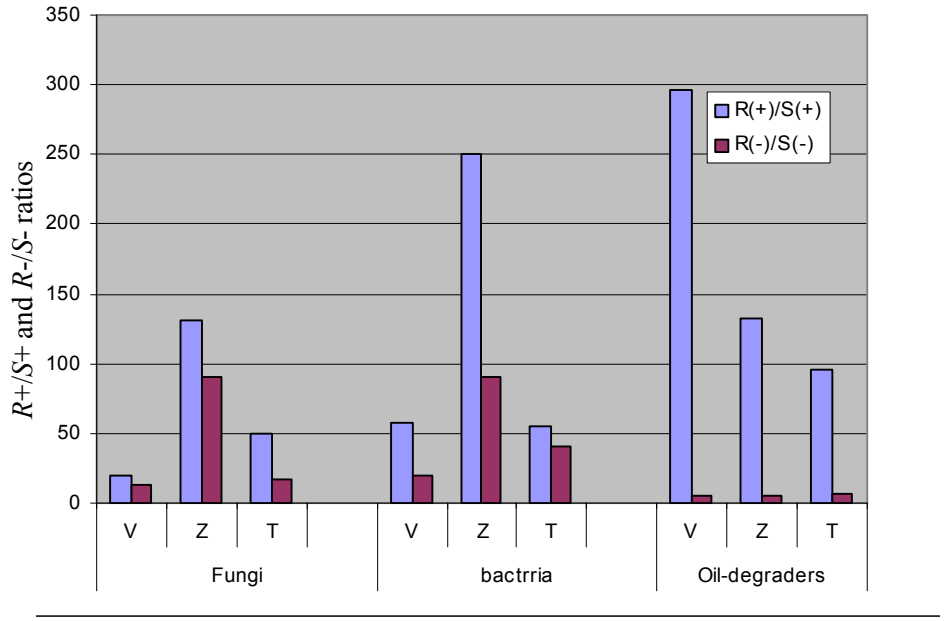

Fig. 1: R+/S+ and R-/S- ratios of fungi, bacteria and oil-degraders of Vicia(V), Zea (Z) and Triticum (T) plots

Table 3: Microbial contents of rhizosphere soil (*R*) and non-rhizosphere soil (*S*) of *Triticum aestivuml* plant after 60 days growth period

	0-time	60 days growth period					
Microorganisms	CFU/g soil	*R*+ CFU/g soil	*S*+ CFU/g soil	R+ / S+	*R*-CFU/g soil	*S*-CFU/g soil	R- / S-
Fungi	$17.3\pm0.6\times10^{2}$	$74.0\pm1.6\times10^{5}$	$15.0\pm0.8\times10^{3}$	49.3	$12.0\pm0.7\times10^{4}$	$7.0\pm0.8\times10^{3}$	17.1
Total bacteria	$29.0\pm1.2\times10^{4}$	$163.4\pm5.1\times10^{8}$	$29.6\pm1.5\times10^{7}$	55.2	$76.7\pm2.5\times10^{6}$	$1.9\pm0.2\times10^{6}$	40.4
Oil-degraders	$22.0\pm0.8\times10^{2}$	$28.7\pm0.9\times10^{8}$	$3.0\pm0.14\times10^{7}$	95.7	$3.6\pm0.2\times10^{5}$	$5.5\pm0.4\times10^{4}$	6.5
Oil-degraders (%)	0.77	17.6	10.1		0.5	2.9	

polluted plots than in the non-polluted one (control). Murotova *et al.* [14] explained that the success of phytoremediation of hydrocarbon contaminated soil is connected with the plants capacity to enhance microbial activity in the rhizosphere.

In the polluted *Vicia faba* plots (Table 1, Fig. 1) (R+/S+) values were in the range of 20 (for fungi) to 296.1 (for oil-degraders). In *Zea maize* plots (R+ / S+) values (Table 2, Fig. 1) were 131.3 (for fungi) to 250.8 (for total bacteria), while in (*Triticum aestivuml*) plots (Table 3, Fig. 1) values of 49.3 (for fungi) to 95.7 (for oil-degraders) were recorded.

In non-polluted plots (R-/S-) values were significantly lower than those of the polluted plots. Generally, addition of 2.2-2.3% (w/w) of crude oil to this type of soil stimulated the development of more microorganisms as compared to the control sample. Schaffner *et al.* [15] reported that when the mean population densities of bacteria in samples from contaminated soil are significantly greater than in background samples, the pollutants are being utilized, they suggested that microbial enumeration is a screening level tool which can be used to evaluate the response of microorganisms to hydrocarbons.

Narino *et al.* [16] reported positive rhizosphere effects of maize and oat on microorganisms of the only contaminated soil in comparison with uncontaminated planted soil. The maize has provided a more stimulatory influence on the microbial community of the polluted soil in comparison to oat plant.

Results of the distribution of oil-degrading microorganisms in the polluted rhizosphere and non-rhizosphere soil of *Vicia faba*, *Zea mays* and *Triticum aestivuml* plots show that the polluted rhizosphere soil of

Table 4: Biodegradation of oil and its fractions in the rhizosphere of *Vicia faba* (*RV*) plant as compared to the non-rhizosphere soil (*S*), after a 60 days growth period

		60 days growth period			
Fractions	0-time mg/100g soil	*S* mg/100g soil	Loss (%)	*RV* mg/100g soil	Loss (%)
Saturates	800±8.0	634.1±4.9	20.7	423.7±5.5	47.0
Aromatics	1080.0±20.0	1005.7±21.0	6.9	797.3±6.4	26.2
Resins	190.0±2.0	192.4±1.7	-	180.0±1.3	5.3
Asphaltenes	180.0±2.0	181.4±3.6	-	176.6±4.1	1.9
Total	2250.0±100.0	2013.6±19.3	10.5	1571.6±13.3	30.2

Table 5: Biodegradation of oil and its fractions in the rhizosphere of *Zea mays* (*RZ*) as compared to the non-rhizosphere soil (*S*), after a 60 days growth period

		60 days growth period			
Fractions	0-time mg/100g soil	*S* mg/100g soil	Loss (%)	*RV* mg/100g soil	Loss (%)
Saturates	840.0±10.0	652.6±2.5	22.3	525.5±11.3	37.4
Aromatics	1070.0±40.0	995.9±3.4	6.9	982.0±2.6	8.2
Resins	180.0±10.0	184.8±2.7	-	181.3±2.9	-
Asphaltenes	240.0±3.0	245.1±4.3	-	250.7±3.8	-
Total	2330.0	2078.3±1.6	10.8	1939.6±15.2	16.8

Table 6: Biodegradation of oil and its fractions in the rhizosphere of *Triticum aestivuml plant* (*RT*) as compared to the non-rhizosphere soil (*S*), after a 60 days growth period

		60 days growth period			
Fractions	0-time mg/100g soil	*S* mg/100g soil	Loss (%)	*RV* mg/100g soil	Loss (%)
Saturates	800±32.0	636.5±6.4	20.4	526.4±3.6	33.2
Aromatics	1020.0±40.0	988.7±2.4	3.1	979.8±8.2	3.9
Resins	170.0±10.0	171.2±2.1	-	186.3±9.9	-
Asphaltenes	250.0±10.0	254.7±3.4	-	241.5±22.3	3.6
Total	2240.0	2057.1±7.5	8.2	1934.2±18.9	13.7

the three plants stimulated the development of higher counts (CFU/g soil) of such organisms as compared to the non-rhizosphere soil (Table 1-3). The percentages of oil degraders also were higher in the rhizosphere soil than in the non-rhizosphere one. *Vicia faba* rhizosphere contained the highest values (62.4%) as compared to *Zea mays* (19.9%) and *Triticum aestivuml* (17.6%) rhizosphere soil. As a comparison the percentages of oil-degraders in the polluted non-rhizosphere soil are in the range of 8.8-12.2%. On the other hand. The non-polluted plots contained significantly lower counts and lower percentages (0.2-2.9%). The above results confirmed the ability of plant roots to neutralize and or to remove the toxic effects of the oil pollutants, this is through the exudates, nutrient and other materials.

Murotova *et al.* [14] explained that the success of phytoremediation of hydrocarbon contaminated soil is connected with the plant's capacity to enhance microbial activity in the rhizosphere. The efficiency of this process is often connected with high number of degrader microorganisms and their degradative activities in the rhizosphere of plants.

Murotova *et al.* [14] also suggested that additional studies are necessary to determine whether the population of hydrocarbon-degrading microorganisms protect the plant from toxic effects of pollutants or whether the plant provides the favorable conditions of this population activity.

Merkl *et al.* [9] tested three legume plants and three grasses for their ability to stimulate microbial degradation in sandy soil contaminated with 5% (w/w) crude oil. They considered legumes to be specifically promising because of their ability to fix atmospheric nitrogen. Radwan *et al.* [17] found that total number of oil-degrading bacteria

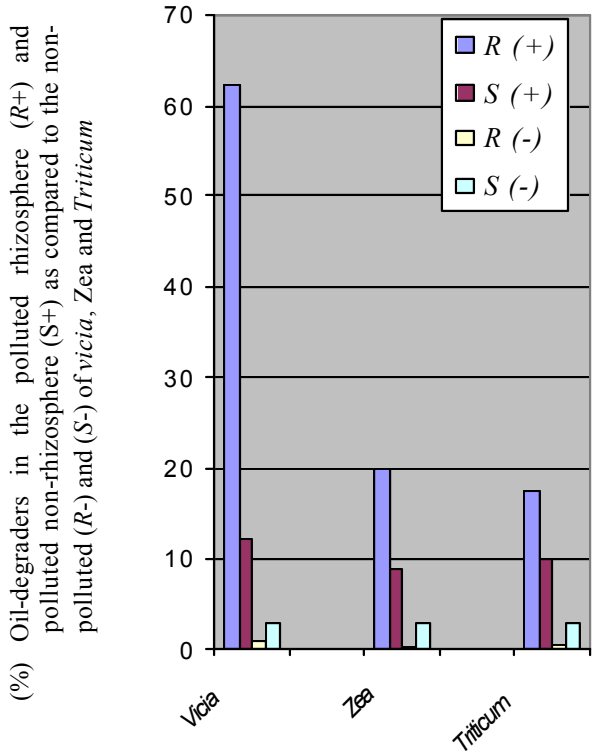

Fig. 2: The percentage of oil-degraders in the polluted rhizosphere (*R*+) and polluted nonrhizosphere (*S*+) as compared to the non-polluted (*R*-) and (*S*-) of *Vicia*, Zea and *Triticum*

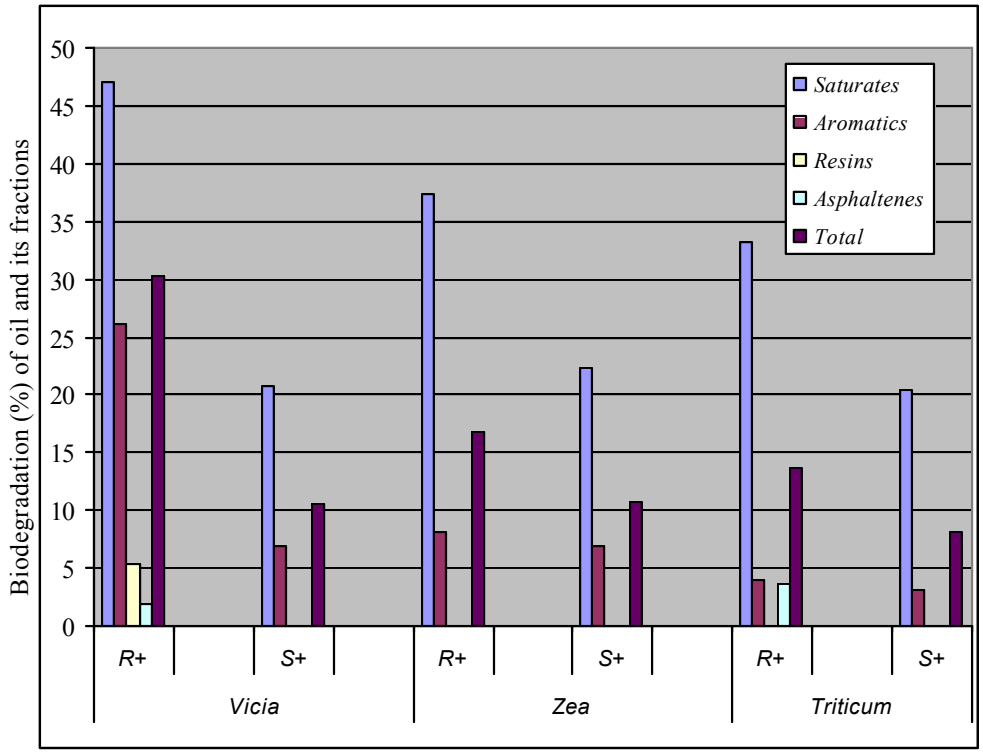

Fig. 3: Effects of plant roots (*R*) on the biodegradation of oil (Total hydrocarbon) and its fracrions, this in contrast to biodegradation in nonrhizosphere soil (*S*)

increased in the rhizosphere of *Vicia faba* plant and more hydrocarbons were eliminated in sand close to the root. The effects of plant roots on the dissipation of organic pollutants has been attributed mainly to increased microbial numbers and selection of specialized microbial communities in the rhizosphere [18,19], but also to improved physical and chemical soil conditions, supply of root exudates for cometabolic processes [20] and increased humidification and absorption of pollutants increasing their bioavailability [21].

Results of the effects of plant roots on the biodegradation of oil and it fractions are found in Table 4-6 and illustrated in Fig. 3. From these results it can be seen that crude oil (Total petroleum hydrocarbons, TPH) was reduced by 30% in the rhizosphere soil of *Vicia faba* plant and by 16.8% and 13.7 in the rhizosphere soil of *Zea mays* and *Triticum aestivuml* plants respectively. This is in contrast to reduction of 10.5%, 10.8% and 8.2% of the non-rhizosphere soil of the above three plants respectively. This shows that TPH biodegradation was enhanced in the rhizosphere soil of the legume plant (*Vicia faba*) as compared to the other two monocot plants (*Zea mays* and *Triticum aestivuml*). Yateem *et al*. [6] investigated the degradation of TPH in the rhizosphere and non-rhizosphere soil of three domestic plants mainly, alfalfa (*Medicago sativa*), broad been (*Vicia faba*) and rayegrass (*Lolium pernne*). They found that TPH degradation in soil cultivated with broad been and alfalfa was 36.6% and 35.8% respectively, compared with 24% degradation in case of rayegrass.

Results of the effects of the roots of *Vicia*, *Mays* and *Triticum* plants on the degradation of the different oil fractions (Table 4-6) show that the most degradable fraction was the saturates followed the aromatics while the calcitrant fractions were resins and asphaltenes. *Vicia faba* roots were able to degrade more of the saturates (47.0%) and the aromatice (26.2%) as compared to the roots of *Zea mays* (37.4% for saturates and 8.2% for the aromatics) and *Triticum aestivuml* (33.2% and 3.9% for saturates and aromatics respectively). It is of interest to observe from this work that 5.3% of the hardly-degradable fraction resin was degraded in the rhizosphere of *Vicia faba*. On the other hand the recalcitrant fraction asphaltene was reduced by 3.6% in the rhizosphere of *Triticum aestivuml* and by 1.9% in the rhizosphere of *Vicia faba*.

The above results lead to the conclusion that the legume plant *Vicia faba* as compared to the other two plants demonstrates successful phytoremediation of the polluted desert soil.

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