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Phytoremediation of polycyclic aromatic hydrocarbon contaminated soil by *Acacia senegal*

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Abstract

Worldwide, contamination of soil is a severe problem. The negative effects of pollutants on the environment and on human health are diverse and depend on the nature of the pollution. Phytoremediation is a promising technology for the cleanup of polycyclic aromatic hydrocarbon contaminated soil. In the present work the rhizosphere of *Acacia senegal* (L.) Willd. Ex. Del. plants were tested for their abilities to stimulate the microbial degradation of soil pollutants in desert soil contaminated with 2.4-2.8% polycyclic aromatic hydrocarbons. The results showed that the roots of the different plants were density associated with total bacteria, fungi and PAH (polycyclic aromatic hydrocarbon)-degrading microorganisms, this is confirmed from the (R^+/S^+) ratios which ranged from 55.7-258.2 (for total bacteria), 20.3-125.1 (for fungi) and 95.6-348.2 (for PAH degraders). Percentages of PAH-degraders were higher in the rhizosphere soil of *A. senegal* (68.5%) as compared to the rhizosphere soil of *A. nilotica* and *A. catechu* plants respectively. The results of the biodegradation of PAH-I, II & III and its fractions showed that great reduction (25.5%) of total polycyclic aromatic hydrocarbons (TPAHs) was observed in the rhizosphere soil of *A. senegal* as compared to reduction in rhizosphere soil of *A. catechu* and *A. nilotica* respectively. It was observed also that in the polluted non-cultivated soil the PAHs were reduced by 8.1 -10.6 % as a result of biostimulation process only (addition of nutrients). The results also showed that *A. senegal* rhizosphere was able to reduce more of the saturated (41.1-41.6 %), more of the aromatics (20.2-20.8%) fractions. It is of interest to find that 6.5 % of the hardly degradable fraction resins were degraded in rhizosphere soil of *A. senegal*. The present results clearly demonstrated that *A. senegal* provided successful phytoremediation process of a contaminated desert soil as compared to the other two legume trees.

Keywords: Phytoremediation, Polycyclic aromatic hydrocarbon, Desert soil contaminated, Rhizosphere

Introduction

Pollution of the soil environment with toxic materials from fossil burning mining and smelting of metalliferous ores, disposal of sewage, fertilizers and pesticides etc has increased dramatically since the onset of industrial revolution. Soil pollution arising from increasing demand for petroleum and its products has become a common problem in recent years. Soils polluted with polycyclic aromatic hydrocarbons differ from unpolluted soils and are not able to support adequate crop growth and development. There is need to treat these soils so as to satisfy the food requirement of the ever increasing world population (1). PAHs are found naturally in the environment but they can also be man-made. Polycyclic aromatic hydrocarbons (PAHs; also known as polyarenes, polynuclear aromatic hydrocarbons) are a product of incomplete combustion. Polycyclic aromatic hydrocarbons are a class of organic compound that are considered as ubiquitous contaminants in the environment.

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 & Sayre (4) estimated phytoremediation clean up costs of $ 162/m3 compare to $ 810/m3 for excavation and incineration.

For successful phytoremediation both plants and microorganisms must survive and grow in PAH 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 PAH contaminated sites, 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 to improve soil quality (5). Yateem et al., (6) investigated the degradation of total polycyclic aromatic hydrocarbons (TPAH) 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% TPAH, 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 & Duncan (7) found that the legume plant (*Vicia sativa*) 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 & 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 (*Trifolium partense*, *Trifolium repens*). 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 polycyclic aromatic 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) PAH. 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 PAH-contaminated soil and enhance PAH degradation.

The objective of the present research is to study the effects of a legume tree species, *A. senegal* on the changes of the rhizosphere microflora and its degradation potential in response to polycyclic aromatic hydrocarbon-contamination of soil. The advantage of the chosen legume plant is their ability to fix atmospheric nitrogen this is in addition to the ability of these legume tree species to tolerate up to 10% (w/w) PAH.

Materials and methods

Field Experiments

Four plots each of 5×5 m^2 were delimited in an area (Western Rajasthan, India) without any history of pollution. The soil in each plot at 0-50 cm depth were ploughed and thoroughly mixed with weathered PAH 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 K2HPO4/kg soil). Plot No. 1, 2 & 3 was planted with 25 seedlings of *A. senegal (PAH-I, II &III)*; and Plot 4 was left without seeding. Another 4 plots received only nutrients (i.e. left unpolluted) to behave as control. The plots were separated by 5m from each other.

After 90 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 noncultivated plots. At the beginning of the experiments soil samples were also collected. Samples were analyzed 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 polycyclic aromatic 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 (10) 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 polycyclic aromatic hydrocarbon and its Fractions

10 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, and the amount of total polycyclic aromatic hydrocarbons (TPAHs) was determined, and the loss (%) of PAH was then calculated. The extracted residual PAH was suspended in hexane and filtered through tared filter paper to remove and to determine the insoluble fraction (asphaltene). The hexanesoluble fraction was fractionated by liquid-solid chromatography into saturates, aromatics and resins. The amount of each fraction was determined according to Chaineau et al (11).

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 polycyclic aromatic hydrocarbons-degrading microorganisms the three tubes mean probable number (MPN) method was used as described by Chaineau et al (11).

Results

The soil sample used in the present study is sandy soil, with pH 7.6 - 7.8. This soil was poor in phosphorus (0.17 ppm) and nitrogen (0.02%) contents. Results of the microbial contents of the polluted and non-polluted plots of *A. senegal* plants are found in figures. The results show that the CFU/g of total bacteria, fungi and PAH-degraders are higher in rhizosphere soil (both polluted and non-polluted) than in the non-rhizosphere soil of the above plants. These results reflect the positive rhizosphere effects of the plants on the microbial communities as indicated from the results of (R/S) ratios (counts in the rhizosphere / counts in the non-rhizosphere) of more than one. The (R^+/S^+) values were more pronounced in the polluted plots than in the nonpolluted one (control). Murotova et al (12) explained that the success of phytoremediation of polycyclic aromatic hydrocarbon contaminated soil is connected with the plants capacity to enhance microbial activity in the rhizosphere.

In the polluted *A. senegal* plots PAH-I, II & III (R^+/S^+) values were in the range of 30-32 (for fungi), value of 59.1-60 (for total bacteria) to 344.1-347.5 (for PAH-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 PAH to this type of soil stimulated the development of more microorganisms as compared to the control sample. Kuiper et al., (13) 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 polycyclic aromatic hydrocarbons. Narino et al., (14) 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 polycyclic aromatic hydrocarbons-degrading microorganisms in the polluted rhizosphere and nonrhizosphere soil of *A. senegal* plots show that the polluted rhizosphere soil of the legume plants stimulated the development of higher counts (CFU/g soil) of such organisms as compared to the non-rhizosphere soil (Figures- 1-3).

The percentages of polycyclic aromatic hydrocarbon degraders also were higher in the rhizosphere soil than in the nonrhizosphere one. *A. senegal* rhizosphere contained the highest values (68.5%) as compared to *A. catechu* and *A. nilotica* rhizosphere soil. As a comparison the percentages of PAH-degraders in the polluted non-rhizosphere soil are in the range of 9.1-11.9%.

Results of the effects of plant roots on the biodegradation of polycyclic aromatic hydrocarbons-I, II & III and it fractions are found in figures. From these results it can be seen that Total polycyclic aromatic hydrocarbons, (TPAHs) was reduced by 30% in the rhizosphere soil of *A. senegal* plant respectively. This is in contrast to reduction of 11.2%, 11.5% and 9.2% of the nonrhizosphere soil of the above plants respectively. This shows that TPAH biodegradation was enhanced in the rhizosphere soil of the *A. senegal* as compared to the other two plants (*A. catechu* and *A. nilotica*). Results of the effects of the roots of *A. senegal* plant on the degradation of the different PAH-I, II & III fractions (Figure 4-6) show that the most degradable fraction was the saturates followed the aromatics while the recalcitrant fractions were resins and asphaltenes. *A. senegal* roots were able to degrade more of the saturates (40-41.5%) and the aromatics (20-20.9%) as compared to the roots of *A. catechu* and *A. nilotica* respectively. It is of interest to observe from this work that 5.5 % of the hardly degradable fraction resin was degraded in the rhizosphere of *A. senegal.* On the other hand the recalcitrant fraction asphaltenes was reduced by 2.4% in the rhizosphere of *A. senegal*.

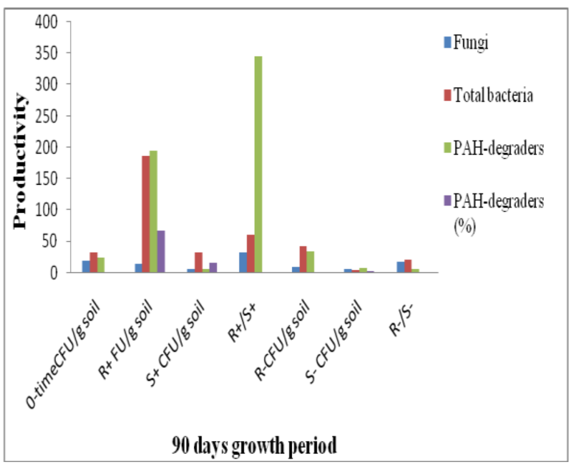

Figure 1: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of *Acacia senegal* (PAH- I) plant after 90 days growth period R^+ = polluted rhizosphere soil, S^+ = polluted non rhizosphere soil, R^- = non-polluted rhizosphere soil, S^- = non-polluted non-rhizosphere soil.

Discussion

Yateem et al., (15) investigated the degradation of Total polycyclic aromatic hydrocarbons in the rhizosphere and nonrhizosphere soil of three domestic plants mainly, alfalfa (*Medicago sativa*), *V. faba* and raye grass (*Lolium perenne*). They found that TPAH degradation in soil cultivated with broad been and alfalfa was 36.4% and 34.7% respectively, compared with 24% degradation in case of rayegrass. Murotova et al., (12) explained that the success of phytoremediation of polycyclic aromatic 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. Radwan et al (16) found that total number of PAH-degrading bacteria increased in the rhizosphere of *A. senegal* plant and more polycyclic aromatic 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 (17,18), but also to improved physical and chemical soil conditions, supply of root exudates for cometabolic processes (19) and increased humidification and absorption of pollutants increasing their bioavailability.

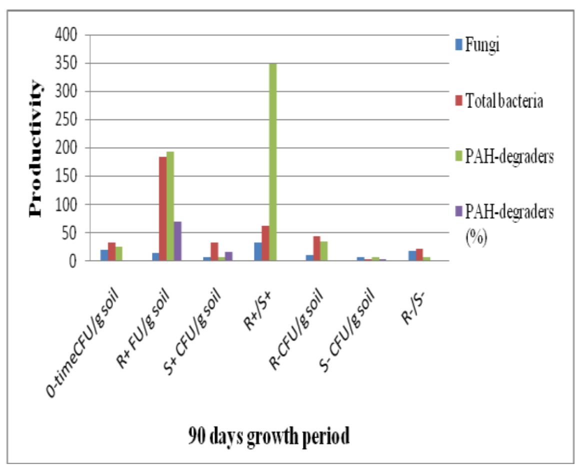

Figure 2: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of *Acacia senegal* (PAH- II) plant after 90 days growth period.

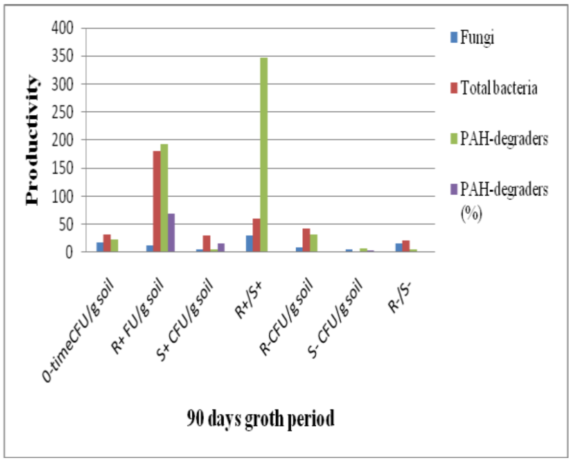

Figure 3: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of *Acacia senegal* (PAH- III) plant after 90 days growth period.

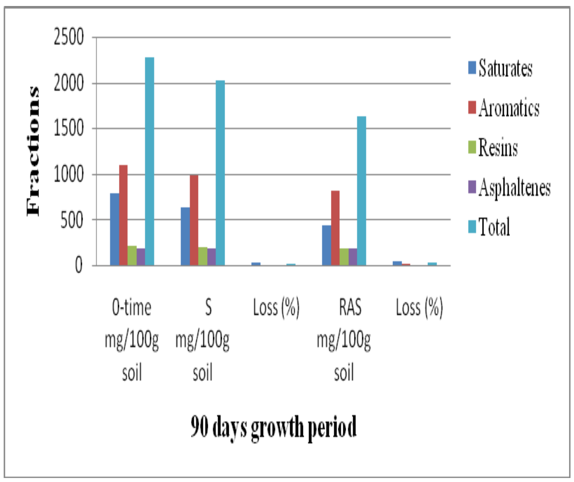

Figure 4: Biodegradation of PAH and its fractions in the rhizosphere of *Acacia senegal* PAH-I (RAS) plant as compared with non-rhizosphere soil (S) after 90 days growth periods.

Conclusion

The above results lead to the conclusion that the plant *A. senegal* as compared to the other two plants demonstrates successful phytoremediation of the polluted desert soil. On the other hand the non-polluted plots contained significantly lower counts and lower percentages (0.3-3.2%). The above results confirmed the ability of plant roots to neutralize and or to remove the toxic effects of the PAH pollutants; this is through the exudates, nutrient and other materials. Merkl et al. tested three legume plants and three grasses for their ability to stimulate microbial degradation in sandy soil contaminated with 5% (w/w) PAH. They considered legumes to be specifically promising because of their ability to fix atmospheric nitrogen.

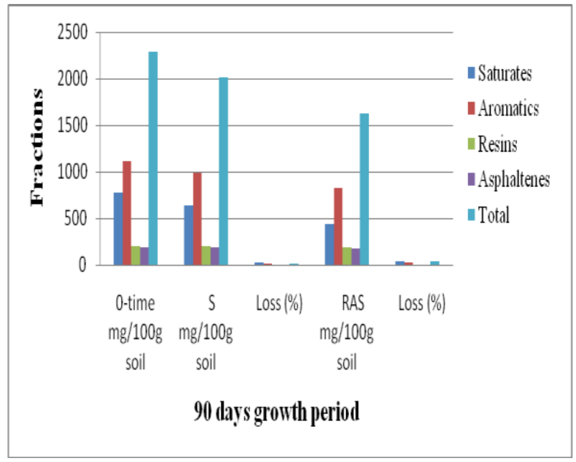

Figure 5: Biodegradation of PAH and its fractions in the rhizosphere of *Acacia senegal* PAH-II (RAS) plant as compared with non-rhizosphere soil (S) after 90 days growth periods

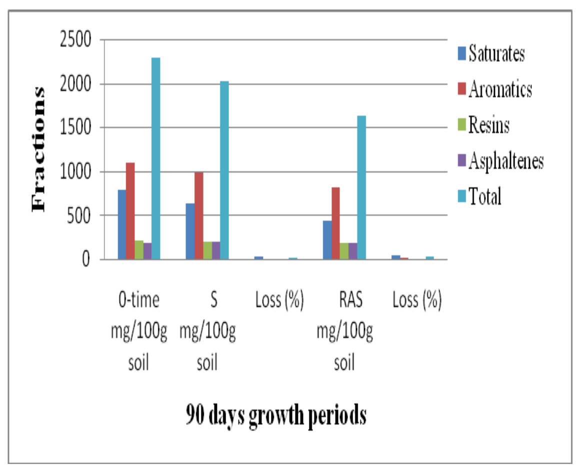

Figure 6: Biodegradation of PAH and its fractions in the rhizosphere of *Acacia senegal* PAH-III (RAS) plant as compared with non-rhizosphere soil (S) after 90 days growth periods.

PLOTS

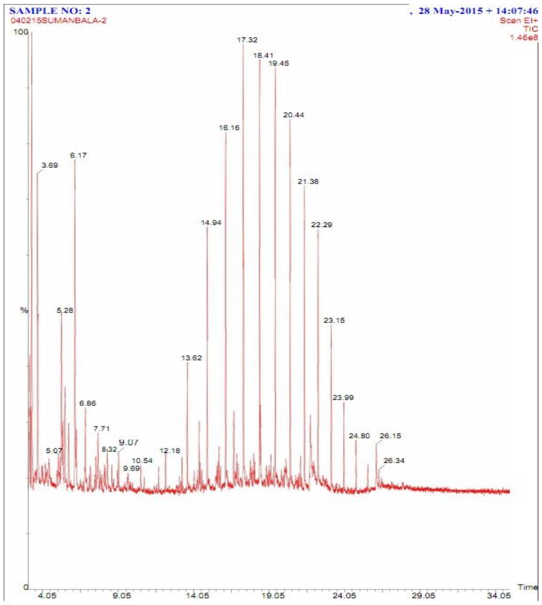

Fig. 1: Conc. of PAH in contaminated soil.

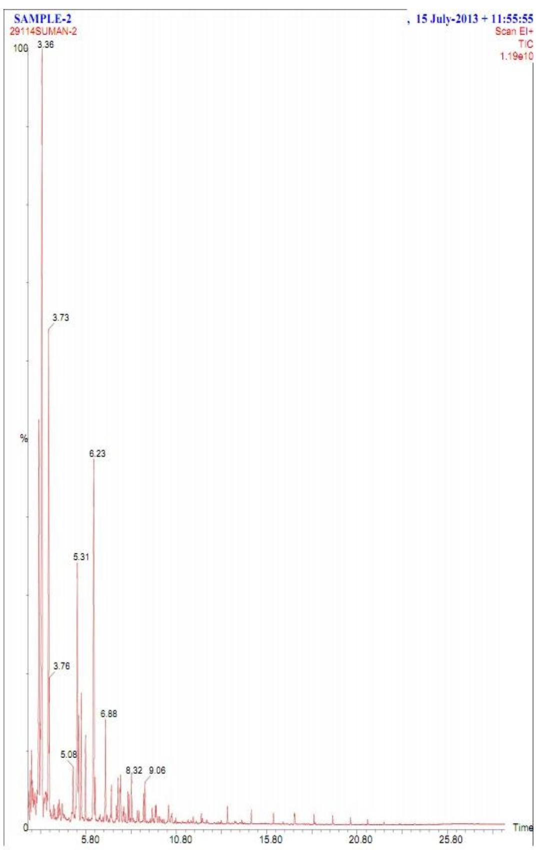

Fig. 2: Degradation of PAH by *Acacia Senegal*.

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