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Mycorhizoremediation of Polycyclic Aromatic Hydrocarbon Contaminated Soil by *Acacia Nilotica*

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Abstract
Soil contamination or soil pollution or it can be also said "Land Pollution" is caused by the presence of xenobiotic (human-made) chemicals or other alteration in the natural soil environment. Phytoremediation is a promising technology for the cleanup of polycyclic aromatic hydrocarbon contaminated soil. In the present work the rhizosphere of *Acacia nilotica* (L.). Ex. Del. plants were tested for their abilities to stimulate the microbial degradation of soil pollutants in desert soil contaminated with 2.1-2.7% 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 55.6-258.2 (for total bacteria), 20-125.1 (for fungi) and 95.7-348.2 (for PAH degraders). Percentages of PAH-degraders were higher in the rhizosphere soil of *A. nilotica* 25.1-25.9 % respectively. The results of the biodegradation of PAH-I, II & III and its fractions showed that great reduction (25.4%) of total polycyclic aromatic hydrocarbons (TPAHs) was observed in the rhizosphere soil of *A. nilotica* respectively. It was observed also that in the polluted non-cultivated soil the PAHs were reduced by 8.1 -10.5 % as a result of bio stimulation process only (addition of nutrients). The results also showed that *A. nilotica* rhizosphere was able to reduce more of the saturated (25-25.9%) and more of the aromatics (3.5-3.8%) fractions. It is of interest to find that 5.2 % of the hardly degradable fraction resins were degraded in rhizosphere soil of *A. nilotica*. The present results clearly demonstrated that *A. nilotica* provided successful phytoremediation process of a contaminated desert soil.

Keywords: Phytoremediation, Polycyclic aromatic hydrocarbon, Desert soil contaminated, rhizosphere.

1. Introduction
Bioremediation is a technique to treat contaminated sites with biological methods and in the context of oil spills it was aimed to stimulate the rate of biodegradation of oil. Polycyclic aromatic hydrocarbons (PAHs) are one of the major groups of these contaminants [1]. PAHs constitute a diverse class of organic compounds consisting of two or more aromatic rings with various structural configurations [2]. Being a derivative of benzene, PAHs are thermodynamically stable. In addition, these chemicals tend to adhere to particle surfaces, such as soils, because of their low water solubility and strong hydrophobicity, and this results in greater persistency under natural conditions. This persistency coupled with their potential carcinogenicity makes PAHs problematic environmental contaminants [3, 4, 5]. PAHs are widely found in high concentrations at many industrial sites, particularly those associated with petroleum, gas production and wood preserving industries [6].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. Yateem *et al* [7] 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 (Viciafaba) and rayegrass (Loliumperenne). 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 [8] 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 [9] 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 (*Trifoliumpartense, Trifoliumrepens*). 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* [10] 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. nilotica* 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.

2. Material and Method

2.1 Field Experiments

Four plots each of $5\times5m^2$ 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. nilotica (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 no cultivated 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*. [11] at the beginning of the experiment and periodically to each plot. The soil in each plot was ploughed weekly for aeration.

2.2 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* [12].

2.3 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) methodwasusedas described by Chaineau *et al* [12].

3. Results

The soil sample used in the present study is sandy soil, with pH 7.6-7.8. This soil was poor in phosphorus (0.17ppm) and nitrogen (0.02%) contents. Results of the microbial contents of the polluted and non-polluted plots of *A. nilotica* plants are presented 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 no polluted one (control). Murotova *et al.* [13] 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.

In the polluted *A. nilotica* plots PAH-I, II & III (fig- 1, 2 & 3) (R^+/S^+) values were in the range of 50-51.4 (for fungi) to 55-56.8 (for total bacteria), to 99-102.4 (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* [14]. 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* [15]. 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. nilotica* plots show that the polluted rhizosphere soil of the plants stimulated the development of higher counts (CFU/g soil) of such organisms as compared to the non-rhizosphere soil.

The percentages of polycyclic aromatic hydrocarbon degraders also were higher in the rhizosphere soil than in the nonrhizosphere one. *A. nilotica* rhizosphere contained the values (25-25.9%) rhizosphere soil. As a comparison the percentages of PAH-degraders in the polluted non-rhizosphere soil are in the range of 5.1-9.3%. On the other hand the non-polluted plots contained significantly lower counts and lower percentages (0.3-3.1%). 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.

Results of the effects of plant roots on the biodegradation of polycyclic aromatic hydrocarbons-I, II & III and it fractions are found in figures (4-6). From these results it can be seen that Total polycyclic aromatic hydrocarbons, (TPAHs) was reduced by 25-25.7% in the rhizosphere soil of *A. nilotica* plants respectively. This is in contrast to reduction of 14.5%, 14.7% and 15.3% of the nonrhizosphere soil of the above three plants respectively. Results of the effects of the roots of *A. nilotica* plants on the degradation of the different PAH-I, II & III fractions (Figures 3-6) show that the most degradable fraction was the saturates followed the aromatics while the recalcitrant fractions were resins and asphaltenes. *A. nilotica* roots were able to degrade more of the saturates (25.4-25.9%) and the aromatics (3.7-3.9%) respectively.

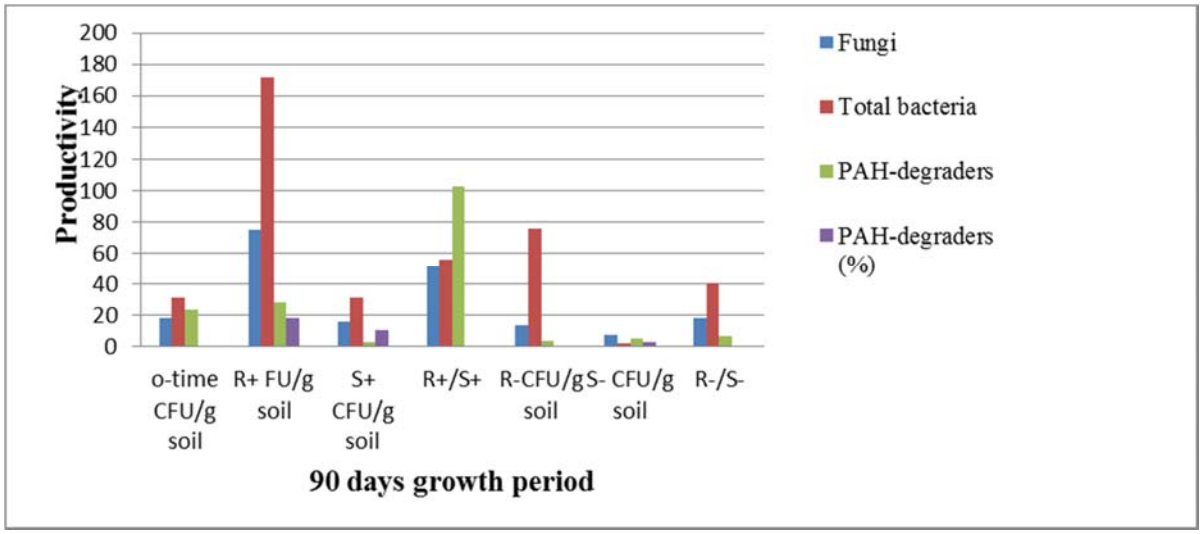

Fig 1: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of *Acacia nilotica* (PAH- I) plant after 90 days growth period R^+=polluted rhizosphere soil, S^+=polluted non rhizosphere soil, R^-=non-polluted rhizosphere soil, S^- = non-polluted non-rhizosphere soil

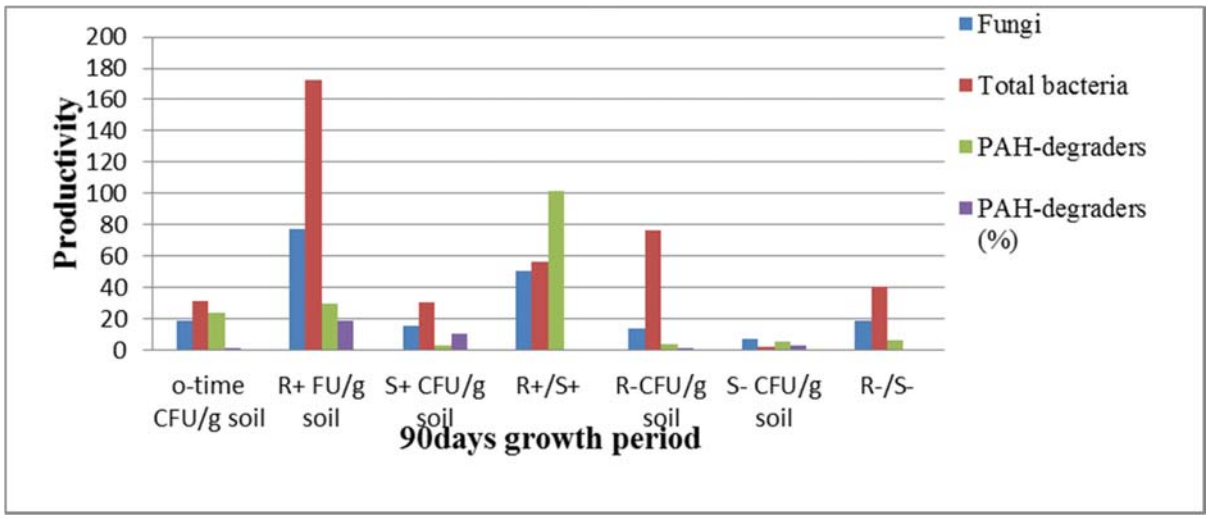

Fig 2: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of *Acacia nilotica* (PAH- II) plant after 90 days growth period.

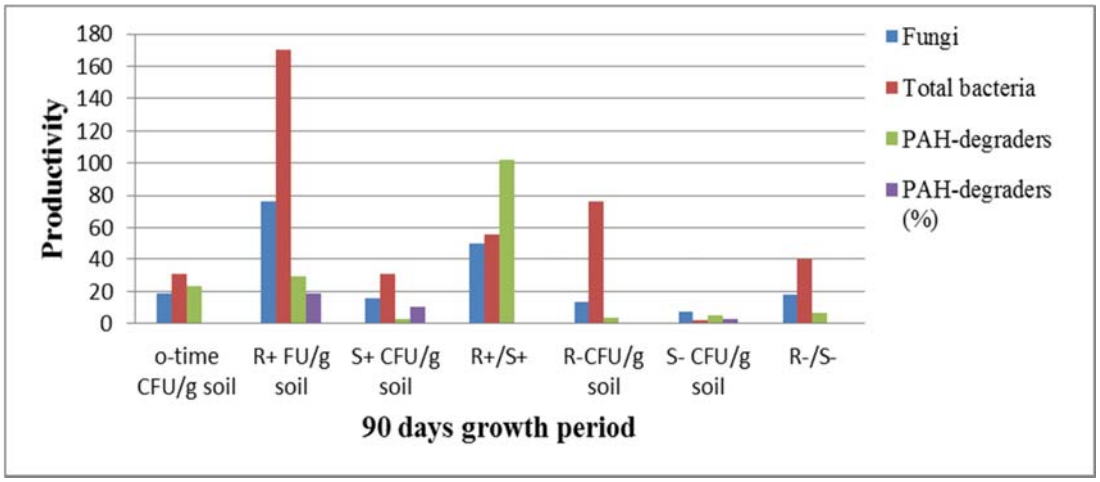

Fig 3: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of *Acacia nilotica* (PAH- III) plant after 90 days growth period

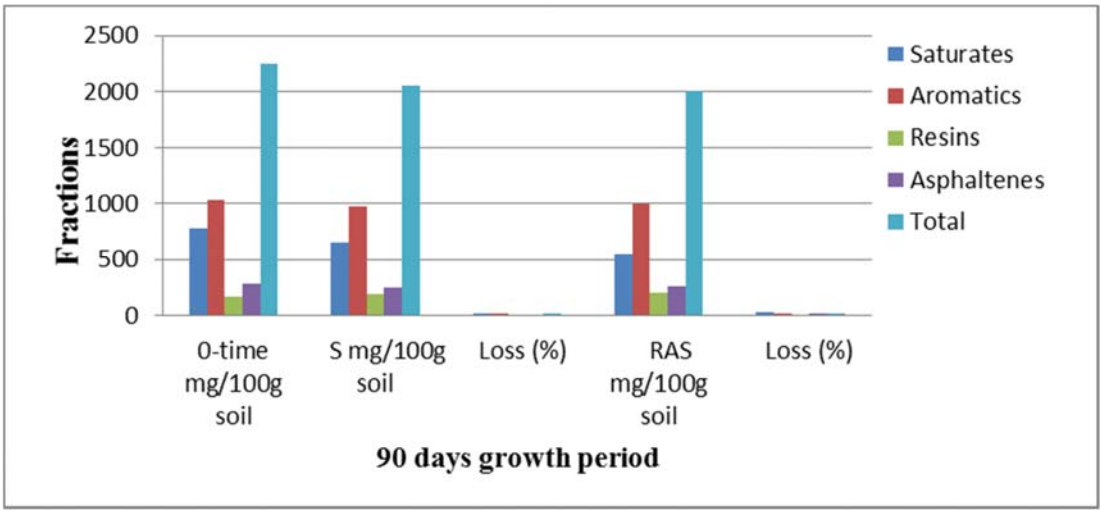

Fig 4: Biodegradation of PAH and its fractions in the rhizosphere of *Acacia nilotica* PAH-I (RAN) plant as compared with non-rhizosphere soil (S) after 90 days growth periods

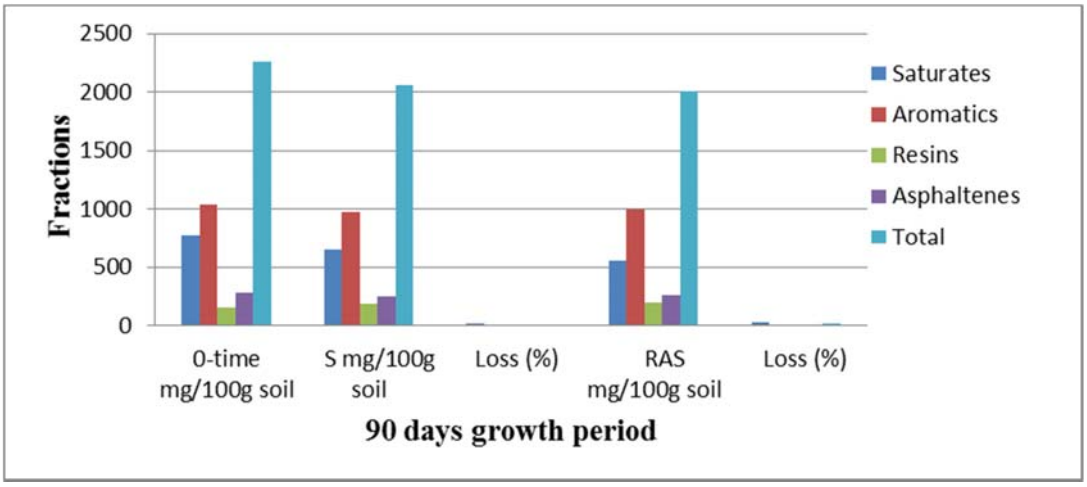

Fig 5: Biodegradation of PAH and its fractions in the rhizosphere of *Acacia nilotica* PAH-II (RAN) plant as compared with non-rhizosphere soil (S) after 90 days growth periods

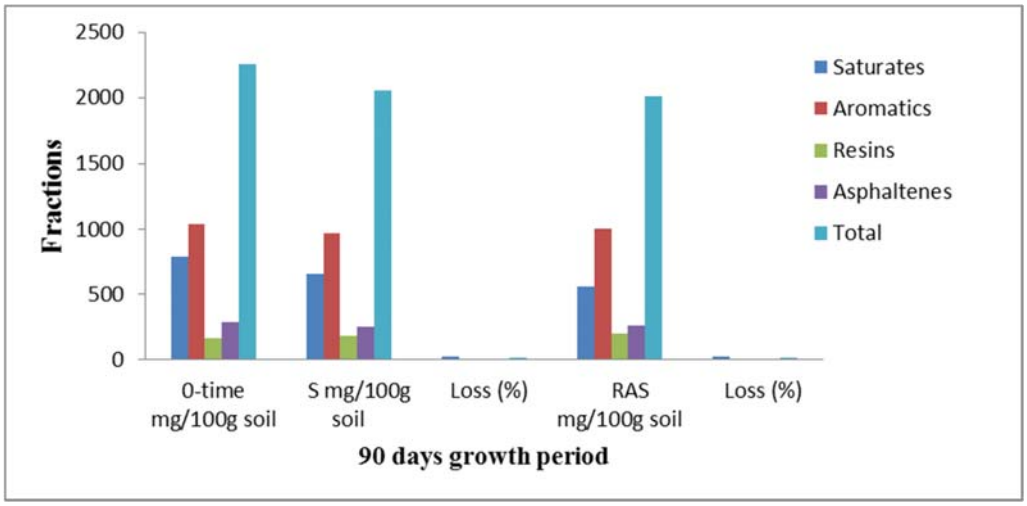

Fig 6: Biodegradation of PAH and its fractions in the rhizosphere of *Acacia nilotica* PAH-III (RAN) plant as compared with non-rhizosphere soil (S) after 90 days growth periods

Plots

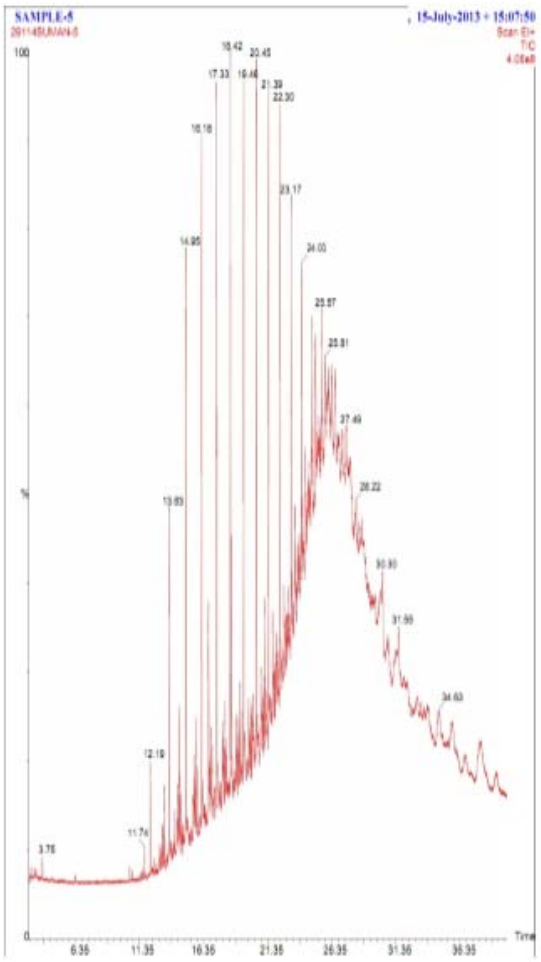

Fig 1: Concentration of PAH in contaminated soil,

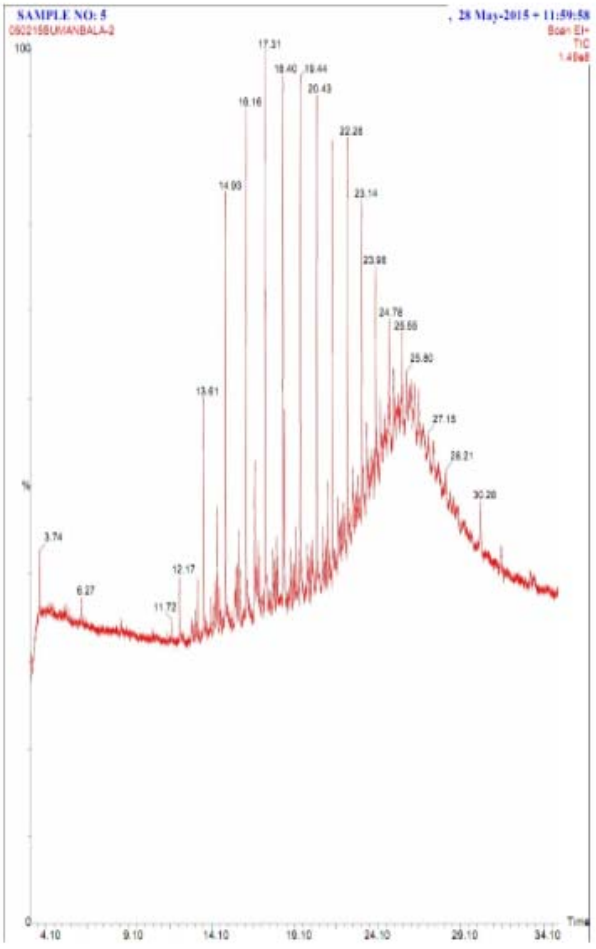

Fig 2: Degradation of PAH by Mycorhizoremediation.

4. Discussion

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. Radwan *et al* [16].Found that total number of PAH-degrading bacteria increased in the rhizosphere of *A. nilotica* 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[19] processes and increased humidification and absorption of pollutants increasing their bioavailability [20].

5. Conclusion

The above results lead to the conclusion that the plant *A. nilotica* plants demonstrates successful phytoremediation of the polluted desert soil. It is of interest to observe from this work that 5.3% of the hardly degradable fraction resin was degraded in the rhizosphere of *A. nilotica*. On the other hand the recalcitrant fraction asphaltenes was reduced by 3.7% in the rhizosphere of *A. nilotica*. Yateem et al., 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 (*Loliumperenne*). 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.

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