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ABSTRACT
The industrial revolution of the past century has resulted in significant damage to environmental resources such as air, water and soil. Phytoremediation is a promising technology for the cleanup of petroleum contaminated soil. This subject is emerging as a cutting edge area of research gaining commercial significance in the contemporary field of environmental biotechnology. Several microbes, including mycorrhizal and non-mycorrhizal fungi, agricultural and vegetable crops, ornamentals, and wild metal hyperaccumulating plants are being tested both in lab and field conditions for decontaminating the metalliferous substrates in the environment. As on todate about 400 plants that hyperaccumulate metals are reported. In the present work the rhizosphere of *Terminalia arjuna* (L.) Druce, *Anogeissus latifolia* (L.) Willd. and *Tecomella undulata* (L.) Willd. Ex. Del. plants were tested for their abilities to stimulate the microbial degradation of soil pollutants in desert soil contaminated with 2.5-2.6% 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 *T. arjuna* (65.5%) as compared to the rhizosphere soil of *A. latifolia* and *T. undulata* plants (22.5 % and 20.2 % respectively). The results of the biodegradation of oil and its fractions showed that great reduction (26 %) of total petroleum hydrocarbons (TPHs) was observed in the rhizosphere soil of *T. arjuna* as compared to 15.6 % and 12.8 % reduction in rhizosphere soil of *A. latifolia* and *T. undulata* plants (22.5 % and 20.2 % respectively). The results also showed that *T. arjuna* rhizosphere was able to reduce more of the saturated (43.0 %) and more of the aromatics (25.7 %) fractions, compared to (35.2 % and 7.9 %) for *A. latifolia* and (31.2 % and 4.1 %) for *T. undulata* rhizospheres. It is of interest to find that 5.3 % of the hardly degradable fraction resins were degraded in rhizosphere soil of *T. arjuna*. The present results clearly demonstrated that *T. arjuna* provided successful phytoremediation process of a contaminated desert soil as compared to the other two trees.

Key words: Phytoremediation, desert soil contaminated, petroleum hydrocarbons, rhizosphere

Introduction
Phytoremediation encompasses the use of plants for the remediation of environments contaminated with hazardous wastes. Plants can be used in site remediation both through the mineralization of toxic organic compounds as well as through the bioaccumulation and concentration of heavy metals

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and other inorganic compounds. 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 [7]. Increased biodegradation of organic contaminants occurs in the rhizosphere, the zone of soil directly adjacent to and under the influence of plant roots [27].

Plants have natural attributes that make them ideal candidates for cleansing contaminated soil environments. The root system represents an enormous surface area that enables plants to absorb and accumulate the water and nutrients essential for growth. Plants have remarkable metabolic and absorption capabilities and possess transport systems that can selectively take up many ions from soils. Plants have evolved a great diversity of genetic adaptations to handle potentially toxic levels of metals and other pollutants that occur in the environment.

Interaction between microorganisms associated with plants and plants is known the main features of this phenomenon. There are three primary mechanisms by which plants and microorganisms remediate petroleum–contaminated soil and ground water. These include degradation, containment and transfer of the hydrocarbons from soil to atmosphere [22,4,20,21].

For successful phytoremediation both plants and microorganisms 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 polishing step to further degrade residual hydrocarbons and to improve soil quality [23]. Yateem et al. [29] 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.

Ojegba and Sadiq [14] also examined effects of spent engine oil on the growth of Amaranthus hybridus and reported significant differences between the control plants and those grown in the oil treated soil. In their experiment the mean height of the control plants was significantly (p < 0.05) greater than those for plants grown in soil treated with 1–5 percent spent engine oil. Vwioko and Fashemi (2005) have investigated growth response of five different plant species in soil supplemented with spent lubricating oil at 1-6 % w/w strengths. The result of this experiment which conducted at six consecutive concentrations level showed that various growth parameters demonstrate an overall dose dependent response although at lower concentration some plant species showed a positive response to the contaminant.

Rosado and Pichtel (2004) 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 partens, 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 oil hydrocarbons.

Metals can be taken up by other plants that do not accumulate the high concentrations of hyperaccumulators, for example, corn (Zea mays), sorghum (Sorghum bicolor), alfalfa (Medicago sativa L.), and willow trees (Salix spp.). The greater biomass of these plants could result in a greater mass of metals being removed from the soil even though the concentrations within the plants might be lower than in hyperaccumulators, since the metal concentration in the plant multiplied by the biomass determines the amount of metal removal.

Studies have shown that trees in particular can be effective in cleaning up ground water and soils contaminated with BTEX, MTBE, and PAHs [9,13,18,28]. Poplars have been used to remediate sites contaminated with nitrates, salts, landfill leachates, heavy metals, pesticides, solvents, explosives, and radionuclides [32]. Willow trees (Salix spp.) have also shown success in removing gasoline from soil and groundwater [13,3,31,25]. These trees are often preferred because they can grow in saturated conditions and produce adventitious roots [19] creating healthier environments for microbial growth [25]. Trees that enhance soil aeration and create macropores should improve the capacity of soil microbes to biodegrade PAHs as well as BTEX.

The objective of the present research is to study the effects of a three tree sps. namely T. arjuna, A. latifolia and T. undulata on the changes of the
rhizosphere microflora and its degradation potential in response to hydrocarbon-contamination of soil. The advantage of the trees is their ability to tolerate up to 10% (w/w) crude oil.

Materials and methods

Field Experiments:

Four plots each of 5×5 m² were delimited in an area (Botanical garden, Department of Botany, J. N. V. University, Jodhpur, India) without any 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 K₂HPO₄/kg soil). Plot No. 1 was planted with 25 seedlings of *T. arjuna*; Plot No. 2 was planted with 25 seedlings of *A. latifolia*; Plot No. 3 was planted with 25 seedlings of (*T. undulata*) and Plot 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 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 non-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 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., [24] 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 8h. The chloroform extract was evaporated in a pre-weighed 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 nhexane 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. [2].

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. [2].

Results and discussion

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 *T. arjuna, A. latifolia* and *T. undulata* plants are found in tables (1-3).

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 polluted plots than in the non-polluted one (control). Murotova et al. (2003) 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 *T. arjuna* plots (Table 1) (R’/S’) values were in the range of 30.5 (for fungi) to 350.0 (for oil-degraders). In *A. latifolia* plots (R’/S’) values (Table 2) were 125.6 (for fungi) to 259.3 (for total bacteria), while in (*T. undulata*) plots (Table 3) values of 50.6 (for fungi) to 102.7 (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. Kuiper et al. [8] 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. [12] 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 nonrhizosphere soil of T. arjuna, A. latifolia and T. undulata plots show that the polluted rhizosphere soil of 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 nonrhizosphere one. T. arjuna rhizosphere contained the highest values (69.2%) as compared to A. latifolia (20.6%) and T. undulata (19.1%) rhizosphere soil. As a comparison the percentages of oil-degraders in the polluted non-rhizosphere soil are in the range of 9.5-15.6%. On the other hand the non-polluted plots contained significantly lower counts and lower percentages (0.7-1.2%). The above results confirmed the ability of plant roots to neutralize and to remove the toxic effects of the oil pollutants; this is through the exudates, nutrient and other materials.

Murotova et al. [11] 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. [11] also suggested that additional studies are necessary to determine whether the population of hydrocarbon-degrading microorganisms protects the plant from toxic effects of pollutants or whether the plant provides the favorable conditions of this population activity.

Merkl et al. [10] 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. [16] found that total number of oil-degrading bacteria increased in the rhizosphere of T. arjuna 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 [17,1], but also to improved physical and chemical soil conditions, supply of root exudates for cometabolic processes [30] and increased humidification and absorption of pollutants increasing their bioavailability [6].

**Table 1**: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of T. arjuna plant after 90 days growth period

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>0-time CFU/g soil</th>
<th>90 days growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R’ FU /g soil</td>
<td>S’ CFU/g soil</td>
</tr>
<tr>
<td>Fungi</td>
<td>18.2 × 10^4</td>
<td>12.2 × 10^4</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>31.0 × 10^4</td>
<td>181.4 + 7.2 × 10^7</td>
</tr>
<tr>
<td>Oil-degraders</td>
<td>23.0 × 10^4</td>
<td>196 + 8.9 × 10^7</td>
</tr>
<tr>
<td>Oil-degraders (%)</td>
<td>0.79</td>
<td>69.2</td>
</tr>
</tbody>
</table>

**Table 2**: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of A. latifolia plant after 90 days growth period

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>0-time CFU/g soil</th>
<th>90 days growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R’ FU /g soil</td>
<td>S’ CFU/g soil</td>
</tr>
<tr>
<td>Fungi</td>
<td>18.2 × 10^4</td>
<td>20.1 + 2.4 × 10^4</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>31.0 × 10^4</td>
<td>829.9 + 20.7 × 10^7</td>
</tr>
<tr>
<td>Oil-degraders</td>
<td>23.0 × 10^4</td>
<td>166.4 + 5.2 × 10^4</td>
</tr>
<tr>
<td>Oil-degraders (%)</td>
<td>0.79</td>
<td>20.6</td>
</tr>
</tbody>
</table>

**Table 3**: Microbial contents of rhizosphere soil (R) and non-rhizosphere soil (S) of T. undulata plant after 90 days growth period

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>0-time CFU/g soil</th>
<th>90 days growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R’ FU /g soil</td>
<td>S’ CFU/g soil</td>
</tr>
<tr>
<td>Fungi</td>
<td>18.2 × 10^4</td>
<td>76.5 + 1.8 × 10^4</td>
</tr>
<tr>
<td>Total bacteria</td>
<td>31.0 × 10^4</td>
<td>172.5 + 4.9 × 10^7</td>
</tr>
<tr>
<td>Oil-degraders</td>
<td>23.0 × 10^4</td>
<td>29.8 + 0.6 × 10^4</td>
</tr>
<tr>
<td>Oil-degraders (%)</td>
<td>0.79</td>
<td>19.1</td>
</tr>
</tbody>
</table>
Results of the effects of plant roots on the biodegradation of oil and it fractions are found in Tables (4-6). From these results it can be seen that crude oil (Total petroleum hydrocarbons, TPH) was reduced by 28.6% in the rhizosphere soil of *T. arjuna* plant and by 16.1% and 11.1% in the rhizosphere soil of *A. latifolia* and *T. undulata* plants respectively. This is in contrast to reduction of 11.6%, 13.2% and 8.9% of the non-rhizosphere soil of the above three plants respectively. This shows that TPH biodegradation was enhanced in the rhizosphere soil of the *T. arjuna* as compared to the other two plants (*A. latifolia* and *T. undulata*). Yateem et al. [29] investigated the degradation of TPH in the rhizosphere and nonrhizosphere soil of three domestic plants mainly, alfalfa (*Medicago sativa*), *V. faba* and raye grass (*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.

Table 4: Biodegradation of oil and its fractions in the rhizosphere of *T. arjuna* (RTA) plant as compared with the non-rhizosphere soil (S) after 90 days growth period

<table>
<thead>
<tr>
<th>Fractions</th>
<th>0-time mg/100g soil</th>
<th>90 days growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S mg/100g soil</td>
<td>Loss (%)</td>
</tr>
<tr>
<td>Saturates</td>
<td>780.0 + 1.2</td>
<td>636 + 4.9</td>
</tr>
<tr>
<td>Aromatics</td>
<td>1102 + 26.2</td>
<td>989 + 20.7</td>
</tr>
<tr>
<td>Resins</td>
<td>210.0 + 3.7</td>
<td>201 + 5.7</td>
</tr>
<tr>
<td>Asphaltenes</td>
<td>190.0 + 3.2</td>
<td>190 + 3.4</td>
</tr>
<tr>
<td>Total</td>
<td>2282</td>
<td>2016 + 11.9</td>
</tr>
</tbody>
</table>

Table 5: Biodegradation of oil and its fractions in the rhizosphere of *A. latifolia* (RAL) plant as compared with the non-rhizosphere soil (S) after 90 days growth period

<table>
<thead>
<tr>
<th>Fractions</th>
<th>0-time mg/100g soil</th>
<th>90 days growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S mg/100g soil</td>
<td>Loss (%)</td>
</tr>
<tr>
<td>Saturates</td>
<td>910 + 9.2</td>
<td>686 + 3.2</td>
</tr>
<tr>
<td>Aromatics</td>
<td>1090.0 + 24.3</td>
<td>978 + 5.2</td>
</tr>
<tr>
<td>Resins</td>
<td>189.0 + 9.8</td>
<td>199 + 2.4</td>
</tr>
<tr>
<td>Asphaltenes</td>
<td>236.0 + 4.2</td>
<td>240 + 4.2</td>
</tr>
<tr>
<td>Total</td>
<td>2425</td>
<td>2103 + 8.4</td>
</tr>
</tbody>
</table>

Table 6: Biodegradation of oil and its fractions in the rhizosphere of *T. undulata* (RTU) plant as compared with the non-rhizosphere soil (S) after 90 days growth period

<table>
<thead>
<tr>
<th>Fractions</th>
<th>0-time mg/100g soil</th>
<th>90 days growth period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S mg/100g soil</td>
<td>Loss (%)</td>
</tr>
<tr>
<td>Saturates</td>
<td>780 + 26.2</td>
<td>650 + 4.1</td>
</tr>
<tr>
<td>Aromatics</td>
<td>1040 + 37.8</td>
<td>971 + 3.2</td>
</tr>
<tr>
<td>Resins</td>
<td>160 + 7.8</td>
<td>184 + 1.7</td>
</tr>
<tr>
<td>Asphaltenes</td>
<td>280 + 9.2</td>
<td>252 + 7.8</td>
</tr>
<tr>
<td>Total</td>
<td>2260</td>
<td>2057 + 6.4</td>
</tr>
</tbody>
</table>

Acknowledgement

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References


