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Implementation of *Parkinsonia aculeata* in Phytoremedation of Crude Oil Contaminated soil in Sudanese Environment

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Phytoremediation is the name given to a set of technologies that use plants to clean contaminated sites. *Parkinsonia aculeata* was tested for it is ability to degrade crude oil contaminated soil in four concentrations 0.5, 1, 1.5 and 2% (w/w) of crude oil. A glasshouse experiment was conducted to investigate effects of crude oil (0, 0.5, 1.0, 1.5 and 2.0 %) on growth of *P. aculeata*, removal of pollutant from soils and the abundance of TPH- degrading bacteria in the rhizosphere. Plant parameters, degradation percentage, retention time and bacterial count were calculated. Results showed that shoot length of *P. aculeata* was not significantly (P≤0.05) affected when seedlings were raised in oil-contaminated soil. Root length in oil - stressed and non –stressed seedlings has been significantly retarded at both four and six month intervals. *P. aculeata* shoot weight has not been significantly reduced by total petroleum hydrocarbon at any of the tested intervals or within any particular stage of growth. No significant differences were observed in root weight of the control

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plants and the treated ones at any of the intervals tested. Degradation percentage was found to be in the range between 49-55%. Penadecane is the first compound appear in most cases and the retention time of the first compound appear was between 19.544 -34.620 min. Number of compounds detected in the rhizosphere of 0.5 % contaminated soil those were 33, 22 , 94 and 106 compound after two, four and six month of growth and at zero time respectively. Viable count of the dominant bacteria showed that there is no significant different between concentrations (P=0.109), but there is significant difference between intervals (P=0.044) in bacterial number. Results indicate that it is possible to use *Parkinsonia aculeata* for the removal of contaminant from soil polluted with crude oil.

Keywords: Crude oil; phytoremedation; Sudan; Parkinsonia aculeate; soil.

1. INTRODUCTION

Phytoremediation is an emerging technology that uses various plants to degrade, extract, contain, or immobilize contaminants from soil and water. This technology has been receiving attention lately as an innovative, cost-effective alternative to the more established treatment methods used at hazardous waste sites EPA [1]. Plants may enhance degradation in the rhizosphere (root zone of influence). Microbial counts in rhizosphere soils can be 1 or 2 orders of magnitude greater than in nonrhizosphere soils. It is not known whether this is due to microbial or fungal symbiosis with the plant, plant exudates including enzymes, or other physical/chemical effects in the root zone. There are, however, measurable effects on certain contaminants in the root zone of planted areas EPA [1]. Another possible mechanism for contaminant degradation is metabolism within the plant. Some plants may be able to take in toxic compounds and in the process of metabolizing the available nutrients, detoxify them.

While numerous studies have been carried out at the lab-scale, very little has been published about field scale implementation of phytoremediation. Nedunuri et al. [2] investigated total petroleum hydrocarbon (TPH) removal at several field sites contaminated with crude oil, diesel fuel, or petroleum refinery wastes, at initial TPH concentrations of 1,700 to 16,000 mg/kg. Plant growth varied by species, but the presence of some species led to greater TPH disappearance than with other species or in unvegetated soil. At a crude oil-contaminated field site near the Gulf of Mexico, an annual rye-soybean rotation plot and a St. Augustine grass-cowpea rotation plot had significantly ($P < 0.05$) greater TPH disappearance than did sorghum-sudan grass or unvegetated control plots, at 21 months Kamath et al. [3]. At a diesel fuel contaminated Craney Island field site in Norfolk, Virginia, the fescue plot had significantly ($P < 0.10$) greater TPH removal than did an unvegetated plot. At a refinery waste site, statistical analyses were not presented due to the short time since establishment of the plots, but Nedunuri et al. [2] reported that qualitatively, the vegetated plots had greater TPH removal than the unvegetated control plots. After investigating the potential to use phytoremediation at a site contaminated with hydrocarbons, the Alabama Department of Environmental Management granted a site, which involved about 1500 cubic yards of soil of which 70% of the baseline samples contained over 100 ppm of total petroleum hydrocarbon (TPH). After 1 year of vegetative cover, approximately 83% of the samples contained less than 10-ppm TPH [4]. Sudan one of the countries suffering from oil discovery and waste water followed therefore the aim of this work is to seek protection of human health and the environment from risks associated with hazardous waste sites, while encouraging development of innovative technologies such as phytoremediation to more efficiently clean up these sites. The main objectives of this study were to evaluate the effect of vegetation establishment on remediation of crude oil – contaminated soil and to determine the effect of the concentration of crude oil finally to analyze soil microorganisms.

2. MATERIALS AND METHODS

The role of the selected tree species and their associated microorganisms in cleaning up oil-contaminated soil was studied in a series of pot experiments and laboratory tests.

2.1 Selection of Plant Species and Collection of Seeds

Parkinsonia aculeata (which dominated the Savanna Zone).Seeds of this tree species were

donated by the Forest Research Center, Soba, Khartoum "Sudan".

2.2 Sowing of Seeds and Transplanting of Seedlings

Seeds of the selected tree species were sown in sand soil contained in 50x70cm trays and watered daily for two months; before they were transplanted to polyethylene bags (29x20cm) containing clay soil contaminated with different crude oil concentrations. Clay soil were air dried, passed through 2 mm-screen sieve and mixed manually with electrical stirrer. The soil was divided into five lots and to each of the first four lots crude oil was added to make a concentration of either 0.5, 1.0, 1.5 or 2.0%. The fifth lot was left as a control in which seedlings of the selected tree species were raised in oil-free soil. The experiment was laid out in Complete Random Design (CRD) with three replicates. In all cases, seedlings were watered daily for eight months.

At two months intervals, seedlings in three out of the nine bags allocated for each tree species at specific concentration were uprooted and measurement such as shoot height, root length and fresh weight of shoot and root were determined. Petroleum hydrocarbon fractions expected to accumulate in tissues and organs of each plant species at each petroleum concentration were determined using GC-MS. Also, at these intervals, total petroleum hydrocarbon was determined in rhizospheric soil contaminated with different concentrations of oil. Moreover, samples of rhizospheric soils taken from underneath each plant species under study at each crude oil concentration were taken for microbial analysis. In each case data were subjected to analysis of variance (ANOVA) using Statistical Package for Social Sciences (SPSS19) [5].

2.3 Chemical and Microbial Soil Analysis

Harvesting of Plant species was conducted every two months, plastic container were opened, plants were air-dried after measurements, soil samples were taken for microbial analysis and stored at 4 ºC. Soil samples were also taken for Total Petroleum Hydrocarbon (TPH) extraction and determination.

2.3.1 Extraction and determination of TPH

After removing the roots, the soil in each container was homogenized and stored at room temperature until further processing. Total oil was determined according to the U S Environmental Protection Agency (EPA) [1]. Fifteen grams of soil were transferred into paper extraction thimbles and placed into a Sochlet apparatus containing dichloromethane for eight hours. The solvent was evaporated through rotary evaporator and the dry weight of the extract was determined. Percentage of total oil was calculated based on soil dry weight as follows:

$$\%\text{Total oil} = \frac{\text{Weight of residual oil} \times 100}{\text{Weight of the initial oil}}$$

2.3.1.1 Fractionation analysis of extracted oil:

Dichloromethane solvent containing hydrocarbon was cleaned up according to Weisman (1998). Four mL n- Hexane was added to 1g silica gel 60A with pore size of 40-63µm mesh and with high purity. The samples were dissolved in more n-Hexane to 10 mL, stirred thoroughly and filtered through Whatman No 1 filter paper. The solvent was allowed to evaporate then one mL more n-Hexane was added and filtered again into GC-MS tube using 0.45 mm syringe filter for GC-MS analysis. Blank sample of crude oil was cleaned up using the same process [6].

Fraction analysis of the degraded hydrocarbon were performed using HP 5890 Gas Chromatography connected to Mass Spectroscopy devise according to EPA 8270 [7]. Chromatographic conditions were: column 25 m×0.2 mm×0.33 ULTRA 2; helium flow rate 1 ml min^{-1}, injector temperature 250ºC and detector temperature 280 ºC. The temperature program was set at: 40 ºC for 5 min, 4 ºC min^{-1} to 130 ºC hold for 2.2 min, increased by 12 ºC min^{-1} to 180 ºC, hold for 2.2 min, increased by 7 ºC min^{-1} to 300 ºC, hold 11:79 min. Qualitative analysis of samples was carried out by scanning the mass range between 35 and 550 amu, one run per sample. The interpretation of each spectrum was performed by comparison with the commercial NIST (National Institute of standard and Technology) database of spectra.

2.3.2 Isolation of bacteria from oil contaminated soil

Starch Ammonium Agar was used for the isolation of potentially oil degrading microorganisms using serial dilution method, one gram of oil- contaminated soil was transferred into nine ml of sterilized distilled water in a test

tube. The contents were shake vigorously to obtain the first dilution (10^{-1}). One ml of this soil water suspension was aseptically transferred to nine ml of sterilized distilled water to give the second dilution (10^{-2}). The third dilution was prepared in a similar way. One ml inoculum from each of dilutions 10^{-1}, 10^{-2} and 10^{-3} was aseptically transferred to a petri dish containing Starch Ammonium Agar for seven days. These media were prepared and used as suggested by Tepper et al. [8].

3. RESULTS

3.1 Effect on the growth of *Parkinsonia aculeate*

3.1.1 Effect on shoot and root lengths

The shoot length of *P. aculeata* was not significantly (P≤0.05) affected when seedlings were raised in oil-contaminated soil Fig. 1. After two months of growth, no significant differences were observed in shoot lengths recorded for oil stressed seedlings, at any tested concentration, when compared to the non –stressed (the control) seedlings. The highest shoot length was recorded for seedlings grown in oil contaminated soil at a level of 1.5% after six months of growth. This highlights the possibility of using this plant species for remediation of oil contaminated sites.

Root length in oil - stressed and non-stressed seedlings has been significantly retarded at both four and six month intervals (Fig. 2). Maximum root growth was observed for the control seedlings at the first interval and for seedlings grown in soil contaminated with 1.0% crude oil at the end of the growth period. Petroleum contamination has been previously reported to reduce root length of Lolium perenne and Schizachyrum scoarium [9]. Also, Zand et al. [10] reported a similar reduction in root length of *Zea mays* and *Festuca arundinacea* due to oil stress.

3.1.2 Effect on shoots and root weight

P. aculeata shoot weight has not been significantly reduced by total petroleum hydrocarbon at any of the tested intervals or within any particular stage of growth (Fig. 3). Actually, the highest values for shoot weight have been scored for seedlings grown in soil-contaminated with 1.5% oil at both two and four months intervals. Similarly, the highest recorded value for *P. aculeata* shoot weight was recorded at the end of the growth period in soil contaminated with 2.0 % crude oil. In contrast to this result, Shirdam et al. [11] reported a significant reduction in shoot biomass due to petroleum hydrocarbon contamination. No significant differences were observed in root weight of the control plants and the treated ones at any of the intervals tested (Fig. 4). Under some oil concentrations and across the intervals, addition of oil to the soil has significantly enhanced root growth and biomass. In conclusion, the enhanced growth of *P. aculeata* in oil- contaminated soil advocate it as strong candidate for cleaning up oil contaminated sites.

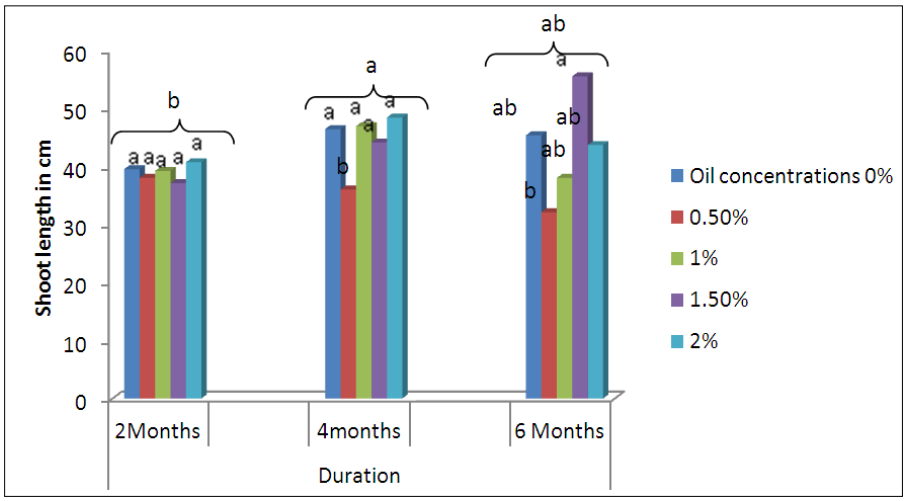

Fig. 1. Shoot length of *Parkinsonia aculeata* seedlings grown at different crude oil concentrations

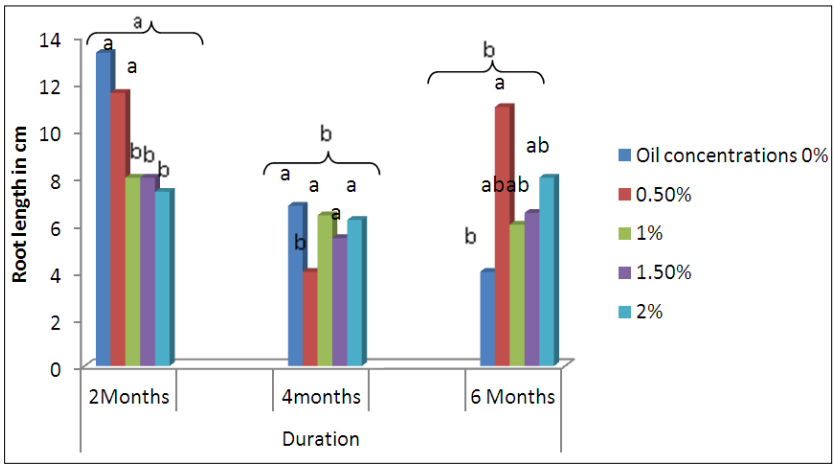

Fig. 2. Root length of *Parkinsonia aculeata* seedlings grown at different crude oil concentrations

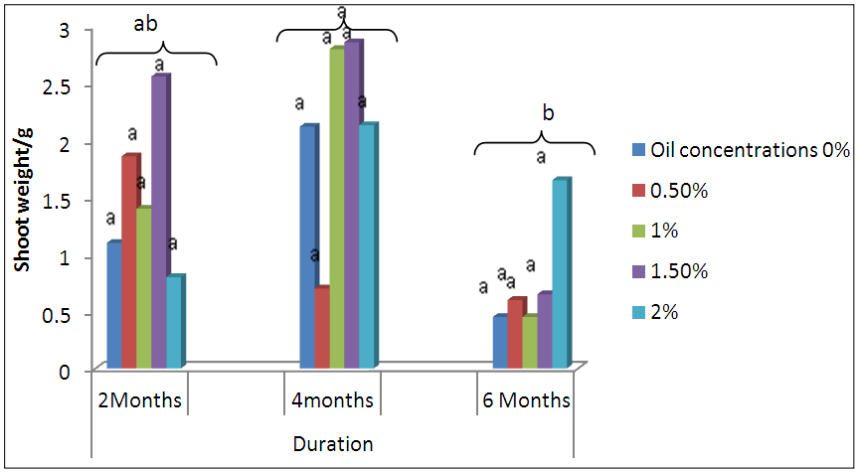

Fig. 3. Shoot weight of *Parkinsonia aculeata* seedlings grown at different crude oil concentrations

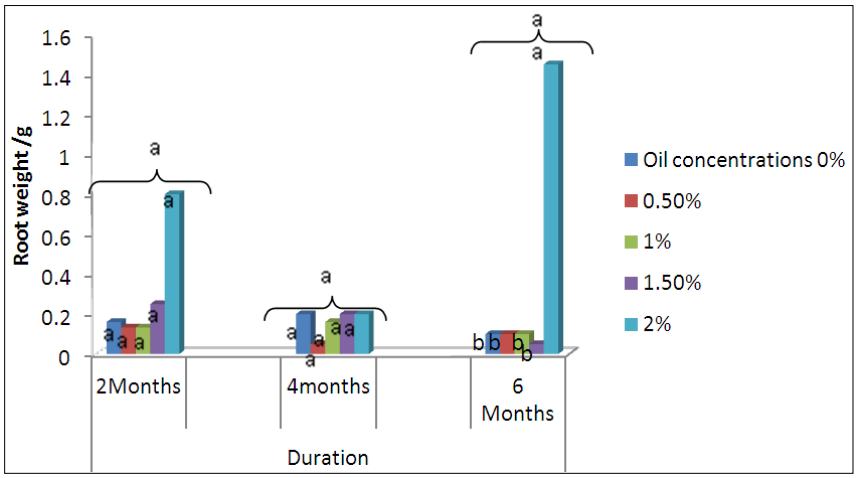

Fig. 4. Root weight of *Parkinsonia aculeata* seedlings grown at different crude oil concentrations

3.2 Chemical Analysis

3.2.1 Extraction and determination of total oil

Petroleum hydrocarbon residue was determined in soil samples collected after 180 days from underneath *Parkinsonia* sp*. seedlings and hydrocarbon degradation percentages was 55% comparing to the control 49%.* Mathur et al. [12] reported that crude oil (Total petroleum hydrocarbons, TPH) was reduced by 30% in the rhizosphere soil of *Prosopis cineraria* plant and by 16.8% and 13.7 in the rhizosphere soil of *Acacia senegal* and *Acacia nilotica* plants respectively.

3.2.2 Fractionation analysis of extracted oil

Individual petroleum hydrocarbons were determined in soil contaminated with 0.5% crude oil and planted with *Parkinsonia aculeata* seedlings. At zero time and then at intervals of 60,120 and 180 days of growth, samples were taken and analyzed by GC-MS. Number of compound detected in the rhizosphere were 33, 22, 94 and 106 compound after two, four and six month of growth and at zero time respectively. Retention time of the first compound appear, Number of compound detected in the rhizosphere, abundance of the compound and first compound appear and degradation percentage were recorded from GC-MS analysis in the three intervals . 60, 120, 180 day as in Table 1.

3.3 Microbial Analysis

Viable count of bacteria in the rhizospheric contaminated soil showed that there is no significant difference between concentrations (P=0.109) but there is significant difference between intervals (P=0.044).

Table 1. Compounds detected at the rhizosphere of *Parkinsonia aculeata* after 2 ,4 and 6 months

Species	R.T	N. Compound	Abundance	First compound	Area %	Occurance percentage	Degradation %
1P5A	34.620	33	200000	Hexadecane	1.67	98	60
2P5A	32.885	22	700000	Pentadecane	2.28	98	60
3P5A	15.509	94	2400000	Decane	0.32	95	46
1P5F	32.914	61	3800000	Pentadecane	0.43	98	60
2P5F	32.891	65	2200000	Pentadecane	1.22	96	20
3P5F	19.544	48	700000	Benzene,1,2,,4,5 tetramethyl	0.97	91	46
1P20A	32.908	62	2000000	Pentadecane	0.2	96	64.7
2P20A	32.885	122	3100000	Pentadecane	0.27	95	64
3P20A	19.445	211	7000000	Benzene,1,2,3,4 tetramethyl	0.04	97	60
1P20F	34.626	50	2000000	Hexadecane	0.6	99	46.7
2P20F	32.891	114	3500000	Pentadecane	0.31	96	66.7
3P20F	11.245	276	7000000	Nonane	0.16	91	64

(1-after 2 months 2 -4 months 3-6 months -P is the latin name-5,20 the concentration of crude oil-A- around the root-F far from the root)

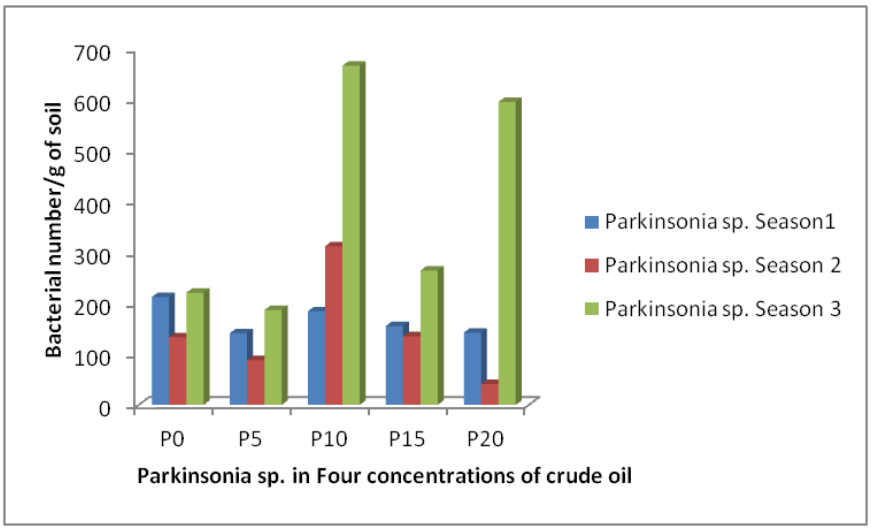

Fig. 5. Number of Colony Forming Units per gram of A. seyal rhizospheric soil in SAA medium at three sampling intervals

4. DISCUSSION

Shoot length of *Parkinsonia aculeata* was not significantly ($p \leq 0.05$) affected when seedling were raised in oil contaminated soil. In contrast to this Zand et al. [10] demonstrated that shoot height of tall fescue (*Festuca arundinacea*) was diminished in presence of petroleum hydrocarbons in soil, but the observed reduction was not significant compared to clean soil ($P > 0.05$). Root length in oil stressed and non stressed seedling has been significantly retarded at both four month and six month intervals. This in line with Kirk et al. [9] observed that Petroleum contamination significantly reduced root length for both (*Lolium perenne* var. "Affinity") and (*Schizachyrum scoparium*) ($P<0.001$) at all levels of contamination. *Parkinsonia aculeata* shoot weight has not been significantly reduced by total petroleum hydrocarbon at any of the tested intervals or within any particular stage of growth. In contrast to this Zand et al. [10] demonstrated that Petroleum hydrocarbon pollution reduced root biomass of maize Zea mays by 28%, while it did not adversely affect root biomass of (*Festuca arundinacea*). *Parkinsonia aculeata* root weight showed no significant differences between the control and the treated ones at any of the intervals under study [13]. Kim et al. [14] reported biomass of *Pinus densiflora* was not significantly decreased in the diesel contamination plot.

5. CONCLUSION

All the Experiment revealed that it is possible to use *Parkinsonia aculeata* for the removal of contaminant from soil polluted with crude oil and the viable number of bacteria revealed that bacterial count differ from month to month.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Environmental Protection Agency EPA. Introduction to phytoremedation National Risk Management Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Cincinnati, Ohio. 2000;45268.
2. Nedunuri KV, Gouindaraju RS, Banks MK, Schwab AP, Z Chen JZ. Environ. Eng. 2000;126:483.
3. Kamath R, Rentz JA, Schnoor JL, Alvarez PJJ. Phytoremediation of hydrocarbon-contaminated soils: principles and applications. In Press; 2004.
4. Hecht D, Badiane G. New Internationalist. 1998;12.
5. Gomez KA, Gomez AA. Statistical procedures for agricultural research (2 ed.). John Wiley and sons, New York. 1984;680.
6. Punin CMO, Lage YMA. Comparison of supercritical fluid extraction and Sochlet extraction for the determination of aliphatic hydrocarbons in seaweed samples. Ecotoxicology and Environmental Safety. 2006;64:400–405
7. Ivancev-Tumbasa I, Trickovioca J, Karlovioca E, Tamasa Z, Roncevioca S, Dalmacijaa B, Petroviocb O, Klasnjac M. GC/MS-SCAN to follow the fate of crude oil components in bioreactors set to remediate contaminated soil. International Biodeterioration and Biodegradation. 2004; 54:311–318.
8. Tepper EZ, Shilinova VK, Perverzeva GI. Mannual of Microbiology 4th edition. Kolas Publishers, Moscow; 1993.
9. Kirk LJ, Klironomos NJ, Lee H, Trevorse TJ. Phytotoxicity Assay to Assess Plant Species for Phytoremediation of Petroleum-Contaminated. Soil Bioremediation Journal. 2002;6(1):57–63.
10. Zand AD, Bidhendi GN, Mehrdadi M. Phytoremediation of total petroleum hydrocarbons (TPHs) using plant species in Iran. Turkey Journal of Agriculture. 2010;34:429-438.
11. Shirdam R, Zand DA, Bidhendi NG, Mehrdadi N. Phytoremediation of hydrocarbon-contaminated soils with emphasis on the effect of petroleum hydrocarbons on the growth of plant species. Phytoprotection. 2008;89: 21-29.
12. Mathur N, Singh J, Bohra S, Bohra A, Chauhan M, Vyas M, Vyas A. Phytoremediation of oil contaminated soil by some arid legume tree species. International Journal of Bioflux Society AES Bioflux. 2010;2(1).
13. Merkl N, Schultze-Kraft R, Infants C. Phytoremediation in the tropics—The

effect of crude oil on the growth of tropical plants. Bioremediation Journal. 2004; 8(3-4):177-184.

14. Kim D, Woo SM, Yim J, Kim T, Thao NNP, Lee J, Kang L, Han G. The feasibility of phytoremediation combined with bioethanol feedstock production on diesel-contaminated soil. 19th World Congress of Soil Science, Soil Solutions for a Changing World; 2010.

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