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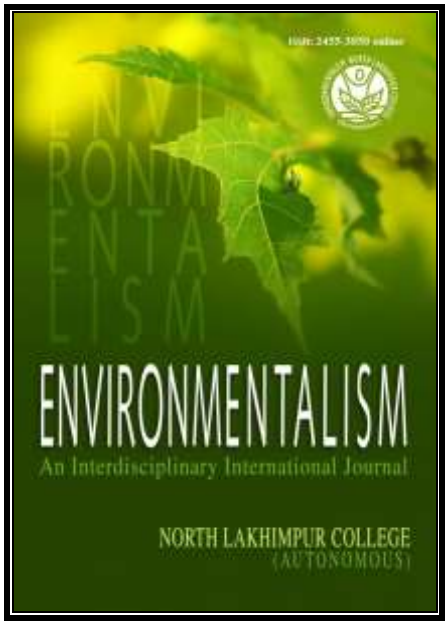

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PHYTOREMEDIATION OF PETROLEUM HYDROCARBON CONTAMINATED SOIL WITH NATIVE PLANTS

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Abstract

Use of native plants for phytoremediation of various contaminants has become important for decontamination of petroleum hydrocarbon contaminated soils. Plants belonging to genus Cyperus and Axonopus are widely being used to study phytoremediation of hydrocarbon contaminated soil. The aim of this experiment was to find out some other native species that could be effective in phytoremediation of petroleum hydrocarbon contaminated soil. Experiments were conducted in net house to determine the tolerance of some plant species and reduction of hydrocarbon components. The soil was spiked with different concentrations of crude oil and reduction of hydrocarbon was monitored for 180 days. Two wild grass species *Cynodon dactylon* and *Mimosa pudica,* were screened for phytoremediation. The estimation of Total Petroleum Hydrocarbon degradation in the root zone of the tested plants showed that *C. dactylon* and *M. pudica,* accelerated cleanup most effectively, degrading some of the contaminant fractions in the vegetated pot than the unvegetated control. Remediation monitoring confirmed the effectiveness of these two species in decreasing hydrocarbon consistently during the experimental period and remained low in comparison with the results of unplanted control pots. This experiment has identified suitable native candidate plant species for further investigation of their phytoremediation potential. However, uses of these plants in relation to petroleum-hydrocarbon contaminated soils are promising for decontamination of polluted sites, to allow other species to get established and for erosion control.

Keywords: Phytotemediation, native plants, petroleum hydrocarbons

1 Introduction:

As raw material for production of petroleum and other chemicals, crude-oil has become one of the most important energy sources in the world. However, contamination of water and soil by crude oil as a result of exploration, production, maintenance, transportation, storage and accidental release, has caused significant environmental impacts presents substantial hazards to human health. Apart from bioaugmentation with oil-degrading microorganisms, phytoremediation is applied to provide long-term rehabilitation of the residual oil-contamination. Searching for the most effective remediation species for a particular compound is a critical step

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in phytoremediation trials. Most studies on the phytoremediation of petroleum hydrocarbon contaminated soil reported the use of grasses (*Poaceae*) and legumes (*Leguminosae*). Grasses have shown to be most effective in enhancing degradation.

Plants with fibrous roots can show high rates of degradation of organic contaminants in soils because their fibrous root system offers an increased root surface for microbial growth and activity (Anderson *et al.* 1993). Furthermore, grasses can effectively stabilize surface soils and hence prevent soil erosion of contaminated soil. Burken *et al.* (1996); Nichols *et al.* (1997) and Siciliano *et al.* (2003) reported that contaminant dissipation in the rhizosphere is most likely due to enhanced microbial degradation. In the phytoremediation of organics which is based on a stimulated microbial degradation in the rhizosphere, fertilization is essential for success. Adequate fertilizer applications may reduce competition between plants and microorganisms for limited nutrients in oil polluted soil, resulting in enhanced Petroleum Hydrocarbon (PHC) degradation rates (Hutchinson *et al.* 2001). The overall goal of this experiment was to evaluate the suitability of *Cynodon dactylon* and *Mimosa pudica* for use in the phytoremediation of crude-oil contaminated soils.

2 Materials and methods

2.1 Potting

Garden soil and sand used in the experiments were air dried and sieved (mesh size 2mm). Three parts of soil and one part of sand were mixed thoroughly, homogenized, oven dried at 60^{O}C for getting constant weight. The soil-sand mixture was weighed and crude-oil was mixed thoroughly in different percentages (2%, 4%, 6%, 8%, and 10%) in w/w basis using a hand held electric mixture. In each alcohol sterilized plastic pot (18-cm diameter) 12kg of soil-sand-crude oil mixture was taken and kept in a net house for two weeks to allow the volatile hydrocarbons to evaporate. Five transplants of each of *Cynodon dactylon* and *Mimosa pudica* were planted in each concentration with nine replications keeping a blank without plant. Prior to this the plant were vegetatively propagated in the net house. The pots were watered twice per day and on sunny days when the rate of evapotranspiration is very high they were sprinkled on the top. Sufficient number of pots (75 for both *Cynodon dactylon* and *Mimosa pudica*) were prepared for three replications and two control (one having crude-oil without plant and one having plant without crude-oil) of each treatment over three sampling periods 60, 120, and 180 days after the crude-oil was mixed. Starting from the date of transplant, the experiment lasted 180 days. Analysis was done for hydrocarbon degradation in different treatments.

2.2Fertilization

Partially decomposed cow dung was used as fertilizer to enrich the experimental pots. Prior to use the organic manure was dried. Plants in all the treatments received basic doses of dry organic manure based on carbon measurement added by crude-oil, in order to support healthy plant growth (Table-1). The total fertilizer amounts were split in to four applications (8, 30, 75, 115 days after planting).

2.3 Evaluation and sampling

Initial soil samples were taken before planting. Three destructive samplings were carried out by using 5 pots per treatment at 2-months interval (up to 6months). Both rhizospheric and bulk soil of each pot was collected and stored at 4 ^{O}C prior to analysis.

2.4 Soil analysis

The *p*H in soil was measured in 1:2.5 soil/DH_2O suspension using a *p*H meter (Elico LI-127) fitted with a glass electrode. Total organic carbon in soil was determined by oxidation with potassium dichromate ($K_2Cr_2O_7$) and titration of excess

dichromate with ammonium ferrous sulphate [$(NH_4)_2Fe(SO_4)_2.6H_2O$]. Total nitrogen in soil was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4 and HgO to digest the sample, soil particle distribution by the hydrometer method. The water holding capacity was measured on water saturated soil samples in a brass-box and left to stand overnight to drain freely and was defined by differences in weight. Available phosphorous was determined by digesting with 0.02N H_2SO_4, ammonium molybdate and $SnCl_2$ and detecting the absorbance in UV-spectrophotometer (Schimadzu UV-1800). Na and K were determined by digesting with ammonium acetate, aqua ragia [HNO_3:HCl; (1:3)] and taking the reading in Flame Photometer-128 (Systronics). Soil Moisture Content was measured by gravimetric method (oven drying until constant weight). *Total Oil and Grease and Fraction analysis*Total Oil and Grease (TOG) and TPH were

Table 1 Experimental details

Treatment (T)	Soil+Crude-oil	Plants[1]	Fertilizer (cow dung) (Mgkg^{-1} soil)
I, VII	Soil+20g crude-oil kg^{-1}	+	200
II, VIII	Soil+40g crude-oil kg^{-1}	+	250
III, IX	Soil+60g crude-oil kg^{-1}	+	350
IV, X	Soil+80g crude-oil kg^{-1}	+	450
V, XI	Soil+100g crude-oil kg^{-1}	+	550
VI, XII	Soil	–	150

[1]+ 5 transplants/pot; – No plants.

Table 2 Characteristic of the soil used in the experiment:

Parameters	before plantation	after 6 month	Oil properties	
Sand [% wt]	81	-	Saturates	28.5
Silt [% wt]	8	-	Aromatics	42.3
Clay [% wt]	11	-	Asphaltenes	1.5
Texture	Sandy loam		Resins	27.7
*p*H	4.8	5.2		
Organic carbon [%wt]	1.4	1.5		
Total N [%wt]	0.09	0.07		
Total P[ppm]	6.1	5.5		
K [ppm]	15	14.6		
Na [pp]	13	13.3		
Moisture content	1.5%	3.1		
Water holding capacity	9%	24 %		

determined by soxhlet extraction method using a modification of EPA method 3540B (USEPA 1994). Of each sample, three 20g replicates were analyzed.

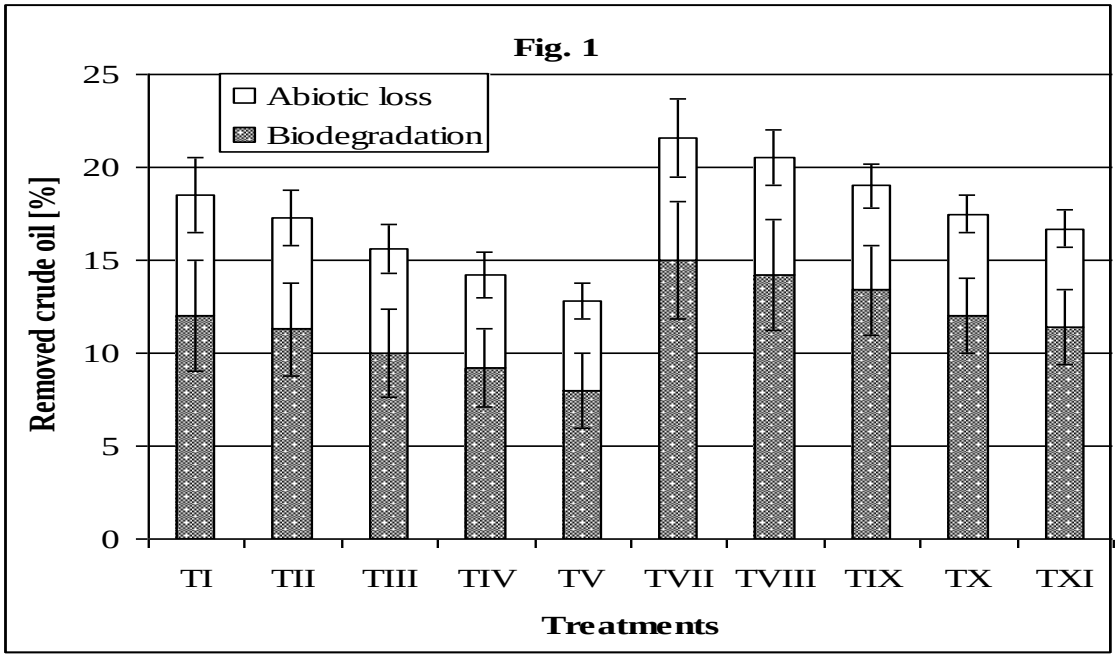

Figure 1 Percent loss of crude-oil in *C. dactylon* (TI to TV) and *M. pudica* (TVII to TXI) in 2 month old pots as a function of time and soil treatment. Values are means ± standard deviation.

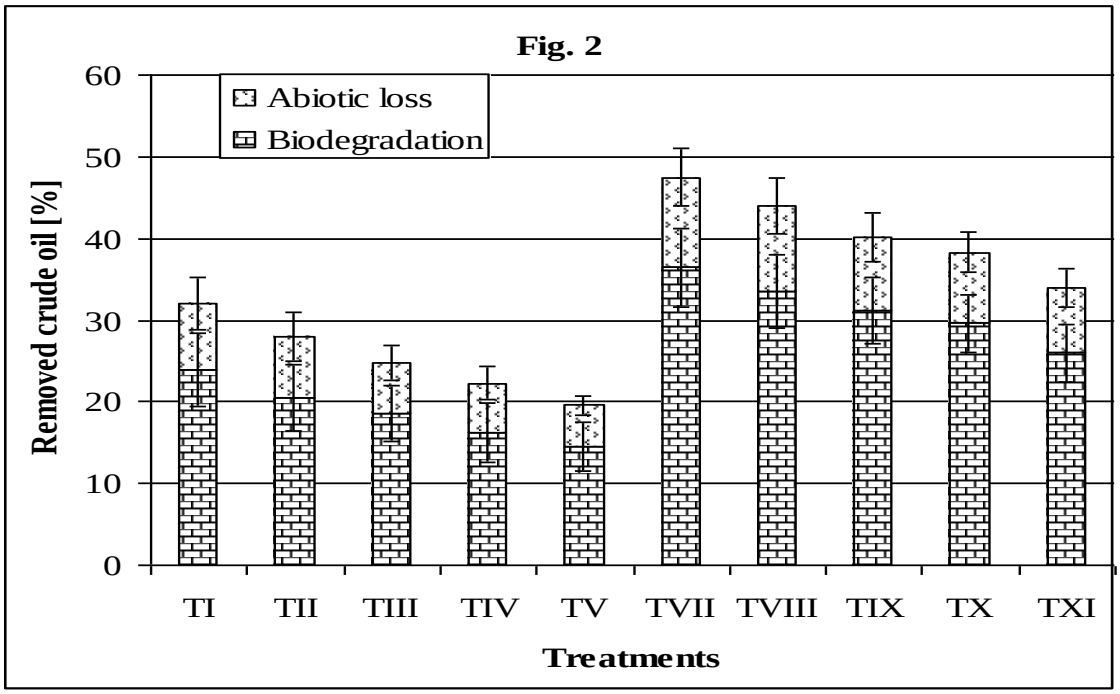

Figure 2 Percent loss of crude-oil in *C. dactylon* (TI to TV) and *M. pudica* (TVII to TXI) in 4 month old pots as a function of time and soil treatment. Values are means ± standard deviation.

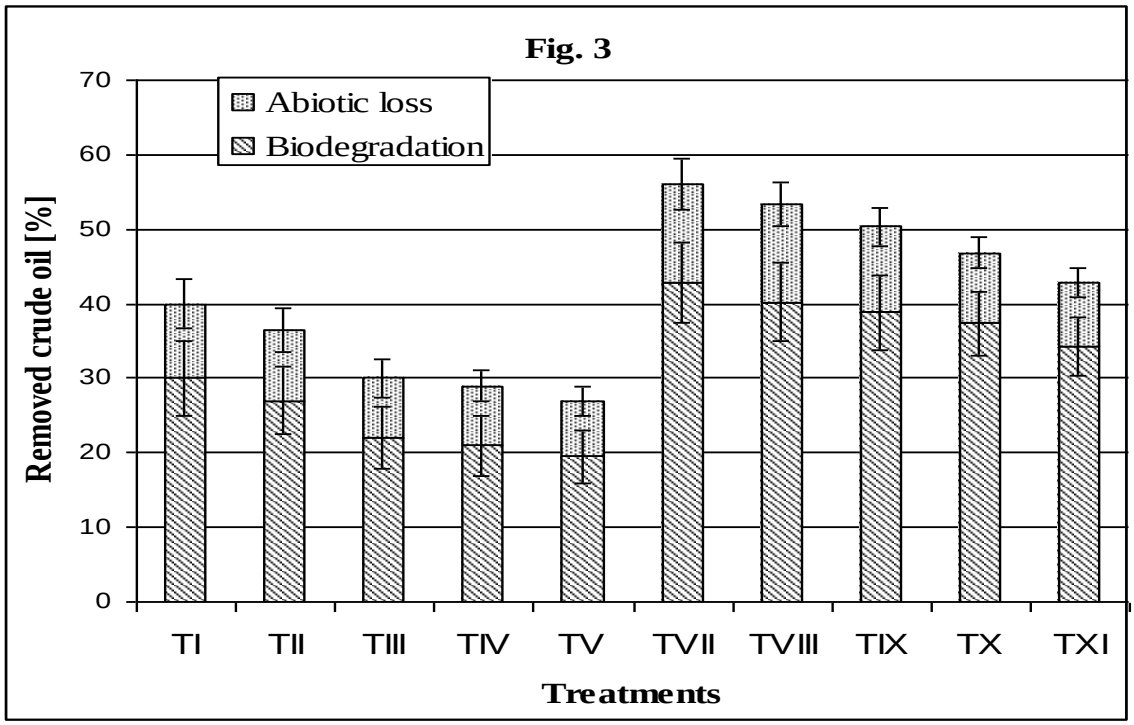

Figure 3 Percent loss of crude-oil in *C. dactylon* (TI to TV) and *M. pudica* (TVII to TXI) in 6 month old pots as a function of time and soil treatment. Values are means ± standard deviation.

The samples were acidified with HCL to pH 2 and dehydrated with $MgSO_4$. Soxhlet extraction with dichloromethane was run for 10h. After passing the extract through a filter paper (Whatman No.4) with approximately 1g Na_2SO_4, the solvent was evaporated and constant weight of the dry extract determined. Percentage of TOG was calculated based on soil dry weight. Saturates, aromatics and polar fraction were performed by thin-layer chromatography combined with a flame ionization detector (Iatroscan). The Chromarods were run with n-heptane to separate the saturated hydrocarbons and subsequently with toluene to collect the aromatic hydrocarbons in a single band. Both fractions, which include resins and asphaltenes, were calculated by difference.

Abiotic loss of TPH was determined from control pot. The soil characteristics is presented in table 2.

3. Results and discussion

The concentration of oil decreased 19.5% in TI, 21% TII, 22% in TIII, 27% in TIV and 30% in TV respectively in the presence of *C. dactylon* and 34.3% in TVII, 37.4% in TVIII, 38.8% in TIX, 40.2% in TX and 42.9% in TXI respectively in the presence of *M. pudica* during the course of six mont time period. However, *M. pudica* is found to be more effective in reducing crude oil in comparision to *C. dactylon*. The slower degradation of crude oil can be attributed to its low solubility and bioavailability which renders it only slowly available for microbial attack initially. The effects of crude-oil on plants are attributed to phytotoxicity, which depends on several factors; concentration of oil in soil, oil type and its content of phytotoxic compounds (e.g., Polycyclic aromatic hydrocarbons, which includes most phytotoxic substances), environmental conditions and plant species (Baker 1970). Despite inhibition of plant growth and root development, the decrease of crude oil was significantly faster in vegetated soil compared to unvegetated soil in 2, 4 and 6 months (P<0.05). As roots grow, they penetrate through the soil, exposing entrapped

contaminants that have been previously inaccessible, increasing their availability to degradation. Moreover, the root increases soil aeration, reducing soil moisture content and changing physicochemical and biological characteristics (USEPA 1999; Bowen *et al.* 1991). TOG degradation was slower in the first two months of growth but increased during 4 months and in 6 months. This might be due to biodegradation of lower molecular weight hydrocarbons in the rhizosphere of *C. dactylon* and *M. pudica* in the presence of some rhizosphere microorganisms.

Apart from biodegradation, a potential weathering process of PHC in soil is volatilization of low molecular weight, aliphatic, and aromatic compounds. Analysis of TOG in experimental soil has revealed that abiotic loss through volatilization of lower molecular weight hydrocarbons was 7.4 to 13.1%. However, it has been found that biodegadation of hydrocarbon was significantly more (P=0.01) responsible than abiotic loss (Fig. 1, 2, 3). Aromatic and polar compounds are less biodegradable than aliphatic (Diaz-Ramirez *et al.* 2003) and asphaltene group is the least biodegradable of all (Harayama, 1997; Garcia-Rivero *et al.* 2002). Rhizosphere microbial populations may enhance plant's adaptation to petroleum hydrocarbons by detoxifying contaminated soils through direct mineralization of these organic contaminants. As root exudates control the quality and quantity of microbial populations in the rhizosphere, an altered plant metabolism caused by pollutants may have an effect. On the other hand, microorganisms also have a strong influence on the health conditions of plants. In our work, a plant promoted degradation of hydrocarbon may be due to the complexity of plant-microorganism interactions. In this study it was found that growth of *C. dactylon* and *M. pudica* was inhibited by contamination with crude oil, but removal of crude oil was accelerated in their rhizosphere and significant degradation was recorded. However, the plant showed effective result in reducing petroleum hydrocarbons and could sustain in crude oil concentration up to 10% (w/w) in the existing laboratory condition. Application of these two plants in the hydrocarbon contaminated sites however, would help erosion control and thus, prevent contaminants from surface spreading.

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