

ORIGINAL ARTICLE

Removal of Hydrocarbon from Crude oil Contaminated Soil by *Cyperus brevifolius* Rottb.

Plabita Baruah¹, Partha Pratim Baruah² and Suresh Deka*¹

¹Resource Management and Environment Division, Institute of Advanced Study in Science and Technology, Paschim Boragaon, Garchuck, Guwahati-35, Assam, India.

²Department of Botany, Gauhati University, Guwahati-14, Assam, India.

ABSTRACT

In this investigation, *Cyperus brevifolius* plants were grown in a bulk soil sample collected locally in earthen pots under 0 ppm (Control), 10000 ppm, 20000 ppm, 30000 ppm, 40000 ppm and 50000 ppm of added crude oil from Noonmati Refinery, Guwahati, India. Results revealed that there is a great impact of crude oil contamination on physico-chemical properties of soil. pH and total organic carbon (TOC) of the soil were increased; but reduced the water holding capacity (WHC). With increasing the crude oil concentration in the soil the growth and development of the plants were decreased significantly. Uptake of hydrocarbon by the plant was increased with increasing the concentration of the crude oil in the soil. Uptake was found maximum in the 50000 ppm concentration of crude oil. Uptake was found more in shoot than root of the plant. Dissipation of total petroleum hydrocarbon (TPH) was also gradually increased with increasing the concentration of crude oil in the soil. Dissipation of hydrocarbon was more after second year harvest of the plant than first year.

Key words: Phytoremediation, Crude oil, Contamination, Soil, *Cyperus brevifolius*

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INTRODUCTION

Phytoremediation is one of the best developed and implemented technologies of bioremediation for cleaning up the environmental pollution which, focuses on the use of living green plants i.e. herbs, trees etc. for the removal of contaminants and metals from soil. Total petroleum hydrocarbon (TPH) contamination is recognized as a serious threat to environmental ecosystems. TPHs are a complex mixture of chemical substances such as alkanes, aromatics and asphaltene fractions [1] and are very toxic to living organisms. Phytoremediation has been proposed as a cost effective, non-intrusive, and environmental friendly technology for the restoration of soils contaminated with TPH [2]. Vegetation may play an important role in the biodegradation of toxic organic chemicals [3, 4, 5, 6, 7, 8, 9, 10, 11]. Plants may indirectly contribute to the dissipation of contaminants in vegetated soil. Soil adjacent to the root contains increased microbial population [12]. The physical, chemical and thermal processes are the common techniques that have been involved in the cleaning up of oil contaminated sites [13]. These techniques not only have some adverse effects on the environment and are expensive also. [13]. Recently biological techniques like phytoremediation are being evaluated for the remediation of sites contaminated with petroleum [14]. Phytoremediation is a viable remediation method for petroleum-contaminated soil. The use of vegetation for remediation of contaminated sites is attractive and eco-friendly [15]. Based on these assumptions the present investigation was carried out to study the phytoremediation potentiality of *Cyperus brevifolius* which is locally available for remediation of petroleum hydrocarbons from the oil contaminated soil.

MATERIALS AND METHODS

Collection and preparation of soil samples

To carry out the experiment, approximately 250 kg soil from agricultural crop field near Guwahati, India was collected. Soil samples were homogenized dried; lumps of the soils were then crushed and sieved through 2mm mesh. Twenty-eight numbers of earthen pots of sized (25 cm x30 cm) were collected and marked them all. About ten liters of crude oil was collected from Noonmati refinery, Guwahati, India for conducting the experiment.

Soil treatment

Three kg of soil was weighted and all the 28 pots were filled with 3 kg of soil. The contamination with the crude oil was done by mixing with their respective plastic buckets uniformly. For this, soil samples in the pots were poured in a bucket and mixed with crude oil to make a concentration of crude oil in soil about 10000 ppm, 20000 ppm, 30000 ppm, 40000 ppm and 50000 ppm by the following process. For preparing 10000ppm concentration, 3000 g previously weighted soil were poured in a plastic bucket followed by adding a mixer of 30 g of crude oil + 1000 ml tap water + 10 ml of biodegradable detergent (Extrain) as emulsifier. The mixing was done with the help of magnetic stirrer. In this way, all the concentrations were made by adding 60, 90, 120 and 150 g crude oil respectively and then the soil samples were allowed to dry in sunlight; crushed the lumps of the soil and sieved through 2mm mesh. The 28 pots were filled with soils according to their markings in the pots. Each prepared soil samples were kept in clean polythene bags for initial analysis. Each treatment including the control (0%) was replicated 3 times. Another control set of soil with detergent but without oil were kept for comparison.

Collection and plantation of the experimental plant

For plantation healthy seedlings of *Cyperus brevifolius* were collected and in each pot 10 numbers of healthy seedlings were planted for 6 months. Experiment was set up during the month of May (2010 and 2011) and harvested the plants in the month of November (2010 and 2011) .

Evaluation of Soil pH

Soil pH was determined by using the Digital pH metre (Elico IL 120) in 1:2.5 soil/water suspensions.

Evaluation of Soil Conductivity

Conductivity of soil was determined by using Conductivity metre (Elico CM 180) in 1:2.5 soil/water suspensions.

Evaluation of Water Holding Capacity of Soil (WHC)

Water holding capacity of the collected soil samples were determined by using circular brass box known weight (a) containing a whatman No. 1 filter paper. A few gram of soil was added to the brass box and reweighed (b). The box with the soil was kept in the petridish containing water, so that about one fourth of the box remains under water. The box was kept overnight. Next day the box was removed and the excess water was allowed to drain off when there was no more water dripping at the bottom of the box, it was reweighed again (c). One whatman No. 1 moist filter paper was weighed (m).

Water holding capacity = $c - (b \times m) / b - a$.

Evaluation of Soil Total Organic Carbon (TOC)

Total organic carbon of the soil samples were determined by Walkley and Black (1974) rapid titration method.

Evaluation of Soil Texture

Soil texture was determined by using the methods of Trivedy and Goel (1986).

Estimation of Oil and Grease

Oil and grease content of the soil and plant samples were estimated by Soxhelets. Petroleum benzene (boiling point 40^oc – 60^oc) was considered as extracted solvent. Extracted solution was poured in to 50 ml beaker and evaporated to dryness in Rotary evaporator. After harvest, the plants were washed and oil and grease content of harvested plants (both in roots and shoots) were extracted with the help of soxhelets.

Estimation of plant Biomass

Root and shoot biomass of the plants were measured by removing and separating the plant parts from soil very carefully. Plant parts were washed and blotted them to remove the excess amount of water and then kept them in an oven at 60 °C temperature till to have constant weight. The biomass of plant roots and shoots were estimated the method mentioned by Trivedy and Goel (1986).

Estimation of TPH

Total Petroleum Hydrocarbon (TPH) present in the soil samples were estimated by taking the weight of the beaker containing extracted solvents of samples till the weights become constant. Then value of the TPH calculated by the following formula:

$$\text{ppm} = X - Y \times 10000 / W$$

Where, X = Final weight of the beaker and oil and grease, Y = Initial weight of the beaker, W = Sample weight

RESULTS AND DISCUSSION

Soil physico-chemical analysis was done before plantation and after harvest of the plants in first year and second year. Results of the physico-chemical analysis of the soil samples were reported in the table 1a, 1b and 1c. Results of the pH revealed that the pH of the contaminated soil samples were initially acidic except control. Before plantation the pH value of the soil samples were found minimum (3.32) in the highest concentration (50000 ppm) and maximum (7.01) in control. The value of pH after first year harvest of the soil samples were found minimum (5.81) in highest concentration and maximum (7.39) in control. Similar trend was found in second year also. Before plantation, the conductivity of the soils were found minimum (0.052) in the highest concentration and maximum (0.110) in control. The conductivity value of the soil after first year harvest were found minimum (0.111) in 50000ppm and maximum (0.367) in control. Similar trend was also found in second year after harvest. The results of the Total Organic Carbon (TOC) revealed that there was slight increase in TOC (%) in contaminated samples than in control. The TOC value (%) of the soil in before plantation were found to be maximum (3.78%) in highest concentration and gradually lower in subsequent concentrations. The value of TOC in first and second year after harvesting shows similar trend than that of before harvesting. The value of TOC shows gradual decrease in year after year. Results showed that Water Holding Capacity (WHC) of soil samples showed variation in results. WHC of oil contaminated soils were lower than the control sample. Before plantation, the WHC of the soils were found to be minimum (10.91%) in highest concentration and maximum (65.05%) in control. Similar kind of trend was found in first and second year after harvest. Results also showed that gradually year after year there was a prominent increase in the WHC of soil along with the decrease in crude oil concentration. Texture of the soil samples were also recorded in the table 2. The results revealed that the percentage of sand was maximum (70.65) than silt (22.99) and clay (6.96). The results of the dissipation of Total Petroleum Hydrocarbon (TPH) in soil samples after 1st and 2nd year harvest were presented in fig 1. It was revealed from the result that the dissipation of TPH were recorded maximum in highest concentrations (50000 ppm) in both the year. Uptake of oil and grease by shoots and roots of the experimental plants were presented in the fig 2 and 3. It was revealed that in 1st year harvest, uptake by shoots of the plant was found minimum in the lowest concentration (10000) and found maximum in the highest concentration (50000 ppm). Uptake by the roots of the plant was also minimum (1683 ppm) in 10000 ppm and found maximum (6680 ppm) in 50000 ppm in 1st year. Similar trend of results were observed in second year after harvest. Uptake was found more in shoots than roots of the plant. Record of the biomass of shoots and roots of *Cyperus brevifolius* were presented in the Fig 4 and 5. It was revealed that both in 1st year and 2nd year after harvest biomass was found maximum in control and gradually decreased according to their concentration gradient. Shoot biomass was maximum than that of root biomass in both the years.

The results revealed that the growth of *Cyperus brevifolius* reduced the acidity of the crude oil polluted soil. On the basis of the above results, it must be said that initially the pH of the soil was 7.01 to 8.01 in both the controls. That means the value of pH was neutral or towards alkaline. But it was observed that the value of pH was acidic when the soil was treated with crude oil (i.e. 3.32-4.71). After planting of *Cyperus brevifolius* it was seen that the pH values increased from acidic towards basic. Similar results were revealed in previous literatures [16, 17]. This decrease in the pH of soil with degradation of crude oil could be due to accumulation of organic acids produced during degradation in the soil. However, since soil bacteria thrive better in neutral than in acidic soils [18, 19], the increase of the soil pH towards neutral condition means more favorable conditions for soil bacteria. Many researchers have reported that bacteria play good role in the degradation of crude oil [20, 21, and 13]. The observed reduction in pH and conductivity was similar to the findings of Osuji and Nwoye [22]. The change in pH towards alkalinity was similar to the findings of Okiemen and Okiemen [23]. Results also revealed that value of conductivity of the soil samples increases after harvest. But the value of conductivity in contaminated soils was lower than the control. The similar findings were reported by Osuji and Nwoye [22]. The record of the total organic carbon of the soil samples were also reported and revealed that before plantation it was seen that, as the concentration of the oil increases the value of organic carbon was also increases than that of control. But after harvest it was decreased in contaminated soils. Similar result was reported in previous findings [24]. Water holding capacity of the soil samples were also increased after harvest. Initially, before plantation maximum WHC 65.05% in control and minimum 10.91% in highest concentration

(50000 ppm) were found. After harvest, the values were increased in the soil in 1st year and in 2nd year soil samples. It was noticed from the above experiment that the WHC increases year after year after harvest. The increase in WHC was may be due to decrease of oil and grease in the soil. Crude oil contamination may produces waxy materials at the top surface of soil which could not allow the water enter into the soil pores, which initially decreases the value of WHC. From the above results, it could be concluded that hydrocarbon contamination adversely alters the soil properties. Percentage of dissipation of oil was more in 2nd year than in 1st year. Highest dissipation was found according to increase in concentration gradient. Similar report was reported from Gighi *et al.*, [25]. Uptake of oil and grease by the plant species was varied depending upon the plant parts. The investigation of the results of the uptake of oil and grease in both the figures revealed that the shoot portion of the plant uptake maximum than the roots in both 1st year and 2nd year. This may be due to presence of naturally occurring microflora in the soil which helps in degradation of crude oil present in the soil. In presence of plant, the population of such microflora is increased in the rhizosphere region. This is because of plant exudates in the root region, which favors the growth and development of the microflora in that particular region. Besides, microbial degradation of hydrocarbon, some amount of hydrocarbon can dissipate by the plant itself by taking up the hydrocarbon from the contaminated soil, as a result of which dissipation of hydrocarbon is enhanced [15]. Uptake of contaminant by the root is a direct function of the pollutant concentration in the soil solution and usually involves chemical partitioning on the root surfaces followed by movement across the cortex to the plant's vascular system [26]. Biomass of the experimental plant showed that after 1st year and 2nd year harvest dry biomass of root and shoots were higher in control and became lower in the subsequent concentrations. It was also revealed that value of 2nd yr biomass was maximum than 1st yr. The reason may be that the crude oil concentration in soil decreased and water holding capacity increased in the 2nd year helping the plants to grow more. It has also been noticed that shoot biomass was maximum than root biomass in both the years. Considerable reduction of plant biomass by the presence of petroleum hydrocarbon was found in some cases. This is in agreement with earlier results reported by Chaineaw *et al.* [27] and Gallegos-Martinez *et al.* [28]. It was also revealed from the result that there is no significant impact of detergent (Extrain) on physico-chemical properties of the soil which was used as emulsifier for mixing soil samples with crude oil.

Table 1a. Physico-chemical characteristics of the soil sample before plantation

Concentration (ppm)	Parameters			
	pH	Conductivity (mS/cm)	Total Organic Carbon (%)	Water Holding Capacity(%)
Control	7.01±1.2	0.110±0.17	0.42±0.65	65.05±2.5
Control with soap	8.10±1.5	0.109±0.71	0.85±0.43	62.84±2.9
10000	4.71±1.9	0.097±0.053	1.27±0.79	45.08±1.1
20000	4.60±1.1	0.083±0.034	1.69±0.94	33.49±1.5
30000	4.52±1.3	0.077±0.028	2.12±1.3	29.58±1.05
40000	4.48±1.8	0.057±0.049	3.39±1.5	22.03±2.0
50000	3.32±2.3	0.052±0.036	3.78±1.3	10.91±1.9

Mean value ± Standard deviation

Table 1b. Physico-chemical characteristics of the soil samples 1st year after harvest

Concentration (ppm)	Parameters			
	pH	Conductivity (mS/cm)	Total Organic Carbon (%)	Water Holding Capacity (%)
Control	7.39±1.95	0.367±0.15	0.79±0.12	66.42±2.2
Control with soap	8.74±1.76	0.211±0.13	0.96±0.52	63.03±2.5
10000	6.41±1.29	0.203±0.16	1.19±0.75	54.79±1.38
20000	6.08±1.1	0.192±0.34	1.46±0.85	45.18±1.2
30000	5.96±1.56	0.179±0.69	2.05±1.4	40.73±1.73
40000	5.92±0.98	0.161±0.37	3.28±1.5	33.35±1.6
50000	5.81±1.16	0.111±0.33	3.55±1.1	25.04±1.2

Mean value ± Standard deviation

Table 1c. Physico-chemical characteristics of the soil samples after 2nd year harvest

Concentration (ppm)	Parameters			
	pH	Conductivity (mS/cm)	Total Organic Carbon (%)	Water Holding Capacity (%)
Control	7.49±1.5	0.401±0.71	0.91 ± 0.45	68.42±2.3
Control with soap	8.94±0.93	0.295±0.23	1.02±0.76	64.14±2.9
10000	6.56±0.82	0.268±0.19	1.10±0.87	62.03±1.9
20000	6.20±1.67	0.243±0.15	1.27±0.18	59.79±1.4
30000	6.12±1.7	0.216±0.87	1.64±1.1	48.28±1.32
40000	6.09±0.97	0.197±0.16	2.55±1.6	41.73±1.1
50000	5.95±0.64	0.145±0.12	2.92±1.32	32.99±1.5

Mean value ± Standard deviation

Table 2. Soil texture of the experimental soil samples

Soil Texture	Value (%)
Sand	70.75 ± 2.89
Silt	22.99 ± 2.32
Clay	6.26 ± 3.19

Mean value ± Standard deviation

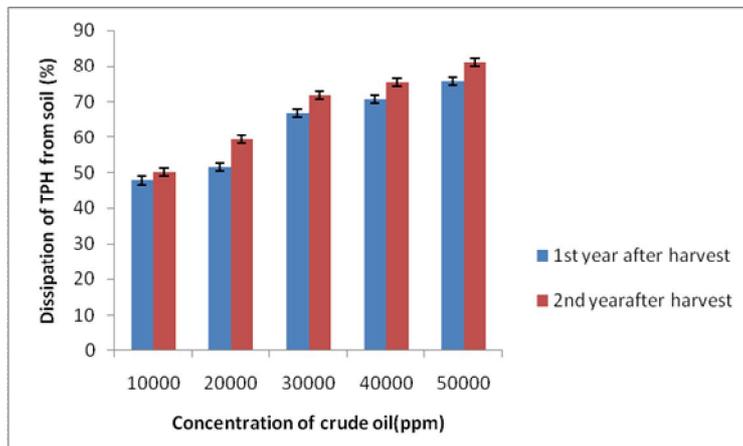


Fig 1. Dissipation of TPH from soil by *Cyperus bravifolius*.

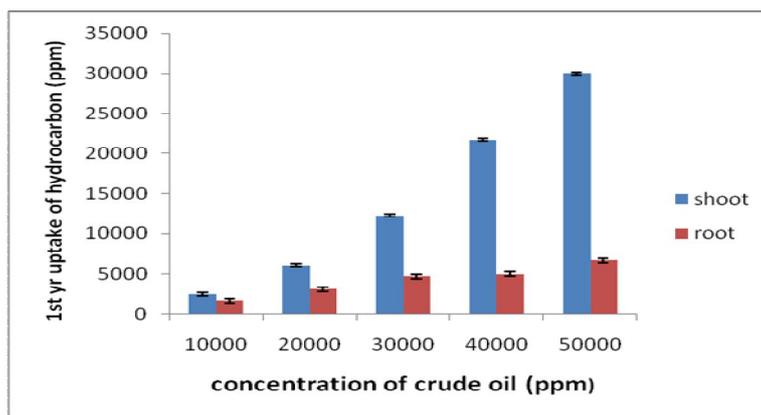


Fig 2. Uptake of hydrocarbon by *Cyperus bravifolius* after 1st yr harvest.

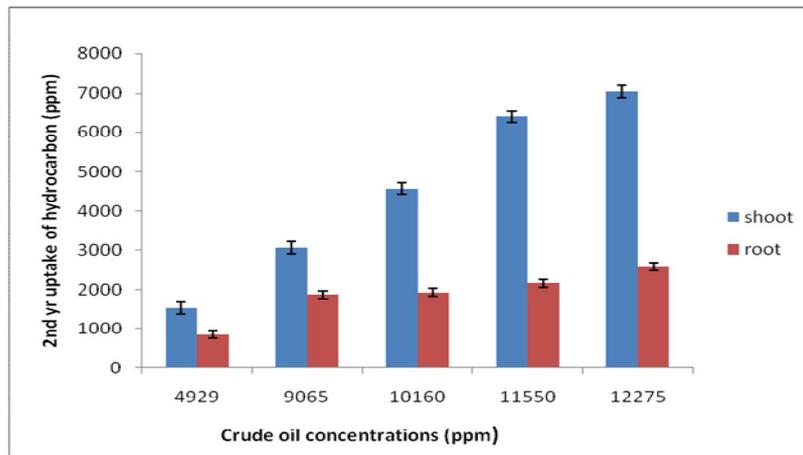


Fig 3. Uptake of hydrocarbon by *Cyperus brevifolius* after 2nd yr harvest

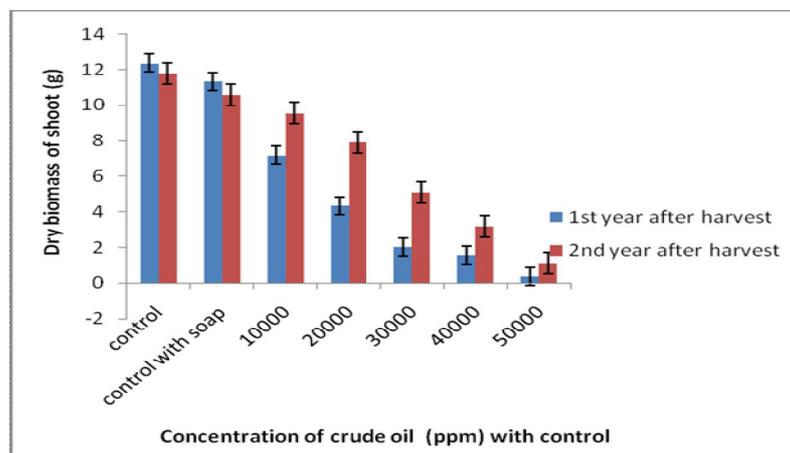


Fig 4. Dry biomass of shoot of *Cyperus brevifolius*.

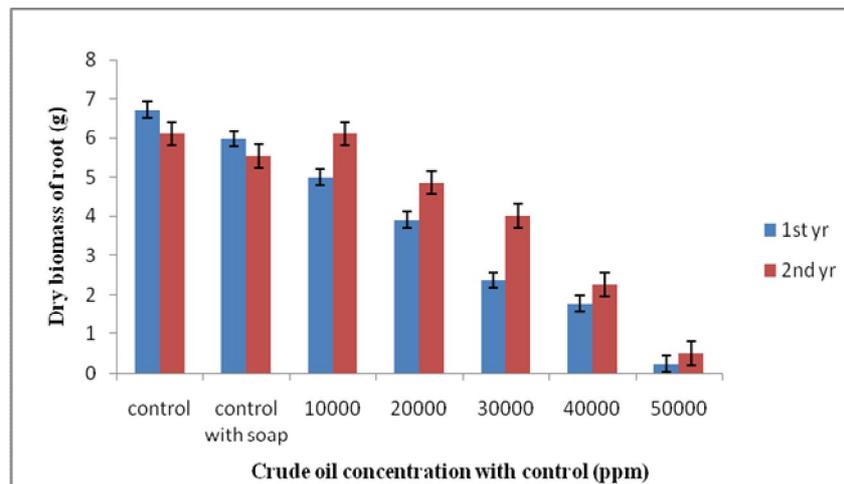


Fig 5. Dry biomass of roots of *Cyperus brevifolius*.

CONCLUSION

Based on the above results, it may be concluded that by planting herbs like *Cyperus brevifolius*, the percentage of oil contamination in soil could be minimized and could make the soil ready for cultivation of other crop and non crop plants. The findings also revealed that the growth of the said plant in crude oil contaminated soils not only reduces the toxicity of the crude oil in the soil, but also

enhances the degradation of crude oil. Since crude oil contamination degrades the quality of soil resulting deterioration in agricultural practices due to their toxic effects, *Cyperus brevifolius* could be recommended as a potent herb to overcome the crude toxicity.

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