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RESEARCH ARTICLE

Phytoremediation of crude oil-contaminated soil employing *Crotalaria pallida* Aiton

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Abstract The purpose of the study was to evaluate the phytoremediation potentiality of a herb named *Crotalaria pallida* which are abundantly grown on crude oil-contaminated soil of oil field situated at upper Assam, India, so that this plant could be used to remediate hydrocarbon from contaminated soil. To evaluate the potentiality of the plant, a pot culture experiment was conducted taking 3 kg of rice field soil mixed with crude oil at a concentration of 10,000 (10 g/kg), 20,000 (20 g/kg), 30,000 (30 g/kg), 40,000 (40 g/kg), 50,000 (50 g/kg), 60,000 (60 g/kg), 70,000 (70 g/kg), 80,000 (80 g/kg), 90,000 (90 g/kg), and 100,000 (100 g/kg) ppm. Ten numbers of healthy seeds of *C. pallida* were sown in three pots of each concentration for germination, and after 15 days of germination, single healthy seedling in each pot was kept for the study. A control setup was also maintained without adding crude oil. The duration of the experiment was fixed for 6 months. The results showed that uptake of hydrocarbon by the plants was increased with increasing the concentration of crude oil in the soil up to 60,000 ppm. After that, uptake of hydrocarbon by the plants was found to be lower with increasing doses of crude oil concentration. Uptake of hydrocarbon by the shoot was found to be maximum, i.e., 35,018 ppm in 60,000 ppm concentration. Dissipation of total petroleum hydrocarbon (TPH) from the soil was also gradually increased with increasing concentration of crude oil in the soil up to 60,000 ppm. Maximum dissipation, i.e., 78.66 %, occurred in 60,000 ppm concentration of crude oil-mixed soil. The plant could not survive in 100,000 ppm concentration of crude oil-mixed soil. The results also demonstrated that there was a reduction in plant shoot and root biomass with an increase of crude oil concentration. Furthermore, results revealed that the shoot biomass was higher than root biomass in all the treatments.

Keywords Phytoremediation · Crude oil · Dissipation · Uptake · Biomass · *Crotalaria pallida*

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Introduction

Phytoremediation is the use of green plants and their associated microbes to remove environmental pollutants or to render them harmless (Kambhampati and Vu 2013). It is a plant-based technology that enhances environmental cleanup (Pilon-Smits 2005; Cook and Hesterberg 2013; Onyema 2013).

Petroleum and its derivates are the mixtures of gaseous, liquid, and solid hydrocarbons. The components of naturally occurring reservoirs of crude oil are therefore named as petroleum hydrocarbons (PHC). The principal constituents of PHC are the elements of hydrogen (10–14 %) and carbon (83–87 %). PHC can be broadly divided into two families: aliphatics (fatty) and aromatics (fragrant) (Chukwuma et al. 2012). Crude oils are colloidal mixtures of a huge number of hydrocarbons ranging from low to high molecular weight. Soil and water represent the first line of recipients of oil pollution (Pathak et al. 2011). Soil pollution by crude oil is a universal problem because of its effect on soil ecosystem

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(Akoto et al. 2008). Petroleum oil pollution exerts adverse effects on plants indirectly by making toxic minerals in the soil available to plants (Adams and Ellis 1960). Crude oil pollution also leads to deterioration of soil structure; loss of organic matter contents; and loss of soil mineral nutrients such as potassium, sodium, sulfate, phosphate, and nitrate, etc. It also exposes soil to leaching and erosion (Palese et al. 2003). The environmental significance of the enhanced levels of these pollutants is judged in terms of the degree of toxicity; the extent of exploitation of the pollutants; and their application, concentration, and consequent mobilization into the soil. The presence of these pollutants obviously has resulted in loss of soil fertility, poor crop yields, and harmful implications on humans and the entire ecosystem.

Crude oil contamination in and around the exploration and spillage sites of oil industries is a burning problem throughout the world. Crude oil releases toxic hydrocarbons in nature which causes pollution. Recently published research data has indicated that vegetation may play an important role in remediation of toxic organic chemicals (Deka et al. 2009). Therefore, phytoremediation is considered as 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. It is a relatively new, efficient, cost-effective, and eco-friendly process. However, selection of suitable plant species is one of the main criteria for success in phytoremediation and for the development of viable phytoremediation technology. The interaction between plant roots and rhizosphere micro-flora significantly enhances degradation of hazardous organic compounds in contaminated soil.

Until now, various works has been done on phytoremediation of hydrocarbon-contaminated soil. The concept of phytoremediation is not new. The use of plants in wastewater treatment technology is over 300 years old (Cunningham and Berti 1993). Noori et al. (2012) studied on some species of Fabaceae family planted in different concentrations of oil pollution in soil and recommended those species as potential phytoremediation plant. Oyedeji et al. (2012) observed that the crude oil-contaminated soil affects the growth performance of *Abelmoschus esculentus* L. as hindered germination, reduced heights, and girths were observed in the crop planted in treated soil. Rahbar et al. (2012) highlighted the ability of some plants at the prevention of hydrocarbon disposal aboveground, particularly photosynthetic tissues. An experiment was conducted by Oyedeji et al. (2013) to study the potentiality of *Cynodon dactylon* and *Eleusine indica*. They observed that biomass of both the plants were gradually increased with increasing the concentration of total hydrocarbon content in the soil. Lotfinasabasl et al. (2013) carried out an experiment to get information on the status of contamination with petroleum hydrocarbons in the mangrove ecosystem of Alibaug and Maharashtra (India). They also studied to assess petroleum hydrocarbon phytoremediation potential of mangrove species *Avicennia marina*. Their reports indicated the potentiality of using *A. marina* for phytoremediation to clean up petroleum hydrocarbons pollutions in the coastal areas. Mathur et al. (2010) clearly demonstrated that *Terminalia arjuna* provided successful phytoremediation process of a contaminated desert soil. Ahalya and Ramachandra (2006) reported that to absorb or degrade the pollutants, phytoremediation is being increasingly adopted where certain plants used are belonging to Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae, and Scrophulariaceae families. Bordoloi et al. (2012) used *Axonopus compressus* for the removal of petroleum hydrocarbons from contaminated soil. They observed that there was a reduction of biomass and height of the plant in the presence of a concentration of crude oil in the soil.

Crotalaria pallida is a native plant of tropical Africa, growing in tropical areas. But its natural distribution is obscured by widespread cultivation. In Asia, it is common in India and Sri Lanka and throughout Southeast Asia. It is an economically and medicinally important genus of the family Fabaceae (Leguminosae). Most of them are source of alkaloids, paper pulp and fibers, ornamentals and green manure, etc. (Ansari 2008). It is an erect shrub, annual or short-lived perennial herb of 1.5 m or more tall. The stout stem is hairy and has longitudinal grooves. Leaves are trifoliate with a long petiole, leaflets elliptical to obovate. Flowers are yellow, often reddish-brown veined, and borne on 15–40-cm-long racemes, each with 20–30 flowers. Fruits are 3–5 × 0.6–0.8 cm in size having 30–40 heart-shaped or kidney-shaped shiny, mottled ochre and dark brown seeds.

This plant is growing abundantly in crude oil-contaminated soil of oil field situated at upper Assam, India. The plant was collected to our laboratory, and the uptake of total petroleum hydrocarbon (TPH) was estimated. The results revealed that this plant could accumulate the highest amount of TPH. Moreover, there are no previous records of phytoremediation study employing this plant. Hence, an effort has been made by cultivating this plant in crude oil-contaminated soil to see the capability of this plant in removing the TPH from the soil.

Materials and methods

Collection and preparation of soil samples

To carry out the experiment, approximately 350 kg of soil from a rice field near Guwahati, India, was collected. Soil samples were homogenized and dried; lumps of the soils were then crushed and sieved through a 2-mm mesh. Thirty-six earthen pots of size 25 cm × 30 cm were collected and marked according to the treatments.

About 10 L of crude oil was collected from Noonmati refinery, Guwahati, India, for conducting the experiment. Three kilograms of soil was weighed for each pot, and the soil was mixed with crude oil in a plastic bucket to make a concentration of crude oil in the soil of about 10,000, 20,000, 30,000, 40,000, 50,000, 60, 000, 70,000, 80,000, 90,000, and 100,000 ppm. For preparing 10,000 ppm concentration, 3000 g previously weighed soil was poured in respective buckets followed by addition of a mixer of 30 g of crude oil + 1000 ml tap water + 10 ml of biodegradable detergent (Extrain) as an emulsifier. The mixing was done with the help of a magnetic stirrer. In this way, all the concentrations were made by adding 60, 90, 120, 150, 180, 210, 240, 270, and 300 g crude oil respectively, and then the soil samples were allowed to dry in sunlight; the lumps of the soil were crushed and sieved through a 2-mm mesh. After that, 3 kg of the above crude oil-mixed soil was filled in each pot. Each prepared soil sample was kept in clean polythene bags for initial analysis. Each treatment including the control (0 %) was replicated three times. Another control set of soil with detergent but without oil was kept for comparison.

Collection and plantation of the experimental plant

For plantation, 10 numbers of healthy seeds of *C. pallida* were sown in each pot for germination, and after 15 days of germination, single healthy seedling in each pot was kept. The experiment was set up during the month of May, 2010, and the plants were harvested in the month of November, 2010.

Evaluation of soil pH

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

Evaluation of soil conductivity

The conductivity of soil was determined by using a conductivity meter (Elico CM 180) in 1:2.5 soil/water suspensions.

Evaluation of water holding capacity of soil

Water holding capacity of the collected soil samples was determined by using circular brass box of known weight (a) containing a Whatman No. 1 filter paper. A few gram of soil was added to the brass box, and the box was reweighed (b). The box with the soil was kept in the petri dish containing water so that about one fourth of the box remains under water. The box was kept overnight. The 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 (c). One Whatman No. 1 moist filter paper was weighed (m).

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

Evaluation of soil total organic carbon

The total organic carbon (TOC) of the soil samples was determined by Walkley and Black titration method. 0.1 g air-dried sample were mixed with 10 ml 1 N $K_2Cr_2O_7$ solution and 20 ml concentrated H_2SO_4 in a 50- ml conical flask. Solid $AgSO_4$ was added to it by gentle stirring so that the $AgSO_4$ goes into the solution completely. The contents were diluted to 200 ml. The color of the solution turned bluish purple on the addition of 1 ml ortho-phosphoric acid and 1 ml diphenylamine indicator. The solution was titrated with ferrous ammonium sulfate till color changes to brilliant green (Deka et al. 2009).

Estimation of TPH

Total petroleum hydrocarbon (TPH) content of the soil and plant samples was estimated by Soxhlet. Petroleum benzene (boiling point 400–600 °C) was considered as an extracted solvent. Extracted solution was poured into a 50-ml beaker and evaporated to dryness in a rotary evaporator. After harvest, the plants were washed and the oil and grease contents of harvested plants (both in roots and shoots) were extracted with the help of Soxhlet.

TPH present in the soil samples was estimated by taking the weight of the beaker containing extracted solvents of samples till the weights become constant. Then, the value of the TPH was 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, and W = sample weight

Plant biomass estimation

Root and shoot biomass of the plants were measured by removing and separating the plant parts from the soil very carefully. After that, plant parts were washed and blotted to remove the excess amount of water. Then, the wet weight of both shoots and roots was taken, and the plant materials were kept in an oven at 60 °C for drying till their constant weight. After that the biomass of the plant material was calculated by as per standard methods (Baruah et al. 2013).

Statistical analysis

Data are presented as mean ± standard error (Figs. 1, 2, and 3) and mean ± standard deviation (Tables 1, 2, 3, and 4) of three replicates ($N = 3$), respectively. Statistical analysis was performed using least significant difference (LSD). The results were calculated by employing PASW Statistics 18 software. $P < 0.05$ compared to the control value.

Results and discussion

The chemical properties of the soil namely pH, conductivity, water holding capacity, total organic carbon, total petroleum hydrocarbon, etc. are the most important parameters for the investigation of phytoremediation potentiality of a plant. The results of the chemical analysis of the soil samples were reported in Tables 1, 2, 3, and 4.

Determination of pH

The results of the pH are presented in the Table 1. It is revealed from the results that the pH of the contaminated soil samples was initially acidic except that of the control. Before plantation, the pH value of the soil samples were found to be minimum (2.67 ± 0.03) in the highest concentration (100,000 ppm) and maximum (7.01 ± 0.25) in the control. The value of pH of the soil samples after harvest was found to be minimum (2.71 ± 0.14) in highest concentration (100,000 ppm) and maximum (7.41 ± 0.37) in the control (0 ppm).

Soil chemical analysis was done before plantation and after the harvest of the plants. On the basis of the results, it can be said that initially, the pH of the experimental soil was 7.01 in the control. That means the value of pH was neutral or towards alkaline. But it was observed that the value of pH was acidic

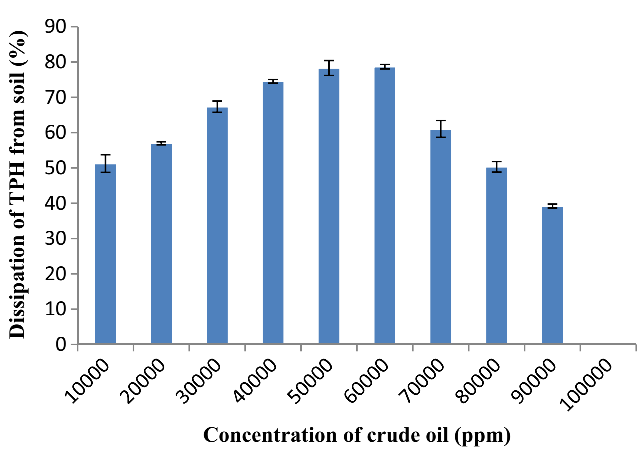

Fig. 1 Dissipation of total petroleum hydrocarbon (%) from different concentrations of crude oil-amended soil where *Crotalaria pallida* was grown. Values are mean ± SE. *Bars* indicates SE

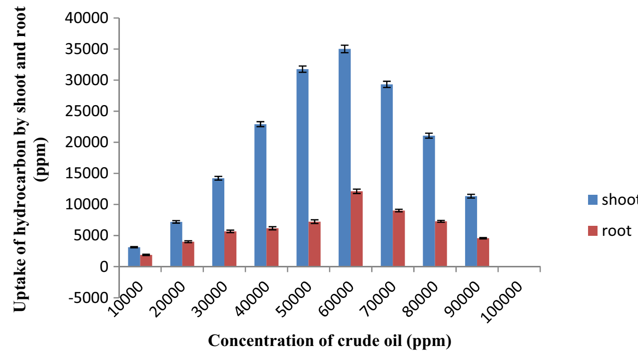

Fig. 2 Uptake of oil and grease by *Crotalaria pallida* plant grown in different concentrations of crude oil-amended soil. Values are mean ± SE. *Bars* indicates SE

when the soil was treated with crude oil (i.e., 2.67–4.71). After planting *C. pallida*, it was observed that the pH values increased from acidic towards basic. Similar results were revealed in previous literature (Merkl et al. 2005). This decreased 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 soil (Song et al. 1986; Phung 1988), the increase of the soil pH towards neutral condition means more favorable conditions for soil bacteria. Many researchers have reported that bacteria play a proactive role in the degradation of crude oil (Atlas and Bartha 1977; Frick et al. 1999). The change in pH towards alkalinity was similar to the findings of Okiemen and Okiemen (2005). Chukwuma et al. (2010) reported that pH increases with the increase of crude oil pollution.

In nature, crude oil-contaminated soil was more acidic. This may be due to the formation of toxic acids in the spilled oils (Barua et al. 2011; Oyem and Oyem 2013; Basumatary et al. 2012). The same condition was observed in the present study. They considered a pH value ranging between 6.5 and 7.5 to be optimum for the growth of many plants. According to Vara et al. (2012), the lowering of the value of pH due to crude oil pollution was responsible for the production of

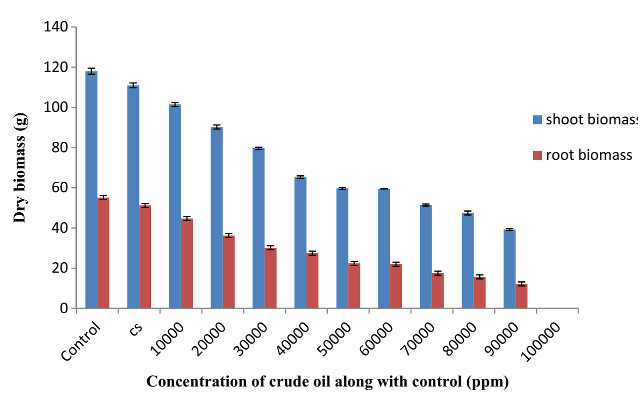

Fig. 3 Shoot and root biomass of *Crotalaria pallida* grown in different concentrations of crude oil-amended soil. Values are mean ± SE. *Bars* indicates SE

Table 1 pH of soil amended with different concentrations of crude oil at the time of plantation and harvesting

Concentrations (ppm)	At the time of plantation	At the time of harvesting
Control	7.01 ± 0.25	7.41 ± 0.37
Control with soap	6.98 ± 0.15	7.36 ± 0.05
10,000	4.71 ± 0.21	6.52 ± 0.26
20,000	4.60 ± 0.13	6.42 ± 0.31
30,000	4.52 ± 0.11	6.23 ± 0.11
40,000	4.48 ± 0.21	6.09 ± 0.15
50,000	3.32 ± 0.15	5.96 ± 0.31
60,000	3.25 ± 0.56	5.84 ± 0.22
70,000	3.17 ± 0.05	5.31 ± 0.25
80,000	3.05 ± 0.14	4.31 ± 0.21
90,000	2.91 ± 0.04	3.84 ± 0.01
100,000	2.67 ± 0.03	2.71 ± 0.14

The results represented are the means ± SD

organic acids by microbial metabolism. On the other hand, in our present investigation, we found that contaminated soil had low pH value compared to the uncontaminated soil which was in conformity with the results of Ujowundu et al. (2011); Onojake and Osuji (2012); Nwazue (2011).

Conductivity

At the time of plantation, the conductivity (Table 2) of the soil was found to be minimum (0.034 mS/cm) in the highest concentration (100,000 ppm) and maximum (0.110 mS/cm) in the control (0 ppm). The conductivity value of the soil after harvest was found to be minimum 0.039 mS/cm in 100,000 ppm and 0.653 mS/cm in control (0 ppm).

Table 2 Conductivity of soil amended with different concentrations of crude oil at the time of plantation and harvesting

Concentrations (ppm)	At the time of plantation	At the time of harvesting
Control	0.110 ± 0.0051	0.653 ± 0.032
Control with soap	0.109 ± 0.0023	0.647 ± 0.027
10,000	0.097 ± 0.0049	0.462 ± 0.023
20,000	0.083 ± 0.0034	0.405 ± 0.02
30,000	0.077 ± 0.0028	0.384 ± 0.019
40,000	0.057 ± 0.0019	0.341 ± 0.017
50,000	0.052 ± 0.0016	0.283 ± 0.014
60,000	0.051 ± 0.0021	0.211 ± 0.01
70,000	0.046 ± 0.0011	0.189 ± 0.005
80,000	0.042 ± 0.0021	0.176 ± 0.003
90,000	0.038 ± 0.0017	0.169 ± 0.006
100,000	0.034 ± 0.001	0.039 ± 0.002

The results represented are the means ± SD

The results also revealed that the value of conductivity of the soil samples increases after harvest. But the value of conductivity in contaminated soil was lower than that in the control. This reduction of conductivity occurs due to crude oil pollution in soil. Reduction in conductivity could be due to the non-polar nature of the crude oil bringing about reduced ionic movement in the soil (Akpoveta et al. 2011; Osuji and Nwoye 2007). It was reported that there was a reduction in the value of conductivity from 61.90 to 34.59 due to crude oil pollution in the soil (Osuagwu et al. 2013). According to Ujowundu et al. (2011), microbes have a tremendous role in determining the conductivity of the soil. Microbial biomass in contaminated soil is lower than that in uncontaminated soil. This may lead to low conductivity value in contaminated soil as compared to uncontaminated soil.

Total organic carbon

The results of the total organic carbon (TOC) revealed that there was a slight increase in TOC (%) in contaminated samples than in control (Table 3). The TOC values (%) of the soil at the time of plantation were found to be maximum 5.17 % in highest concentration (100,000 ppm) and gradually lower in subsequent concentrations. The value of TOC after harvesting showed similar trend than that of before harvesting. The value of TOC showed a gradual decrease with increased time. TOC after harvest was found to be 5.14 % in 100,000 ppm and 0.46 % in 0 ppm concentration. There was a slight increase in TOC in contaminated samples than in control. The TOC of the soil before plantation were found to be maximum in the highest concentration and gradually lower in subsequent concentrations. The value of TOC after harvesting showed similar trend than that of before harvesting. However, after harvesting, it decreased in contaminated soils. Similar results were reported by Pathak et al. (2011). The increase in crude oil pollution of soil leads to increase in high organic carbon content. It was reported that in the crude oil-contaminated sites, the percentage of organic carbon was found to be eight times more than that in the uncontaminated sites. This may be because of the carbon from the spilled crude oil (Ali et al. 2009; Chukwuma et al. 2010; Barua et al. 2011; Nwazue 2011). This increase of organic carbon is directly proportional to the increase of crude oil addition to the soil. Investigation on total organic carbon revealed that during the remediation treatment, the organic carbon content decreases. The parallel observation was also reported by Ayotamuno et al. (2006).

Water holding capacity

Water holding capacity (WHC) of soil samples showed variation in the results (Table 4). WHC of oil-contaminated soils was lower than that of the control sample. At the time of plantation, the WHC of the soils were found to be minimum, i.e., 5.12 %,

Table 3 Total organic carbon content (%) of soil amended with different concentrations of crude oil at the time of plantation and harvesting

Concentrations (ppm)	At the time of plantation	At the time of harvesting
Control	0.52 ± 0.016	0.46 ± 0.023
Control with soap	0.45 ± 0.011	0.38 ± 0.019
10,000	1.27 ± 0.053	1.09 ± 0.054
20,000	1.69 ± 0.075	1.24 ± 0.062
30,000	2.12 ± 0.11	1.86 ± 0.093
40,000	3.39 ± 0.16	2.72 ± 0.126
50,000	3.78 ± 0.19	3.43 ± 0.171
60,000	3.96 ± 0.02	3.68 ± 0.184
70,000	4.25 ± 0.01	3.87 ± 0.193
80,000	4.63 ± 0.023	4.15 ± 0.21
90,000	4.86 ± 0.01	4.61 ± 0.231
100,000	5.17 ± 0.12	5.14 ± 0.24

The results represented are the means ± SD

in highest concentration (100,000 ppm) and maximum, i.e., 65.05 %, in control (0 ppm), and after harvest, it was 5.18 % in 100,000 ppm and 66.77 % in 0 ppm concentration.

The increase in WHC was may be due to a decrease of oil and grease in the soil. Crude oil contamination may produce waxy materials at the top surface of the soil which could not allow the water to 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. Studies have showed that crude oil-polluted soil was characterized by lower WHC compared with unpolluted soils (Chibuike and Obiora 2013). The result suggested that the percentage of WHC in crude oil-affected areas

Table 4 Water holding capacity (%) of soil amended with different concentrations of crude oil at the time of plantation and harvesting

Concentrations (ppm)	At the time of plantation	At the time of harvesting
Control	65.05 ± 2.5	66.77 ± 1.2
Control with soap	64.84 ± 2.9	65.99 ± 1.5
10,000	45.08 ± 1.1	58.35 ± 1.3
20,000	33.49 ± 1.6	47.27 ± 0.22
30,000	29.58 ± 1.05	44.63 ± 1.3
40,000	22.03 ± 1.10	36.89 ± 0.69
50,000	10.91 ± 0.54	26.84 ± 1.34
60,000	8.75 ± 0.0.43	24.76 ± 0.23
70,000	7.01 ± 0.35	22.45 ± 0.59
80,000	6.56 ± 0.0.32	19.12 ± 0.11
90,000	5.74 ± 0.28	13.94 ± 0.58
100,000	5.12 ± 0.25	5.18 ± 0.29

The results represented are the means ± SD

was found to be lower compared to the uncontaminated sites. This may be due to the formation of a thick crude oil coating above the soil surface of crude oil spilled areas. This type of observation was also reported by Barua et al. (2011) and Vara et al. (2012). The results confirmed that crude oil pollution has a great impact on WHC.

It was revealed from the investigation that the crude oil contamination has a great impact on chemical properties namely pH, conductivity, water holding capacity, total organic carbon content, and total petroleum hydrocarbon of the soil. These parameters have a great role on the general welfare of any plants. On the other hand, the potentiality of phytoremediation also depends upon the general welfare of the plants. There is a relation between phytoremediation and general welfare of the plants. The plant *C. pallida* could survive up to 90,000 ppm concentration of crude oil-mixed soil; however, their growth gradually decreased with increasing the concentration of the crude oil in the soil, and at 100,000 ppm concentration, the plant could not survive. It was also revealed from the experiment that the chemical properties namely pH, conductivity, water holding capacity, total organic carbon content, total petroleum hydrocarbon, etc. of the soil are gradually changed after the harvest of the plant. The results establish the fact that phytoremediation can help to rehabilitate the soil chemical properties and make the soil fit for cultivation of other crops.

Dissipation of total petroleum hydrocarbon

The results of the dissipation of total petroleum hydrocarbon (TPH) in soil samples after harvest are presented in Fig. 1. It was revealed from the result that the dissipation of TPH was recorded to be maximum, i.e., 78.66 %, in 60,000 ppm concentration of crude oil-mixed soil, whereas it was found to be minimum, i.e., 51.23 %, in 10,000 ppm concentration.

Statistical analysis of the data revealed that the values of dissipation percentage for all the concentrations were significantly different where $F_{9,47} = 62.029$, $P = 0.000$).

Dissipation of TPH from the soil was gradually increased with increasing the concentration gradient of crude oil from 10,000 to 60,000 ppm, and the highest dissipation was observed in 60,000 ppm, and thereafter, it was gradually decreased. It was revealed from the investigation that there is a relation between dissipation and uptake of crude oil by the plant. As the uptake of crude oil was found to be maximum in 60,000 ppm concentration of crude oil-mixed soil, the dissipation was also found to be maximum at this concentration of crude oil. Moreover, dissipation and uptake of crude oil are plant dependent. In our earlier study, it was revealed that the *Cyperus brevifolius* plant could dissipate a maximum amount of crude oil in 50,000 ppm concentration, and in the same concentration, uptake was found to be maximum (Baruah et al. 2013). TPH level in shoot tissue showed an upward trend with contaminant concentration (Nwaichi and Ayalogu 2011).

According to Nwoko et al. (2013), TPH concentration was increased with increasing the concentration of crude oil. It was also seen that uptake of hydrocarbon was found more in the shoot as compared to the root. TPH uptakes of the plants were varying depending on plant parts along with its physiology and the nature of the contaminant (Deka et al. 2009).

Uptake of TPH by the plants

Uptake of TPH by shoots and roots of the experimental plant is presented in Fig. 2. Shoot uptake was found to be maximum, i.e., 35,018 ppm, in a concentration of 60,000 ppm, whereas root uptake was found to be maximum, i.e., 12, 125 ppm, in a concentration of 60,000 ppm. Uptake was found more in shoots than in roots.

Statistical analysis of uptake of TPH showed that uptake was significantly different ($F_{1,52}=37.006$, $P=0.000$). Concentration-wise uptakes by shoots and roots were different, i.e., each individual value was significantly different from the other where $F_{17,36}=1.690\times10^{8}$, $P=0.000$.

Uptake of TPH by the plant species was varied depending upon the plant parts. The investigation of the results of the uptake of TPH in the Fig. 2 revealed that the uptake of crude oil by shoot portion of the plant was maximum than that of the roots. Uptake of the contaminant by the root was a direct function of the pollutant concentration in the soil solution and usually involved chemical partitioning on the root surfaces followed by movement across the cortex to the plant's vascular system (Crowdy and Jones 1956). Uptake of TPH in plants was an indication of the capability of those particular plants which could tolerate stress condition. In this study, it was observed that uptake of TPH was increased with increasing the concentration of the crude oil in the soil and it was found to be maximum in 60,000 ppm; afterwards, it was decreased gradually. This gradual decrease may be due to the stress tolerance of the plants to the crude oil contamination. That means the plant could tolerate the oil stress in 60, 000 ppm concentration where it could uptake 78.66 % crude oil. Xiao-Dong et al. (2004) acknowledged that a successful phytoremediation process could be facilitated through a selection of more contaminant-tolerant plant species. They proposed that some physiological characteristics can be used as indicators for enhancement of the tolerance to contamination. There was a direct relation between the contaminant tolerance of a plant species and its physiology and biochemistry.

Plant biomass

The record of the biomass of shoots and roots is presented in the Fig. 3. After harvest, shoot biomass was recorded as 118.01 g in 0 ppm and 39.25 g in 90,000 ppm. Root biomass of both the plants was also analyzed. Root biomass was recorded as 55.13 g in 0 ppm and 12.14 g in 90,000 ppm. It was revealed that after harvest, plant biomass was found to be the maximum in 0 ppm (i.e., control) and gradually decreased according to their concentration gradient. Shoot biomass was maximum than root biomass.

Statistical analysis of shoot revealed that the concentration-wise change in biomass was statistically significant ($F_{10,22}=2077.036$, $P<0.000$). Pairwise comparisons (LSD) revealed that this difference was significant between each and every concentration, except for 70,000 and 80,000 ppm, where the values were not statistically significant. Root biomass for concentration gradient values were significantly different (($F_{10,22}=664.523$, $P<0.000$).

Investigation on biomass (Fig. 3) of the experimental plant revealed that after harvest, the dry biomass of root and shoots was higher in control and became lower in the subsequent concentrations. The reason may be that the crude oil concentration in soil decreased and water holding capacity increased helping the plants to grow more. Considerable reduction of plant biomass by the presence of petroleum hydrocarbon was found in some cases. This was in agreement with earlier results reported by Chaineau et al. (1997) and Baruah et al. (2013). Chaineau et al. (1997) studied on bean and wheat and recorded 80 % reduction of growth rate occurred due to petroleum hydrocarbon concentration in soil. The plant grown in oil-polluted soil generally had retarded growth and showed chlorophyll deficiency on leaves, which attributed to water deficiency in the plants. As a result of which, there remains a hindrance of transpiration and photosynthesis consequence that retarded plant growth and ultimately leads to reduction of plant biomass (Akapo et al. 2011; Odjegba and Okunnu 2012). Njoku et al. (2012) also reported that the acidity of petroleum-polluted soil is increased with increasing the concentration of pollution, and as a result of which, the growth and development of the plant are retarded. Due to the action of contaminants in lowering the fertility of contaminated soils and decreasing nutrient uptake by roots, plant production was reduced (Nwaichi and Ayalogu 2011). Crude oil contamination of soil has a highly significant effect of reducing the biomass accumulation in *Jatropha curcas* seedlings (Agbogidi 2011). Mukasheva et al. (2013) reported that at a concentration of 30 g of oil/kg soil, plant shoot biomass was reduced significantly. Similarly, they observed that root biomass was reduced at a concentration of 20 g of oil/kg soil. The results revealed that as the concentration of crude oil present in the soil was increased, a gradual decrease in the biomass of a plant had taken place. This was similar with the investigation of Oje et al. (2012), Udeh et al. (2013), and Ogbo et al. (2009). It was also reported that hydrocarbon pollution significantly reduced the biomass of *Telfairia occidentalis* (Ekpo et al. 2014). Investigation of Otitoju et al. (2014) showed that crude oil pollution in soil was often characterized by increased water retention capacity, poor aeration, and low nutrient bio-availability which may lead to the low biomass of the plants.

Conclusions

From the above investigation, it may be concluded that by planting herbs like *C. pallida*, the contamination of crude oil in soil could be minimized and could make the soil ready for the cultivation of other crop and non-crop plants. The plant could be recommended as a potent herb for phytoremediation. So far, in our knowledge, this is the first to report that this plant could survive up to 90,000 ppm concentration of crude oil-mixed soil and could dissipate the highest amount of crude oil at 60,000 ppm concentration of crude-contaminated soil.

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