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Crude Oil-Contaminated Soil Phytoremediation by Using *Cyperus brevifolius* (Rottb.) Hassk

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Abstract The degradation of total oil and grease (TOG) in crude oil-contaminated soil in the presence of *Cyperus brevifolius* (Rottb.) Hassk was investigated in a net house study. *C. brevifolius* plants were transplanted in to spiked soil containing 8% (*w*/*w*) crude oil. The capability of plant for enhancing the biodegradation process was tested in pots containing fertilized and unfertilized soil over a 360-day period. Analysis of the degradation of hydrocarbon contaminants, plant growth, and biomass was conducted at 60-day interval. In the presence of contaminants, plant biomass and height were significantly reduced. The specific root surface area was reduced under the effects of crude oil. Concerning TOG content in soil, *C. brevifolius* could decrease up to 86.2% in TA (crude oil-contaminated soil with fertilizer) and 61.2% in TC (crude oil-contaminated soil without fertilizer). In the unvegetated pots, the reduction of TOG was 13.7% in TB (crude oil-contaminated soil with fertilizer) and 12.5% in TD (crude oil-contaminated soil without fertilizer). However, biodegradation was significantly more in vegetated pots than in unvegetated pots ($p=0.05$). The addition of fertilizer had positive effect on TOG degradation in the presence of *C. brevifolius* compared to the unfertilized treatments. Thus, there was evidence of *C. brevifolius* enhancing the biodegradation of crude oil in soil under the conditions of this experiment.

Keywords Crude oil-contaminated soil · *Cyperus brevifolius* · Petroleum contamination · Phytoremediation · Total oil and grease (TOG)

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1 Introduction

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. Phytoremediation requires no excavation and transportation of contaminated media or cumbersome physical, chemical, or thermal procedures; thus it is more cost-effective (Cunningham et al. 1995). Roots possess soluble and wall-bound oxidative enzymes that may be directly involved in the dissipation of polycyclic aromatic hydrocarbons (PAHs) and can also be taken up by plants and transformed or stored in a nonphytotoxic form (Siciliano and Germida 1998; Macek et al. 2000). In particular, plants with deep root, fibrous roots having faster growth rate, such as grasses, are useful in phytoremediation. The property of plants to tolerate stress conditions is another important required characteristic (Siciliano and Germida 1998).

Cunningham et al. (1995) showed accelerated petroleum hydrocarbon degradation in rhizosphere region compared to bulk soil. Several studies report on the use of gramineae, e.g., prairie grasses, in the phytoremediation of hazardous organic compounds. Miya and Firestone (2000) observed greater percentages of phenanthrene degrading bacteria in rhizosphere soil than bulk soils and suggested the rhizosphere selected for PAH degraders. Merkl et al. (2005a) showed that some tropical grasses and legumes to be resistant to petroleum pollution and root surface were increased in graminoids, namely *Brachiaria brizantha*, *Cyperus aggregatus*, and *Eleusine indica* in petroleum-polluted soils.

Apart from bioaugmentation with oil-degrading microorganisms, phytoremediation is applied to provide long-term rehabilitation of the residual oil contamination (Panchenko et al. 2002). The successful application of plant species to the bioremediated sites having oil-contaminated soil has been shown in a number of studies (Günther et al. 1996; Carmen et al. 1998; Pichtel and Liskanen 2001; Banks et al. 2003; Jones et al. 2004; White et al. 2006; Kaimi et al. 2006; Brandt et al. 2006; Muratova et al. 2008; Al-Surrayai et al. 2009; Ayotamuno et al. 2010). Several comprehensive reviews are available (Frick et al. 1999; Hutchinson et al. 2003; Hall et al. 2011) on this subject. In general, the selection of phytoremediation for specific sites is empirical and based on preliminary results from pot experiments (Kirkpatrick et al. 2006; Liste and Prutz 2006; Euliss et al. 2007). Most studies on the phytoremediation of petroleum hydrocarbon-contaminated soil reported the use of grasses (*Poaceae*) and legumes (*Leguminosae*; Aprill and Sims 1990; Günther et al. 1996; Qiu et al. 1997; Merkl et al. 2005b; Kirkpatrick et al. 2006; Schwab et al. 2006; Kaimi et al. 2006; Kechavarzi et al. 2007). In the phytoremediation of organics which is based on a stimulated microbial degradation in the rhizosphere, fertilization is very much essential. 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).

There are no reports available on the use of *Cyperus rotundus* for phytoremediation of hydrocarbon-contaminated soil. In general, phytoremediation must be managed by using indigenous plants, especially those growing in the contaminated sites, instead of foreign or genetically modified species (Lopez-Martinez et al. (2008). Prior to phytoremediation field trials a plant should be evaluated for its suitability in the laboratories and greenhouses. *Cyperus brevifolius* is a commonly distributed species of North East India and also found growing in oil-contaminated areas. It is a species that propagates by rhizome and seeds, grow in wet, disturbed, and altered areas thus, it could be vegetatively established in contaminated soil. It has fibrous, perennial root system with vigorous deep root rhizomes which can control soil erosion and surface spreading of hydrocarbon contaminants. Considering this, it was selected to evaluate its suitability for use in the phytoremediation of hydrocarbon-contaminated soils. The overall goal of this experiment was to evaluate the suitability of *C. rotundus* for use in the phytoremediation of crude oil-contaminated soils in Assam, India. The particular objectives were to study the influence of oil on plant growth, the effect of plants on oil degradation, and the influence of fertilizer on plant growth and oil degradation.

2 Materials and Methods

2.1 Soil, Crude Oil, and Chemicals

The soil used in the contaminated pots (Table 1) was collected from the oil-contaminated pit of Oil India Limited, Duliajan (27°20′29″ N 90°28.57′92″ E), Assam, India containing crude oil concentration of 4% *w/w*. Crude oil (average organic composition:

Table 1 Physico-chemical analysis of base materials

Parameter	Contaminated soil	Uncontaminated soil
pH	4.4±0.06	4±0.06
Sand [%]	73±1.2	75±2.1
Silt [%]	15±0.35	16±0.8
Clay [%]	12±0.16	9±0.5
Texture	Sandy loam	Sandy loam
Moisture (%)	6.9±0.4	9±1
Total organic carbon [%]	11±0.4	5.5±0.02
Total N [%]	0.06±0.02	0.07±0.01
C/N ratio	400:1	3:1

Values represent mean±standard deviation of three replicates

27.5% saturated, 43.4% aromatics, 1.8% asphaltenes, and 27.3% resins) was obtained from Research and Development Department, Oil India Limited, Duliajan, Assam (India). The chemicals were purchased from Merck.

2.2 Experimental Setup

The experiment was conducted in a net house of IASST, Assam, India. The experimental site is located Northern Latitude 26°10′45″ Eastern Longitude 91° 45′ Climate with a mean annual rainfall of 1,600 mm, mean relative humidity of 76.6%, and minimum temperature of 6°C and maximum temperature of 38°C. (http://kamrup.nic.in/ghy.html).

2.3 Preparation of Soil

The soil was air-dried and sieved through a 2-mm screen. The soil for PHC-contaminated treatments was mixed by hand with crude oil to the concentration of 80,000 mg kg^{-1} of dry soil weight. Continuous checking was done to ensure the uniformity of crude oil in soil during mixing. The uncontaminated soil, with similar pedologic characteristic of the contaminated one, was used as control. The physicochemical characteristics are reported in Table 1. The crude oil-contaminated soil was allowed to stand for 6 weeks to allow for partial aging of the contamination and adsorption of oil to the soil particles. Plastic pots were filled (approximately 15 kg per pot) using contaminated or uncontaminated soil.

2.4 Preparation of Plant

C. brevifolius was used for phytoremediation study. It is a ubiquitous plant growing wild and widely distributed throughout North-Eastern India. Five transplants were planted in each pot after soil contamination with crude oil. Prior to this, the plants were propagated in the net house. A total of 90 pots were maintained in a half open net house without environmental control. The mean monthly temperatures were 22–25°C (from a daily minimum average of 19°C to maximum 38°C). The mean monthly relative humidity was 71–76.6% (from a daily minimum average of 35% to maximum 98%). The daily photoperiod was characterized by 12 h of daylight with low variation during the experimental period. 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. Replacement of plants with healthy transplants was done in case of plants died in 10 days. During replacement, soil particles adhering to the roots were carefully collected to prevent release of the contaminated soil. Starting from the date of transplant, the experiment lasted 360 days om7th May 2010 to 7th May 2011). Analysis was done for hydrocarbon degradation in different treatments.

2.5 Treatment Details

TA: contaminated soil+fertilizer+plants, TB: contaminated soil+fertilizer+no plants, TC: contaminated soil+without fertilizer+plants, TD: contaminated soil+without fertilizer+no plants, TE: uncontaminated soil+fertilizer+plants.

2.6 Fertilization

Basic dose of fertilizer [nitrogen (N), phosphorus (P), and potassium (K) 120, 60, and 60 mg kg^{-1} soil] was applied to TA, TB, and TE, respectively to support healthy plant growth. TC and TE were maintained without fertilizer amendment. The total fertilizer amounts were split in to six applications (8, 30, 75, 115, 180, and 280 days after planting).

2.7 Evaluation and Sampling

Initial soil samples were taken before planting. Weekly monitoring of plant was done during the experimental period to ensure healthy plant growth. Three destructive samplings were carried out by using 5 pots per treatment at 60-day interval (up to 360 days). Plants were cut at their base and the length of shoot was determined. Roots were carefully separated from soil; both roots and shoots were rinsed and stored at 50% isopropyl alcohol under refrigeration (4°C). Both shoots and roots were oven dried at 65°C until constant weight and dry-weight of biomass was determined. The specific root length, root diameter, and root surface area were analyzed with WinRhizo 2008a, Régent Instruments Inc., for determination of root diameter classes (x<2 mm (fine roots) and x>2 mm). The soil of each pot was homogenized and one 500-g composite soil was stored at 4°C for 1–2 weeks prior to analysis.

2.8 Analytical Methods

The pH in soil was measured in 1:2.5 soil/DH_2O suspension using a pH meter (Elico LI-127) fitted with a glass electrode (Thomas and Sparks 1996). 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 [$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$] (Kalembasa and Jenkinson 1973). Total nitrogen in soil was measured by the Kjeldahl method using concentrated H_2SO_4, K_2SO_4, and HgO to digest the sample (Bremner and Sparks 1996), soil particle distribution by the hydrometer method as described by Gee et al. (1986). Soil moisture content was measured by gravimetric method (oven drying until constant weight). Bacterial counts were determined by using plate count agar (Alexander et al. 2005).

2.9 Total Oil and Grease Analysis in Soil

Total oil and grease (TOG) in soil was determined by Soxhlet extraction method using a modification of EPA method 3540B (USEPA 1994). Of each sample, three 20 g replicates were analyzed. The samples were acidified with HCL to pH 2 and dehydrated with $MgSO_4$. Soxhlet extraction with dichloromethane was run for 10 h. After passing the extract through a filter paper (Whatman No.4) with approximately 1 g 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.

2.10 Statistical Analysis

The statistical analyses were conducted using the Superior Performance Software System 15.0 for Windows. Concentration of TOG in soil, plant dry weight, root, and shoot length was subjected to one way analysis of variance to test for significant difference between treatments ($p<0.05$). Moreover, the bivariate correlation between TOG in soil, plant dry weight, root, and shoot length in the contaminated treatments with plants was evaluated with Pearson's correlation-coefficient for normal-distributed variables at $p=0.05$.

3 Results

3.1 Physicochemical Characteristics

Tables 1, 2, 3, 4, and 5 represent the selected properties of soil used in the experiment. The contaminated and uncontaminated soil used in the experiment was acidic. The pH range in the contaminated soil in vegetated pots during the study period was 4.5 to 6 and that of unvegetated pots was 4.4 to 5.1. Moreover, the pH intervals recorded in uncontaminated soil were 4.2 to 5.4. The texture of soil was sandy-loam. The moisture content of contaminated soil was 5.6% to 20.5% while that of uncontaminated soil was 11% to 25.6% during the course of study. It has been observed that moisture content of TA and TC at 180 and 360 days was about 5% to 13% higher than that in TB and TD. The uncontaminated soil had higher moisture content (25.6%) during 360 days. Petroleum hydrocarbons (PHCs) usually have a very high carbon–nitrogen ratio. After mixing crude oil, the soil had a C/N ratio of 670:1 in TA and TB and 550:1 in TC and TD, respectively. During 360 days, C/N ratio was 110:1 in TA and 120:1 in TC and that of TB and TD was 590:1 and 440:1, respectively. In 360 days, the total nitrogen increased from 0.14% to 0.25% in TA and 0.1% to 0.2% in TC

Table 2 Selected properties of soil at time 0

Parameter	TA	TB	TC	TD	TE
pH	4.5±0.06	4.4±0.09	4.5±0.06	4.4±0.05	4.2±0.07
Moisture (%)	5.7±0.35	5.6±0.25	5.6±0.4	5.6±0.4	11±0.8
TOC [%]	13±0.5	12±0.5	11±0.4	12±0.4	5±0.03
Total N [%]	0.14±0.02	0.14±0.01	0.1±0.05	0.1±0.05	0.15±0.09
C/N ratio	670:1	670:1	550:1	550:1	6:1
THB ($\times 10^6$ CFU/g)	5.3±0.07	5.3±0.05	5.3±0.06	5.3±0.08	6.2±0.08

Values represent mean±standard deviation of three replicates

while that in TB it increased from 0.14% to 0.18% and in TD it increased from 0.1% to 0.12%.

3.2 Plant Height and Biomass

Experimental transplants had an initial height of about 6 cm. Height of tillers in the contaminated soil was slower and shorter than in uncontaminated soil (Fig. 1). Significant difference of plant height (p=0.05) was observed between contaminated and uncontaminated soil throughout the experimental period. The biomass yield per pot increased in all the pots during the experimental period (Fig. 2). There was significant difference of biomass yield (p=0.05) between the contaminated and uncontaminated soil during the course of the study. Concerning fertilizer effects in contaminated treatments, no significant difference of plant height and biomass was observed between the fertilized and unfertilizer soil throughout the study.

3.3 Root Structure

The difference between the root structure of contaminated and uncontaminated soil was significant (p=0.05) during the study period. Roots growing in uncontaminated soil were finer and longer than that of contaminated soil. The average specific root length (cm g^{-1}) of *C. brevifolius* was 1350.5 in TA, 980.7 in TC in comparison to 2420.4 in TE. The average dry weight of root was 53.5 and 32.7 g in TA and TB respectively whereas, that of TE is 98.5 g during the study of 360 days. Similarly, 64% of root surface in fertilized (TA) and 58% in unfertilized (TC) on contaminated soil belonged to fine roots in comparison to 96% in uncontaminated soil (TE).

3.4 Microbial Population

The bacterial population increased with time. The initial counts went from 5.3×10^6 cfu/g in contaminated soil and 6.2×10^6 cfu/g. In 180 days, the bacterial colony counts increased to 31.5×10^6 in TA, 8.65×10^6 in TB, 26.8×10^6 in TC, 8.3×10^6 in TD, and 36.5×10^6 in TE, respectively. The final count bacterial population at 360 days increased to 35.2×10^6 in TA, 9.6×10^6 in TB, 32.4×10^6 in TC, 9.2×10^6 in TD, and 38.5×10^6 in TE. There was significant difference of bacterial colony count between vegetated and unvegetated pots. Concerning fertilizer effect, no significant influence of fertilizer was observed on bacterial population.

3.5 Concentration of TOG in Soil

The soil in the contaminated treatments had 8% TOG content of the total soil dry weight. There was significant difference of TOG degradation between the vegetated and unvegetated pots (Fig. 3). Reduction of TOG in vegetated soil was rapid during the first 60 days in the presence of *C. brevifolius* (TA 31.2%; TC 15%). The unvegetated soil showed slight decrease during 60 days (TB 5%; TD 3.7%). TOG degradation became relatively slower and stable in vegetated treatments during 240 days (TA 78.7%; TC 57.5%) and became almost stable during 300 (TA 83.7%; TC 60%) to 360 days (TA 86.2%; TC 61.2%). In the unvegetated treatments, TOG reduction was very slow and became almost stable in 60 days. Significant difference (p=0.05) of TOG degradation was observed between fertilized soil and unfertilized soil in the presence of *C. brevifolius*. In case of fertilized soil (TA), TOG degradation was relatively faster, continued sharp reduction until day 240, continued to be slow,

Table 3 Selected properties of soil at 60 days

Parameter	TA	TB	TC	TD	TE
pH	4.8±0.1	4.6±0.05	4.7±0.09	4.5±0.07	4.4±0.06
Moisture (%)	6.8±0.4	5.8±0.08	6.6±0.25	5.7±0.3	15±1.2
TOC [%]	12.5±0.5	11.9±0.08	10. 5±0.6	11.8±0.16	5.5±0.2
Total N [%]	0.16±0.02	0.15±0.02	0.13±0.05	0.1±0.08	0.17±0.06
C/N ratio	540:1	640:1	480:1	520:1	5:1
THB (× 10^6 CFU/g)	19.5±0.7	6.8±0.06	18±0.5	5.8±0.03	21.5±0.8

Values represent mean±standard deviation of three replicates

Table 4 Selected properties of soil at 180 days

Parameter	TA	TB	TC	TD	TE
pH	5.4±0.07	4.7±0.04	5.2±0.04	4.7±0.08	4.8±0.05
Moisture (%)	13.5±0.8	6.4±0.06	11.5±0.05	6.2±0.07	19.5±0.75
TOC [%]	9.1±0.1	11.2±0.05	9.5±0.07	11.5±0.08	4±0.05
Total N [%]	0.2±0.04	0.17±0.04	0.17±0.07	0.11±0.02	0.22±0.06
C/N ratio	180:1	605:1	255:1	500:1	3:1
THB ($\times 10^6$ CFU/g)	31.5±1.2	8.65±0.06	26.8±0.3	8.3±0.04	36.5±1.5

Values represent mean ± standard deviation of three replicates

and became almost stable at day 360. In unfertilized soil (TC), TOG reduction was comparatively slower than the fertilized soil and continued to be stable at day 180. The bivariate correlation (r) between TOG degradation and root biomass in TA and TC was significant (p=0.05).

4 Discussion

The pH of vegetated soil was more than in unvegetated soil during the study period. Plants provide root exudates of carbon, energy, nutrients, enzymes, and sometimes oxygen to microbial population in the rhizosphere (Campbell 1985; Cunningham et al. 1996; Vance 1996). These exudates provide sufficient carbon and energy to support large number of microbes in the rhizosphere (Erickson et al. 1995). This plant-induced enhancement of the microbial population is called rhizosphere effect (Atlas and Bartha 1998) and is believed to result in enhanced degradation of PHC contaminants in the rhizosphere. With sufficient oxygen, soil moisture, and an acclimated population of microorganisms, the soil column acts as a natural biofilter within which PHC vapors are degraded at sufficient fast rates (USEPA 2011). This dissipation of PHCs might have increased moisture content in soil. Increase in N level and bacterial utilization of PHCs might have declined the C/N ratio. The decrease in total organic carbon in the vegetated pot might be due to utilization of hydrocarbon by plants and microbes. As an additional compartment, plant roots can interact with both microbes and organic pollutants (Bossert and Bartha 1984). Root proliferation of the plant can support a flourishing microbial consortium, thus accelerating biodegradation of PHCs (Cai et al. 2010). In turn, the healthier microbial consortium can benefit better growth of the plant, thus improving phytoremediation efficiency and characteristic of soil. However, due to the complexity of rhizosphere, more information about relationships between *C. brevifolius* root–microbial interactions in respect to TOG degradation needs to be further extracted.

Several studies have shown that crude oil has an inhibiting effect on plant and root growth (Wiltse et al. 1998; Brandt et al. 2006). Brandt et al. (2006) observed

Table 5 Selected properties of soil at 360 days

Parameter	TA	TB	TC	TD	TE
pH	6±0.08	5.1±0.05	5.6±0.04	5±0.08	5.4±0.06
Moisture (%)	20.5±1.2	7±0.06	15.5±1.3	6.8±0.08	25.6±2.5
TOC [%]	7.5±0.5	11.1±0.7	8.2±0.08	11.2±0.08	3.4±0.04
Total N [%]	0.25±0.05	0.18±0.04	0.2±0.08	0.12±0.01	0.28±0.05
C/N ratio	110:1	590:1	120:1	440:1	2:1
THB ($\times 10^6$ CFU/g)	35.2±1.3	9.6±0.5	32.4±1.3	9.2±0.5	38.5±1.6

Values represent mean±standard deviation of three replicates

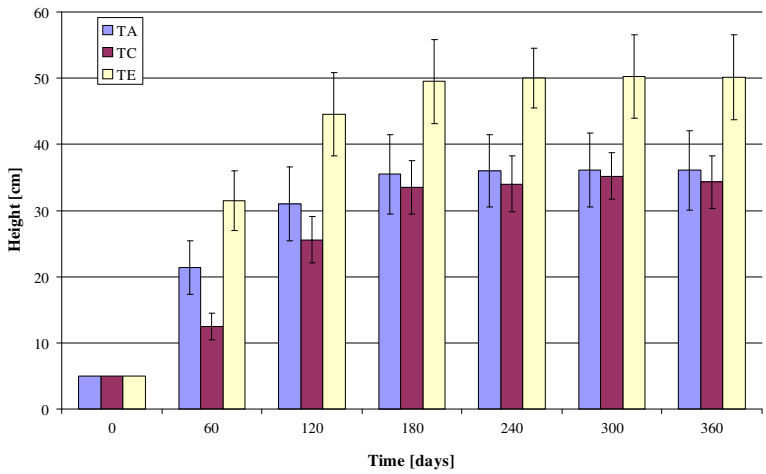

Fig. 1 Plant height (cm) of *C. brevifolius*. Values are means±SD of three replicates. *TA* contaminated soil+fertilizer+plants, *TC* contaminated soil+without fertilizer+plants, *TE* uncontaminated soil+fertilizer+plants

50% decrease in total biomass and 40% decrease in plant height in 5% crude oil-contaminated soil compared to uncontaminated soil in 6 month old *Vetiveria zizanioides* (L.) Nash. In the study reported here, the same phenomenon was found for *C. brevifolius*. The average yields were significantly lower in contaminated soil than in uncontaminated soil ($p=0.05$). In the crude oil-contaminated soil, average reduction of plant height and biomass during 360 days was TA 27.9% and 46.3% and TC 31.5% and 56.5%, respectively compared to uncontaminated soil, regardless of the fertilizer in the contaminated soil compensating for the higher C/N ratio. High rates of plant mortality and reduction in height and biomass are typical reactions caused by oil contamination (Lin and Mendelssohn 1998). The root structure of *C. brevifolius* was reduced by the toxicity of crude oil. This plant is herbaceous with extensive fibrous root system, can multiply both vegetatively as well as sexually. Despite these characteristics, growth of plant was inhibited by addition of crude oil during the initial stage of growth. After 60 days, the species showed adaptability to the toxic environment, as shown by the high rates of tillering and biomass production. However, there are reports on large differences of PHC tolerance among plants growing in contaminated soil. For instance, Radwan et al. (1998) reported that Fava bean (*Vicia fava* L.) could tolerate up to 10% (*w*/*w*); Brandt et al. (2006) observed 5% (*w*/*w*) crude oil tolerance of vetiver grass (*V. zizanioides* (L.) Nash). In the present study, *C. brevifolius* could tolerate 8% TOG concentration.

The concentration of oil dropped sharply in the vegetated soil than in unvegetated soil during the course of experiment. Despite inhibition of plant

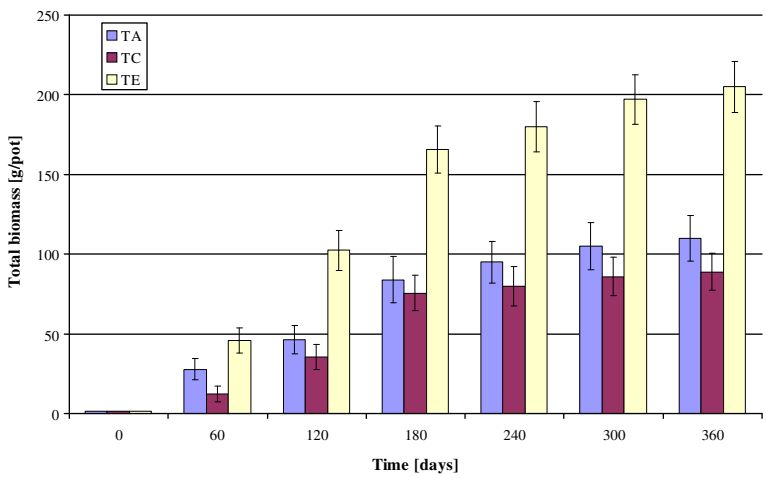

Fig. 2 Total biomass (g/pot) of *C. brevifolius*. Values are means±SD of three replicates. *TA* contaminated soil+fertilizer+plants, *TC* contaminated soil+without fertilizer+plants, *TE* uncontaminated soil+fertilizer+plants

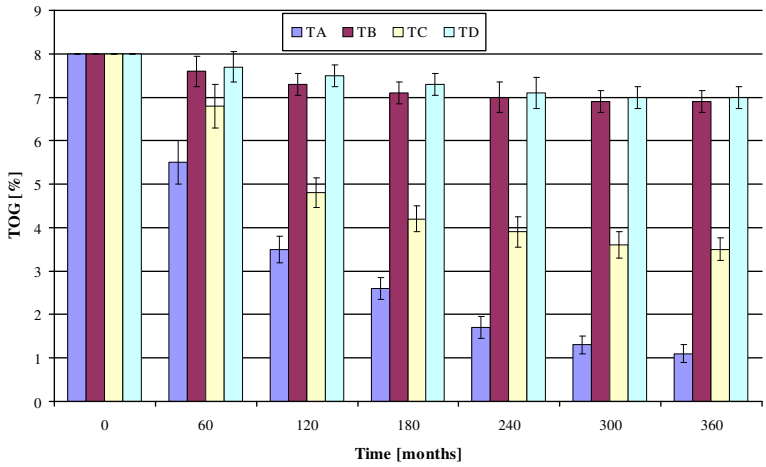

Fig. 3 TOG reduction in soil. Values are means±SD of three replicates. *TA* contaminated soil+fertilizer+plants, *TB* contaminated soil+fertilizer+no plants, *TC* contaminated soil+without fertilizer+plants, *TD* contaminated soil+without fertilizer+no plants

growth and root development, the decrease of crude oil in vegetated soil was significantly more than in unvegetated soil. Merkl et al. (2005a) and Merkl et al. (2005b) showed enhanced degradation of crude oil under the influence of a tropical grass after only a few months, Muratova et al. (2008) showed total petroleum hydrocarbon (TPH) reduction up to 52% during 3 years of rye cultivation. Diab (2008) recorded 30%, 16.8%, and 13.8% reduction of TPH in rhizosphere soil of broad bean, corn and wheat respectively. In addition, Peng et al. (2009) noted 41.61–63.2% removal of TPH by *Mirabilis jalapa*. In the present study, *C. brevifolius* could decrease significant amount of TOG (TA 86.2%; TC 61.2%) in comparison to uncontaminated soil (TB 13.7%; TD 12.5%) during 360 days (Fig. 3). In the same experiment system conducted, *Axonupus compressus* (SW.) P. Beauv. decreased 70% of TPH in the fertilized soil compared to 40% in unfertilized one in 60,000 mg kg^{-1} crude oil-contaminated soil during the study of 360 days (Bordoloi et al. 2012). Furthermore, the degradation of TPH by *Cyperus odoratus* (78%) and *Cyperus laevigatus* (73%) in the fertilized soil was significantly more than that in unfertilized one (*C. odoratus* 45%; *C. laevigatus* 43%) during the study of 360 days in 80,000 mg kg^{-1} crude oil-contaminated soil (Basumatary et al. 2012). In the present study, the degradation might be due capability of plant to tolerate and degrade crude oil. Lopez-Martinez et al. (2008) also found significant reduction of TPH by *Cyperus laxus* Lam. in 24 months when plants were cultivated on hydrocarbon-contaminated soil and spiked perlite. The slower and less degradation of TOG in unvegetated soil can be attributed to its lack of plants. Volatilization of lighter fractions of crude oil might have contributed to the rapid degradation. In addition, microbial degradation might have also contributed to reduction in crude oil in soil. Both these action constitute part of the natural attenuation phenomenon (Margesin and Schinner 2001; Pichtel and Liskanen 2001; Bento et al. 2005; Chaîneau et al. 2005; Sarkar et al. 2005; Scow and Hicks 2005; Atagana 2010). The slow rate of removal after day 60 might be due to removal of volatile components and the remaining components of the crude oil required intervention to be removed from the soil. A comparison of TOG removal in the vegetated treatments (TA 86.2% and TC 61.2%) and unvegetated treatments (TB 13.7% and TD 12.5%) clearly showed that phytoremediation by *C. brevifolius* was responsible for removal of more than 72.5% (TA) and 48.7% (TC) of the oil (Fig. 3). However, the removal of TOG in vegetated treatments was significantly higher than the unvegetated treatments during the study of 360 days. Further decrease of TOG was not observed after 360 days of plant growth. In the present study, removal of TOG in vegetated treatments is believed to be due to the interaction of rhizosphere microorganisms and plants which is similar to the findings of Liste and Prutz (2006), Ho et al. (2007), and Muratova et al. (2008). As roots grow, they penetrate through the soil, exposing entrapped contaminants that have been previously inaccessible, increasing their availability to degradation (Fava et al. 2004; Bogan and Sullivan 2003).

Fertilizers increase plant growth in oil-polluted soils in the case of nutrient deficiency (Lin and Mendelssohn 1998; Hutchinson et al. 2001). However, over fertilizing usually leads to yield depressions (Brandt et al. 2006). In our study, there was significant difference of plant growth between the fertilized and unfertilized soil during the first 60 days but the differences diminished towards the end of the experiment. Concerning oil degradation, there was significant influence of fertilizer application during the experiment. However, the fertilized soil could show significant amount of TOG degradation in comparison to unfertilized soil. For the soil used, the unfertilized soil seemed to be insufficient to meet the nutrient demands of plants. The fertilized soil could compensate the nutrient demands of plant for phytoremediation. Hutchinson et al. (2001) also observed better degradation of TPH using grasses with N/P amendments than without inorganic amendments. In addition, Merkl et al. (2005a) found enhanced degradation of crude oil by using *B. brizantha* in NPK-fertilized soil in comparison to control.

Rhizosphere microbial populations may enhance a plant's adaptation to petroleum hydrocarbons by detoxifying contaminated soils through direct mineralization of these organic contaminants (Siciliano and Germida 1998; Jeffries et al. 2003; Barea et al. 2005). In the present study, the bacterial population was found to be 6 times in vegetated pots during the study of 360 days. Sabaté et al. (2004) also found similar levels of soil bacteria in hydrocarbon-contaminated soil. Ghazali et al. (2004) reported a tenfold increase in soil microorganisms. In the unvegetated pots, no significant increase of bacterial population was observed in this study. The increase in bacterial population in the vegetated pots might have positive impact on degradation of TOG. The increased microbial numbers are primarily due to the presence of plant exudates and sloughed tissue, which serve as sources of energy, carbon, nitrogen, or growth factors (Lee and Banks 1993; Banks et al. 2003). Huang et al. (2004) reported on the enhanced phytoremediation of organic contaminants using grass species inoculated with a mixture of beneficial bacteria such as *Pseudomonas putida*, *Azospirillum brasilense*, and *Enterobacter cloaceae*. Microorganisms 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.

5 Conclusions

This study has shown the suitability of *C. brevifolius* in phytoremediation of crude oil-contaminated soil. Though this plant showed reduced plant height, biomass and root growth in the contaminated soil, the results are significant in terms of degradation. The average degradation percentage of PHC by *C. brevifolius* (86.2%) is comparatively more than the degradation percentage of *A. compressus* (70%), *C. odoratus* (78%), and *C. laevigatus* (73%) during the study of 360 days. However, C. *brevifolius* showed superiority in the degradation of PHC in the crude oil-contaminated soil. This study suggests that cultivation of *C. brevifolius* on oil-polluted sites in Assam, India is considered to be useful. It is a green technology, also provides erosion control and, thus, prevents contaminants from surface spreading. Moreover, if planted on up to 80,000 mg kg^{-1} crude oil-contaminated soil, it could polish crude oil contaminants.

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