

A comparative assessment of the crude oil-remediating potential of *Cynodon dactylon* and *Eleusine indica*

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Abstract

The study was performed to compare the crude oil-remediating potential of *Cynodon dactylon* and *Eleusine indica* in a greenhouse. The experimental design was completely randomized. Treatments used were crude oil concentrations: 0 (control), 2.5, 5.0, 7.5, 10.0 and 12.5 mL in 3 kg of soil, denoted as T₀, T_{2.5}, T_{5.0}, T_{7.5}, T_{10.0} and T_{12.5}, respectively. Twelve weeks after planting, plants were harvested, weighed fresh and oven-dried at 80 °C to constant mass. Total hydrocarbon content in plant and soil samples, and soil physicochemical parameters were determined. Student's *t*-test was used for comparison between grasses while ANOVA and Duncan Multiple Range Test were used to test significant differences between treatment mean values at 5% level of significance. Fresh and dry mass were significantly higher in *E. indica* than *C. dactylon*. Total hydrocarbon content in soil and plant were significantly lower in soil under the *E. indica* than *C. dactylon*. Soil pH and exchangeable acidity did not significantly differ in soil under the grasses, except in treatment T_{2.5}. Soil organic carbon was higher in the soil where *C. dactylon* was planted (36.86 to 46.45 g kg⁻¹) than in soil under *E. indica*. N, P and cations did not significantly differ in soil under the grasses. It was concluded that *E. indica* has a higher crude oil pollutant remediating potential on soil than *C. dactylon*.

Key words: *Cynodon dactylon*, *Eleusine indica*, hydrocarbon, oil contamination, phytoremediation, soil properties.

Abbreviations: CEC, cation exchange capacity; PAHs, polycyclic aromatic hydrocarbons; SOC, soil organic carbon; THC, total hydrocarbon content; WAP, weeks after planting.

Introduction

Ever since the discovery of oil in Nigeria in the 1950s, the country has been suffering the negative environmental consequences of oil exploration and exploitation. The growth of the country's oil industry, combined with a population explosion and a lack of enforcement of environmental regulations has led to significant contamination of the environment (Nwilo, Badejo 2005). Healthy survival of human beings depends on the quality of the physical environment (Riaz et al. 2002).

In developed countries where pollution monitoring and legislation have been established, engineering techniques based on physical, chemical and thermal methods have been traditionally used for clean-up of oil-contaminated soils and waters (Frick et al. 1999). However in Nigeria where environmental degradation or contamination from oil exploration is left at the mercy of the inhabitants, phytoremediation proves a cost-effective and non-intrusive means of remediation of soils.

Phytoremediation uses vegetation and associated microbiota, soil amendments and agronomic techniques to remove, contain, or render soil contaminants harmless (Helmisaari et al. 2007). Plants are used successfully in the

remediation of a wide range of contaminated soils, mainly due to their two-way functioning: creating favourable conditions for microbial degradation and uptake of the contaminants by plant roots (Kathi, Khan 2011). Various plants have been identified that have potential to facilitate remediation of sites contaminated with petroleum hydrocarbons (Frick et al. 1999). In the majority of studies, grasses and legumes have been singled out for their potential in this regard (e.g. Aprill, Sims 1990; Merkl et al. 2005; Parrish et al. 2005; Gunther et al. 1996; Reilley et al. 1996; Smith et al. 2006; White et al. 2006; Abedi-Koupai et al. 2007; Qiu et al. 1997). Grasses are considered to be superior for phytoremediation because they have extensive, fibrous root systems and inherent genetic diversity, which may give them a competitive advantage in becoming established in oil-contaminated soils (Frick et al. 1999).

Aprill and Sims (1990) established a mix of eight prairie grasses (*Andropogon gerardii*, *Schizachyrium scoparium*, *Sorghastrum nutans*, *Panicum virgatum*, *Elymus canadensis*, *Bouteloua curtipendula*, *Bouteloua gracilis* and *Pascopyrum smithii*) in sandy loam soils to determine whether the degradation of four polycyclic aromatic hydrocarbons (PAHs) was stimulated by plant growth. Qiu et al. (1997) found that prairie buffalograss accelerated the reduction

of naphthalene in clay soil compared to unplanted clay soil and also conducted a parallel experiment to assess the ability of 12 warm season grass species to remove various PAHs from contaminated soil. Gunther et al. (1996) found that soil planted with ryegrass lost the greater amount of a mixture of hydrocarbons than soil that was unplanted. Merkl et al. (2005) reported that legumes died within six to eight weeks in heavily crude-oil contaminated soil, while grasses showed reduced biomass production. Furthermore, a positive correlation between root biomass production and oil degradation was found. Parrish et al. (2005) observed that root maturity in ryegrass, tall fescue and yellow sweet clover contribute to the reduction in the bioavailability of PAHs. Smith et al. (2006) found that fescue, ryegrass, birdsfoot-trefoil and clover significantly reduced naphthalene and other PAH concentrations in the rhizosphere. White et al. (2006) documented the biodegradation of recalcitrant alkylated polycyclic aromatic hydrocarbons in crude oil-contaminated soil; increased population size of total bacterial, fungal, and PAH degrader was associated with the increased rhizosphere soil volume of *Lolium arundinaceum*, *Lolium multiflorum* and *Cynodon dactylon*. Abedi-Koupai et al. (2007) in their study on rhizosphere effects in *Agropyron* sp., *Lolium* sp., *Festuca arundinacea* and *Piccinellia distans*, observed that only tall fescue (*F. arundinacea*) survived harsh conditions with crude oil-contaminated concentrations.

Cynodon dactylon and *Eleusine indica* are widely distributed warm-season grasses in Nigeria, which persist even in highly polluted soils (Anoliefo et al. 2006). There are a few studies on the crude oil remediating potential of *C. dactylon* (White et al. 2006; Onwuka et al. 2012) and *E. indica* (Merkl et al. 2005), but no attempt has been performed to compare their potential remediation effect. This study was carried out to compare the crude oil remediating abilities of *C. dactylon* and *E. indica* in a screenhouse trial.

Materials and methods

A screenhouse study was conducted in Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Nigeria, to determine and compare a potential of *Cynodon dactylon* L. and *Eleusine indica* L. (Gaertn) in the decontamination of crude oil-contaminated soils. Top soil used for the experiment was collected from the University Biological Garden (N 07° 51' 958", E 04° 52' 630"). The garden is relatively undisturbed and occupies about 0.6 km² within Obafemi Awolowo University estate and supporting lush plantation of tropical trees including *Azadirachta indica*, *Hildegardia barteri*, *Draceana mannii*, *Pycanthus angolensis*, *Sterculia tragacantha*, *Terminalia catappa* and *Voacanga africana*. The soil of Ile-Ife area has been mapped and grouped as Iwo Association (Symth, Montgomery 1962). The soils are generally deep and well-drained, sandy loam and rich in humus. The collected soil was air-dried for 7

days and sieved using a 2 mm mesh to remove the debris. A sample of 3 kg each of the sieved soil were weighed into pots (0.30 m by 0.12 m, diameter by depth) and perforated at the bottom to ease soil aeration. Five crude oil treatments made of 2.5, 5.0, 7.5, 10.0 and 12.5 mL in 3 kg of soil were set up for each grass species and denoted as T_{2.5}, T_{5.0}, T_{7.5}, T_{10.0} and T_{12.5} respectively. The control soils without crude oil were set up for each plant and denoted as T0. All treatments were arranged in a completely randomized design and replicated three times. The crude oil used for the experiment was obtained from the Nigeria National Petroleum Corporation (NNPC), Eleme, Rivers State, Nigeria.

Five 2 cm rhizomes of *C. dactylon* and *E. indica* were planted in each pot one week after contamination with crude oil. Twelve weeks after planting, the plants were harvested, washed thoroughly with distilled water, weighed fresh and after oven-drying at 80 °C to constant mass.

Total hydrocarbon concentration (THC) in plant and soil samples was determined by colorimetric method using a Spectronic 21D (Milton Roy) spectrophotometer at 650 nm.

Soil chemical parameters [pH, total organic carbon, nitrogen and phosphorus concentration, exchangeable acidity, as well as cations such as potassium, sodium, calcium, magnesium and cation exchange capacity (CEC)] were determined. Soil pH was measured with a glass electrode pH meter in 1:1 water suspension. Soil particle size analysis was conducted by hydrometer method in 5% hexametaphosphate as outlined by Bouyoucos (1951). Exchangeable cations (Ca, Mg, K and Na) were determined using 1 M NH₄OAc buffered at pH 7.0 as extractant (Thomas 1982). Na concentration in the soil extract was read on a Gallenkamp flame photometer; Ca, Mg and K were determined using an atomic absorption spectrophotometer (Bulk Scientific – 210/211 VGP). Exchangeable acidity in soil samples was determined in 1 M KCl extract (Thomas 1982). Solution of the extract was titrated with 0.05 M NaOH to a permanent pink endpoint using phenolphthalein as an indicator. The amount of base (NaOH) used is equivalent to the total amount of exchangeable acidity in the aliquot taken (Odu et al. 1986). Total organic carbon concentration was determined using the wet digestion method (Walkley, Black 1934). The Kjeldahl method was used for the determination of total nitrogen (Bremner, Mulvaney 1982). Available P was determined by Bray P1 method (Olsen, Sommers 1982) using a Spectronic 20 spectrophotometer.

The Student's *t*-test was used for comparison between grasses, while ANOVA and Duncan Multiple Range Test were used to determine significant differences between treatments at 5% probability.

Results

Fresh mass range in *C. dactylon* was 41.0 to 51.0 g while that of *E. indica* was 76.2 to 179.0 g (Fig. 1A). Dry mass ranges

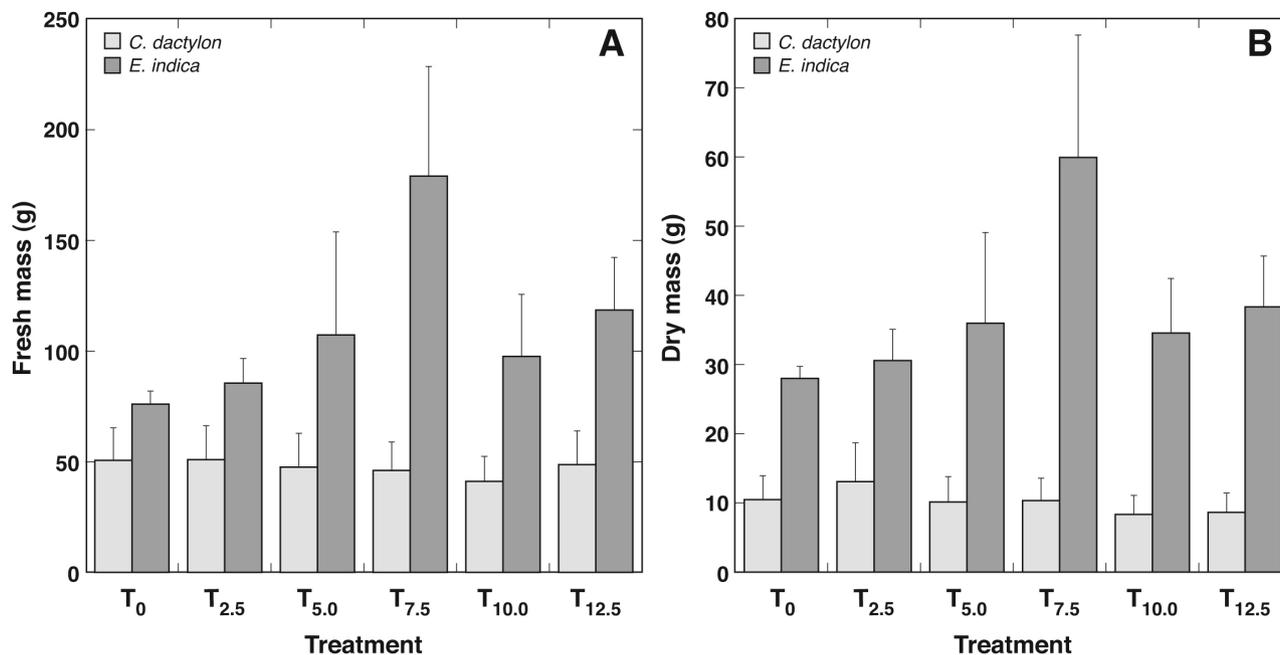


Fig. 1. Fresh (A) and dry (B) mass of *Cynodon dactylon* and *Eleusine indica* grown for 12 weeks at soils treated with different levels of crude oil. The data are means \pm SD.

were 8.3 to 10.4 g and 28.0 to 59.9 g in *C. dactylon* and *E. indica*, respectively (Fig. 1B). Fresh and dry mass of *E. indica* across the treatments were significantly higher than these of *C. dactylon* ($p < 0.05$). There were no significant differences in fresh and dry mass among the treatments with different levels of crude oil in *C. dactylon*. In contrast, for *E. indica* significant differences were evident in both fresh and dry mass. *E. indica* plants grown on crude oil-contaminated soil produced more biomass, and this effect was statistically significant for the 7.5 mL crude oil treatment.

The content of THC in plant tissues increased in parallel with soil crude oil concentration (Table 1). Unexpectedly, THC in *C. dactylon* in the T_{7.5} treatment was less than that of T_{5.0}. In general, *C. dactylon* accumulated higher concentration of THC in comparison to *E. indica*, with significant differences for the T_{2.5} and T_{5.0} treatments. There were no traces of crude oil (as indicated by zero level of THC) in soil in all treatments with *E. indica*. In contrast, in *C. dactylon* treatments T_{7.5}, T_{10.0}, and T_{12.5}, THC concentrations were 0.03, 0.10 and 0.04 mg kg⁻¹, respectively (Table 1).

Growth of plants in oil-contaminated soil resulted in changes of several soil parameters in comparison to control

soil (Table 2). pH in all crude oil-treated soils under *C. dactylon* did not significantly differ from the control (T₀). Excepting in the T_{2.5} treatment, all crude oil-treated soils under *E. indica* had significantly lower pH than the control. There was no significant difference in the pH of soils between planted species for all treatments, except T_{2.5}. Soil organic carbon (SOC) in soil under both *C. dactylon* and *E. indica* was significantly higher only in T_{2.5} than that in the control. SOC in all treatments with *C. dactylon* were significantly higher than in those with *E. indica*. Soil nitrogen was significantly higher than the control for both grass species only in T_{2.5}. Nitrogen concentration in the T_{5.0} soil of both grass species was similar (1.38 g kg⁻¹). Soil N was higher in *E. indica* soils than in *C. dactylon* but the difference was not significant. Soil phosphorus ranged from 23.30 to 25.99 mg kg⁻¹ and from 22.41 to 24.12 mg kg⁻¹ in soil under *C. dactylon* and *E. indica*, respectively. P concentration in the crude oil treatments did not significantly differ from those in the control for both plants. There was also no significant difference in P concentration in soil under *C. dactylon* and *E. indica* in all treatments (Table 2). Soil exchangeable acidity was significantly higher in the control than in oil-

Table 1. Total hydrocarbon concentration (mg kg⁻¹ dry mass) in tissues of *Cynodon dactylon* and *Eleusine indica*, grown on crude oil-contaminated soil, and in the respective soil samples. Means with the same letter(s) across the row are not significantly different ($p \geq 0.05$)

Sample	Plant species	T ₀	T _{2.5}	T _{5.0}	T _{7.5}	T _{10.0}	T _{12.5}
Plant tissues	<i>C. dactylon</i>	0.00c	12.79b	16.46b	11.49bc	16.34b	39.26a
	<i>E. indica</i>	0.00e	5.08de	8.73cd	12.81bc	16.31b	28.73a
Soil	<i>C. dactylon</i>	0.00a	0.00a	0.00a	0.03a	0.10a	0.04a
	<i>E. indica</i>	0.00	0.00	0.00	0.00	0.00	0.00

Table 2. Soil properties after harvest of *Cynodon dactylon* and *Eleusine indica* in soil treated with different levels of crude oil. Mean values with the same letter(s) across each row are not significantly different. SOC, soil organic carbon; EA, exchangeable acidity; CEC, cation exchange capacity; *, indicates a significantly higher/lower value of *C. dactylon* for the test parameter in comparison to *E. indica*

Soil parameter	Plant species	T0	T2.5	T5.0	T7.5	T10.0	T12.5
pH (units)	<i>C. dactylon</i>	5.78a	5.64a	5.68a	5.60a	5.54a	5.58a
	<i>E. indica</i>	5.93a	5.81ab*	5.72bc	5.63bcd	5.51d	5.59cd
SOC (g kg ⁻¹)	<i>C. dactylon</i>	40.07b*	46.45a*	39.46b*	39.91b*	36.86b*	39.08b*
	<i>E. indica</i>	23.94b	34.21a	19.23d	22.64bc	21.86c	22.73bc
N (g kg ⁻¹)	<i>C. dactylon</i>	1.52b	2.03a	1.38b	1.39b	1.34b	1.25b
	<i>E. indica</i>	1.57b	2.23a	1.38bc	1.41bc	1.37bc	1.31c
P (mg kg ⁻¹)	<i>C. dactylon</i>	23.73a	23.34a	23.30a	24.70a	23.64a	25.99a
	<i>E. indica</i>	22.98a	22.47a	22.41a	23.74a	22.86a	24.12a
EA	<i>C. dactylon</i>	0.88a	0.36b*	0.42b	0.38b	0.38b	0.33b
	<i>E. indica</i>	0.85a	0.30b	0.41b	0.37b	0.33b	0.36b
K (cmol kg ⁻¹)	<i>C. dactylon</i>	0.46ab	0.34b	0.40ab	0.30b	0.39b	0.51a
	<i>E. indica</i>	0.49ab	0.32d	0.41c	0.30d	0.42bc	0.53a
Na (cmol kg ⁻¹)	<i>C. dactylon</i>	0.78a	0.80a	0.68ab	0.59b	0.62b	0.68ab
	<i>E. indica</i>	0.77ab	0.80a	0.66bc	0.57c	0.61c	0.67bc
Ca (cmol kg ⁻¹)	<i>C. dactylon</i>	24.47ab	24.62ab	22.71ab	21.54b	25.20a	22.04ab
	<i>E. indica</i>	24.45ab	24.69ab	23.04bc	21.49c	25.38a	23.02bc
Mg (cmol kg ⁻¹)	<i>C. dactylon</i>	2.53a	2.56a	2.45a	2.30a	2.43a	1.30b
	<i>E. indica</i>	2.38bc	2.53ab	2.31bc	2.29c	2.56a	1.32d
CEC (cmol kg ⁻¹)	<i>C. dactylon</i>	28.24a	28.33a	26.24ab	24.73b	28.64a	24.52b
	<i>E. indica</i>	28.09ab	28.34ab	26.42bc	24.65c	28.97a	25.54c

treated soils for both plants. Acidity in T_{2.5} for soil planted with *C. dactylon* was significantly higher than in that with *E. indica*. Soil potassium (K⁺) concentration in control was not significantly different from that in crude oil treatments in both plants. Sodium (Na⁺) concentration in the control soils under both plants was significantly higher than in those in T_{7.5} and T_{10.0}. Calcium (Ca²⁺) concentration was highest in T_{10.0}, although did not significantly differ from the control treatment for both grass species. Magnesium ion concentration in all crude oil treatments with *C. dactylon* did not significantly differ from that in control, excepting for T_{12.5}. In soils under *E. indica*, Mg concentration was significantly higher in T_{10.0} was significantly higher than in the control, but significantly lower in T_{12.5}. Cation exchange capacity (CEC) was higher in the control soil for both plants than in T_{7.5} and T_{12.5}. Soil K⁺, Na⁺, Ca²⁺, and Mg²⁺ concentrations, and CEC in the treatments with *C. dactylon* did not significantly differ from those under *E. indica*.

Discussion

E. indica out-yielded *C. dactylon* in all treatments considered in this experiment. This result is similar to that of Strickland (1973) on yield of *C. dactylon* in comparison with *Digitaria decumbens* and *Digitaria didactyla*, but in contrast to the yields of *C. dactylon* obtained by Juraimi et al. (2005). The high degree of stomatal conductance of *E. indica* (Sharma 1984) and increased net photosynthesis

favoured the growth and emergence of tillers and net biomass (Kobayashi, Hori 2000). Limited radiation similar to that in the screenhouse may have negative effect on the yield of *C. dactylon*, in contrast to effect on *E. indica*, which can tolerate 70% irradiance (Singh 1967). Crude oil contamination at optimum concentration (7.5 mL) increased dry matter production of *E. indica*, but not of *C. dactylon* (non significant difference from the control). The lower dry mass in the T_{10.0} and T_{12.5} treatments could be due to a decrease in plant growth. Contamination and perturbations in the environment can have significant effect on shoot growth, causing plants to allocate much of its resources to roots. This ability to change carbon fluxes in the plant is an essential tool for phytoremediation, as it indicates that plants can respond to changes in the environment. This finding corroborates Merkl et al. (2005) report on the tolerance of *E. indica* to crude oil treatments.

The differences in THC concentration in tissues of both grass species among the treatments indicate uptake of oil from the soil. This was confirmed by the zero level of THC in tissues of *E. indica* and *C. dactylon* in the control treatment (T₀), in the *Eleusine*-planted soil in the crude oil treatment, and also the reduction in crude oil levels in the *Cynodon*-planted soils. This corroborates studies by Merkl et al. (2005) on *E. indica* and Onwuka et al. (2012) on *C. dactylon*, in which significant reduction in crude oil levels was reported. Plant species that are capable of growing in soils polluted with crude oil hydrocarbons participate

in their degradation through the rhizosphere. Their roots favour the growth of several microorganisms (Quinones-Aquilar et al. 2003) and increase biomass and microbial activity, accelerating degradation processes (Schwab, Banks 1994). High root biomass as in *E. indica* translates to a larger rhizosphere, which stimulates soil microorganisms and their biodegradative activity by increase of diffusion, mass flow and concentration of nutrients, thus facilitating the degradation of crude oil hydrocarbons (Merkl et al. 2005). This can explain the lower levels of THC in *E. indica* and *Eleusine*-planted soils in this experiment.

The absence of significant difference in pH of crude oil treatments compared to the control in the *Cynodon*-planted soil is similar to the effect described by Miller and Pesaran (1980), Bauder et al. (2005) and Kisić et al. (2010). The difference in pH in *Cynodon*- and *Eleusine*-planted soils in treatment T_{2.5} reflects the effect of crude oil levels on the structure of the root zone in the two grass species. The lower organic carbon concentration in the soils in all crude oil treatments, except T_{2.5}, compared to that in the control could be due to delayed senescence of older tissues (both root and shoot) in the soils containing large amount of crude oil and uptake of hydrocarbons by the plant. A similar result was reported by Osuji and Nwoye (2007). Crude oil contamination can lead to an alteration in plant development and a postponement of senescence (Merkl et al. 2005). An explanation for this is that the life cycle of species in crude oil-contaminated soil is delayed by petroleum hydrocarbons. Itai and Birnbaum (2002) remarked that perturbations in the environment can change the pattern and amount of produced endogenous plant growth regulators, thus affecting plant growth. The low carbon concentration in the higher level of crude oil contamination found in the present study contradicts the results of Benka-Coker and Ekundayo (1995), Ekundayo and Obuekwe (1997) and Shukry et al. (2013). According to them, the carbon concentration in crude oil contributes to the overall soil organic carbon content coupled with slow decomposition of organic matter by soil microbes due to poor aeration in crude oil-contaminated soil. The significantly lower SOC in the *Eleusine*-planted soil could be due to its higher carbon use efficiency, due to its higher growth rate and environmental tolerance (Singh 1967). The results on soil nitrogen concentration corroborate those of Shukry et al. (2013); the decrease in total nitrogen concentration with increase of crude oil levels may be due to temporal immobilization of this nutrient by microorganisms.

The reduction in soil N concentration in the crude oil treatments, excluding T_{2.5}, is in conformity with that of Osuji and Nwoye (2007). The reduction in the concentration of NO₃-N in the contaminated site suggests that the nitrification rate might have reduced following oil spillage. According to Odu et al. (1985), after oil spillage, hydrocarbon-utilizing microorganisms, such as *Azobacter*

spp., normally become more abundant while nitrifying bacteria, such as *Nitrosomonas* spp., become reduced in number. Ekpo et al. (2012) confirmed that crude oil pollutants reduce soil P. Soil P concentration in treatments T_{7.5} and T_{12.5} were inconsistent with this report.

Crude oil contamination is known to improve soil content with some nutrient elements, including K, Na, Ca, Al (Ekpo et al. 2012), Mg and cation exchange capacity (Agbogidi et al. 2007). However, the concentration of soil cations in this experiment were inconsistent with the reported trend. This could be due to differences in the rate of utilization of the nutrients caused by differential growth rates of the plants.

In conclusion, *C. dactylon* and *E. indica* showed the ability to remediate the crude oil levels used in the study, confirmed by the insignificant concentration of oil remains in the soil after harvest. *E. indica* had higher yield and lower levels of crude oil in the tissues and soil indicating its better remediating potential over *C. dactylon*. Since *C. dactylon* and *E. indica* are widely distributed and have proved successful in phytoremediation of crude oil contaminated soils, they can be beneficial for many other tropical countries facing the problem of crude oil spillage.

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