STUDIA UNIVERSITATIS BABEŞ-BOLYAI BIOLOGIA, LXII, 1, 2017 (p. 43-55)
doi:10.24193/subbbiol.2017.1.03

Assessment of the phytoreclamation of an oil-contaminated soil cultivated with *Cynodon dactylon*, *Eleusine indica* and *Eragrostis tenela*

Beckley Ikhajiagbe [1,✉], Boniface O. Edegbai[1], Gloria O. Omoregie[2], and Amenze M. Eweka[1]

SUMMARY. The interactions between *Cynodon dactylon, Eragrostis tenela* and *Eleusine indica* in the phytoremediation of petroleum hydrocarbon were investigated. Top soil was collected from a marked plot and polluted with spent engine oil (SEO) to obtain a constant 5% w/w concentration. Thereafter, the soils were sown with *Cynodon dactylon, Eragrostis tenela* and *Eleusine indica* singly and in combination of two's and three's in separate bowls. The set up was left for three (3) months in a screen house. The results revealed that there were reductions in soil concentrations of total petroleum hydrocarbons, from 26523.76 mg/kg to 19959 mg/kg in the oil-polluted soil. *Bacillus subtilis, Micrococcus* sp.*, Proteus vulgaris* were the prevalent bacteria species found in the soils, while prevalence fungi species included *Aspergillus niger, Geotricium* sp.*, Penicillium* sp., *Rhizopus* sp., *Aspergillus flavus,* and *Fusarium solani*. Morphological parameters of the three grasses were better enhanced when sown singly than when they were in combinations of two's and three's. Remediation was however best when they were sown altogether as one.

Keywords: *Cynodon, Eragrostis*, *Eleusine,* hydrocarbon, rhizoremediation.

Introduction

One of the vulnerabilities associated with oil exploration results mainly from oil spills. In some case, these spills may be accidental; other times they may just result from indiscriminate disposal of both new and used petroleum products. Over the years, snowballing levels of oil spills on both land and aquatic environments continue to pose momentous threat to the environment. This, in fact, has always

[1] *Environmental Biotechnology and Sustainability Research Group, Dept of Plant Biology and Biotech., Univ. of Benin, Benin City, Nigeria.*
[2] *Department of Environmental Management and Toxicology, Fed. Univ. of Petroleum Resources, Effurun, Nigeria*
✉ ***Corresponding author: Beckley Ikhajiagbe**, Environmental Biotechnology and Sustainability Research Group, Dept of Plant Biology and Biotech., Univ. of Benin, Benin City, Nigeria., E-mail: beckley.ikhajiagbe@uniben.edu*

been one of the greatest environmental and health concerns in contemporary oil and Gas Industry. Oil spills can cause enormous damage to the soil, plants and animals as well as cause serious human hazards and destruction of economic and social activities. This implies that if left unchecked, oil spills may spell doom for human populations. The critical goal for cleaning up any contaminated site is to eliminate any current or potential threat to human health and the environment from the chemicals that have been released into the soil, air or water. One of such measures is the reliance on plant populations, or phytoremediation.

Obviously, certain plants are better at removing contaminants than others. Plants that are utilized for contaminated land reclamation must be able to tolerate the types and concentrations of contaminants present. They also must be able to grow and survive in the local climate. Most studies on the phytoremediation of petroleum hydrocarbon (PHC) contaminated soil reported the use of grasses and legumes (Qui *et al*., 1997; Merkl *et al*., 2005; Schwab *et al*., 2006; Kecha varzi *et al*., 2007).

Grass species have far-reaching fibrous root structures, which possess pointedly greater root surface area compared to other species, and have been reported to penetrate the soils to as deepm as 3 meters (Qui *et al*., 1997). This characteristic confers on these grasses some level of resilience and persistence in unfavourable soil conditions. Several other studies have been conducted on the phytoremediative abilities of grasses, including the work of Gunther *et al.* (1996) on rye grass (*Lolium perenne L.*), Reilley *et al*., (1996) on alfafa (*Medicago sativa L.*), tall fescue (*Festuca arundinacea schreb.*), Sudan grass (*Sorghum vulgare L.*) and Switch grass (*Panicum virgatum*).

The degradation of polycyclic aromatic hydrocarbons in contaminated soils by a mix of *Andropogon gerardii, Schizachyrium scoparium, Sorghastrum nutans, Panicum virgatum, Elymus canadensis, Bouteloua curtipendula, Bouteloua gracilis* and *Pascopyrum smithii* was reported by Aprill and Sims (1990). White *et al.* (2006) acknowledged the biodegradation of intractable alkylated PAH compounds in oil-contaminated soil grassed with *Lolium arundinaceum, Lolium multiflorum* and *Cynodon dactylon*. Similarly, Abedi-Koupai *et al.* (2007) have reported the capacity of selected grass species to subsist under harsh environmental conditions occasioned by crude oil pollution.

The study plant species - *Cynodon dactylon, Eleusine indica* and *Eragrostis tenela,* are widely dispersed warm-season grasses in Nigeria, with persistence in oil- and metal-contaminated soils (Anoliefo *et al.,* 2006). In separate studies by Anoliefo *et al.* (2006, 2008); Ikhajiagbe and Anoliefo (2012), these species were persistent within and around workshops owned by automechanics, welders, vulcanizers, generator repairers, and other artisans over a large area spanning more than 10 Local Government Area Councils in MidWestern Nigeria put together. These workshops were usually notable for their deposition of heavy metals and hydrocarbons to soils and the surroundings (Anoliefo *et al*., 2006, 2008; Daniel *et al.*, 2016; Ikhajiagbe and Anoliefo, 2012). This study therefore evaluates the ability of grasses (*Eleusine indica, Eragrostis tenela, Cynodon dactylon*) in the phytoremediation of oil-polluted soil.

Materials and methods

The grasses used for the study were *Eleusine indica, Eragrostis tenela* and *Cynodon dactylon.* These were collected from an open field beside the Faculty of Agriculture, University of Benin, Nigeria, and their identities confirmed at the Department of Plant Biology and Biotechnology Herbarium. The grasses were thereafter regrown in a nursery from tillers.

Garden top soil was collected, air-dried to constant weight and measured into open boxes before amendment with the oil contaminant. The soil was thoroughly mixed with waste engine oil obtained as pooled to get constant 5% w/w oil in soil concentration.

There were 3 sets of experimental boxes (S-box, D-box, and T-box) arranged according to the number of grass species that would be sown thereon. The S-box was designed to cater for only single grass applications. The dimension was 60 cm (L) x 30 cm (B) x 15 cm (H) and would contain 20kg soil. The D-box had a dimension double that of the S-box since it would contain 2 grass species sown together; whereas the T-box was thrice the dimension of S-box. The reason for this disparity dimension of holding boxes was to ensure that plants were exposed to equal amounts of oil in soil. Pollution was done gradually with little soil samples being polluted at a time until the whole sample was thoroughly mixed. After contamination, the soil was left for about two weeks to attenuate.

Eleusine indica, Cynodon dactylon and *Eragrostis tenela,* initially raised from a pre-designated nursery, were sown singly and in combinations of two's and three's in the S-, D- and T-boxes respectively in three replicates each. Three months after plant exposure to oil-polluted soils, total petroleum hydrocarbon contents of soil were determined using GC-2010 (Shimadzu) Gas Chromatograph (GC) equipped with a split/splitless injector and a flame ionization detector (FID) from Agilent Technologies Inc., and according to the method of Dean and Xiong (2000). Remediation efficiency herein defined as percentage reduction in total assayed aliphatic hydrocarbons over the experimental period of three months was determined according to Ikhajiagbe *et al.* (2013). Culturable fungal and bacterial composition of rhizospheric soils of the treatment plants collected and assayed as pooled composite sample respectively was determined according to the procedure proposed by Cheesebrough (2001).

The grasses were also carefully observed for morphological changes including increase in height, stem width, internode length, peduncle length, flag leaf blade length, and flag leaf blade width. Tolerance index was determined at 5 weeks, where plant height was used as the determining parameter. Tolerance index was computed according to Iyagba and Offor (2013);

$$\text{Tolerance index} = \frac{\text{Parameter in contaminant}}{\text{Parameter in control}} \times \frac{100}{1}$$

Results were therefore presented as mean of 5 replications. Least significant differences were used to separate the means at 95% confidence limit.

Results and discussion

Soil contamination ensuing from petroleum exploration activities as well as from indiscriminate disposal of petroleum wastes has become a major source of concern as continuous oil pollution not only threatens food security, but also has negative impact on the health and wellbeing of the environment. In other to address the situation, plants are used in the restoration of contaminated lands. This is even predicated on the fact that the use of plants offers the advantage of being inexpensive, environmentally friendly and not destructive for soil matrix. This study investigated the interactions between *Eleusine indica, Eragrostis tenela,*and *Cynodon dactylon* in the phytoremediation of petroleum hydrocarbon and their plant microbial interactions. *Eleusine indica* and *Eragrostis tenela* has been shown to grow in oil contaminated sites and *Eleusine indica* been able to survive in crude oil and Pb contaminated sites (Anoliefo *et al*., 2006).

Three months after oil-polluted soils were sown with the test grass species singly and in double and triple combinations, there was a 45.65% reduction in TAH in the soil on which *E.* indica was sown, compared to 18.08% reduction in the control (Table 1a,b). TAH remediation efficiency was higher in the soils on which all three plants were sown > single or double > no plant.

Remediation of the compounds was better in the soil with grasses, than soil without. Grasses are generally required for phytoremediation activities particularly because they offer enhanced root zone parameters compared to other plants. Their numerously branched root systems offer them added advantage particularly with enhanced microbial activity and processes (Aprill and Sims, 1990). Accordingly, *Panicum maximum* and *Brachiara brizantha* have been reported to degrade oil and grease in contaminated soils (Merkl *et al*., 2004). Grass species have been previously reported in the remediation of oil-contaminated soils (Shirdam *et al*., 2008; Bordoloi *et al*., 2012; Ikhajiagbe and Anoliefo, 2012). Shirdam *et al.* (2008) studied the outcome of hydrocarbon contamination on selected growth features *Sorghum bicolor* and *Linum usitatissumum*, and reported that the species exhibited significant remediation efficiency even in vastly polluted soil. *Axonopus compressus, C. dactylon,* as well as *E. indica* have also been reported to colonize petroleum hydrocarbon-contaminated soils, with consequent remediation (Bordoloi *et al*., 2012; Ikhajiagbe and Anoliefo, 2012).

It is noteworthy that the restoration of degraded soils by mixed plant communities requires an understanding of the mechanisms responsible for community structure and dynamics (Holmes and Richardson, 1999). Although McCutcheon and Schnoor (2003) reported that *C. dactylon* showed improved performance in remediation of TPH and PAHs in soil where mixed with other grasses, the fact remains that growing more than one plant species at a spot would sometimes lead

to inter or intraspecific competition for nutrients and space. Competition between native plants and invasive species often restricts the success of restoration efforts. In a number of reports, competition among native species have negatively affected the success of restoration projects (Dyer and Rice, 1999; Brooks, 2000; Brown and Rice, 2000; Carlsen *et al.,* 2000; Green and Galatowitsch, 2002). However, in the present study, results showed that remediation of the compounds was better in the soil with *Cynodon dactylon, Eleusine indica* and *Eragrostis tenela* (CEE) altogether. Therefore, the effects of close proximity of planted grasses showed significant remediation. Anoliefo *et al.* (2006) reported earlier that *Cynodon dactylon* and *Eleusine indica* were widely distributed warm-season grasses in many tropical countries like Nigeria; these grasses persevere even in highly polluted soils. There are a number of other reports on the oil-remediating prospects of *C. dactylon* (White *et al*., 2006; Onwuka *et al*., 2012) and *E. indica* (Merkl *et al.,* 2005).

The removal TPH in contaminated soils is assumed to be rhizodegradation, the stimulation of rhizobacteria in the rhizosphere zone to degrade and enhance removal of TPH (Cai *et al*., 2010). An affirmative correlation exists between root biomass production and plants' capability for oil degradation. This is supported by earlier reports of Anoliefo and Ikhajiagbe (2011); Ikhajiagbe and Anoliefo (2011); Ikhajiagbe *et al.* (2012); Ikhajiagbe (2016).

Table 2 provides information on culturable microbial composition of the rhizospheric soils of the test plants after 3 months. Bacteria species which were present in the rhizosphere soil included *Bacillus subtilis, Micrococcus* sp. and *Proteus vulgaris,* whereas fungi species such as *Aspergillus niger, Fusarium solani, Geotricum* sp.*, Penicillium* sp.*, Rhizopus* sp.*,* and *Aspergillus flavus* were also present. Bacteria count after 3 months ranged from 4.2 to 8.0×10^5 cfu/g, compared to 2.4 - 6.9×10^5 cfu/g for fungi. For unpolluted and oil-polluted soils with no plant grown on them, *Bacillus subtilis, Micrococcus* sp. and *Proteus vulgaris* were present. However, *B. subtilis* appeared to be common to all treatments other than *Eragrostis tenela.* The fungi *Aspergillus niger* and *Penicillium* sp. were the most prevalent fungi species in the study after 3 months. Although both *Bacillus subtilis* and *Proteus vulgaris* was reported in the rhizosphere of *Cynodon dactylon*, *Eleusine indica and Eragrostis tenela* (EE), however, in trying to interact rhizospherically, *Bacillus subtilis* may have shown antagonism. However, *Bacillus subtilis* is a hydrocarbon degrading bacterium. The reason why remediation was not successful may be that *Bacillus subtilis* could have been antagonistic to *Micrococcus* sp. and *Proteus vulgaris*. This was similarly suggested According to Radha *et al.* (2010). They reported that *Bacillus subtilis* is antagonistic to *Proteus vulgaris, Candida albican, Staph. aureus, Pseudomonas aureginosa, E.coli* and *Aspergillus niger*. It is therefore suggested that the reason why remediation has been slow may have been the antagonism effect.

Morphological parameters of *Eleusine indica* after 9 weeks of exposure to oil contamination alone or sown with the other two test plants have been presented on Table 3. Before transplanting, the height of *Eleusine indica* was 41.20 cm. Nine

weeks after been sown alone in polluted soil, the height was 44.60 cm, compared to 42.10 cm when sown together with *Eragrostis tenela.* However, it was noticed that *Eleusine indica* grew best when planted alone.

Table 1a.

Total petroleum hydrocarbon contents of soil at 3 months after application of treatments

	1 day after pollution	No plant	ET	CD	LSD (0.05)
Nonane	3143.78	2577.90	1949.14	243.79	112.11
Decane	4326.36	3547.62	2682.34	2466.03	212.31
Dodecane	5526.11	4531.41	3426.19	3149.88	423.41
Tetradecane	3276.43	2686.67	2031.39	1867.57	302.31
Hexadecane	39.67	12.69	4.25	33.75	8.54
Octadecane	2543.55	2085.71	1577.00	1119.16	311.97
Nonadecane	835.84	685.39	518.22	702.11	285.65
Eicosane	2041.56	1674.08	449.14	489.97	113.42
Docasane	1053.21	863.63	126.39	326.50	323.23
Tetracosane	783.49	642.46	250.72	102.56	102.43
Hexacosane	237.83	195.02	<0.001	42.84	25.33
Tricosane	303.61	248.96	89.53	59.12	32.04
TAH	24111.44	19751.55	13104.31	10603.27	ND
TVAH	2412.32	207.45	12.98	9.97	ND
TPH	26523.76	19959	13117.29	10613.24	ND
TAHef (%)	---------	18.08	45.65	56.02	ND

TAH total aliphatic hydrocarbons; TVAH total volatile aromatic hydrocarbons; TPH total petroleum hydrocarbons; TAHef TAH remediation Efficiency; ET *Eragrostis tenela,* EI *Eleusine indica,* CD *Cynodon dactylon;* LSD (0.05) least significant difference at 95% confidence limit; ND not determined

Table 1b.

Total petroleum hydrocarbon contents of soil at 3 months after application of treatments

	EI	CD+ET	EI+ET	EI+CD	EI+CD+ET	LSD (0.05)
Nonane	2137.77	1226.07	185.42	1065.54	69.16	112.11
Decane	2941.92	1687.28	3724.11	1006.45	1384.44	212.31
Dodecane	3757.75	2155.18	3021.83	984.86	1768.36	423.41
Tetradecane	2227.97	584.34	542.72	709.43	1048.46	302.31
Hexadecane	8.21	21.48	17.80	<0.001	1.74	8.54
Octadecane	1220.90	1067.43	743.32	869.64	142.68	311.97
Nonadecane	854.37	483.79	789.53	515.98	521.91	285.65

	EI	CD+ET	EI+ET	EI+CD	EI+CD+ET	LSD (0.05)
Eicosane	799.64	812.05	1183.83	905.43	812.45	113.42
Docasane	74.87	622.71	583.95	428.12	51.74	323.23
Tetracosane	94.12	201.38	41.67	98.04	43.43	102.43
Hexacosane	101.65	89.66	38.22	48.54	<0.001	25.33
Tricosane	21.57	31.14	19.45	65.83	<0.001	32.04
TAH	14240.76	8982.52	10891.85	6697.86	5844.36	ND
TVAH	1.54	1.98	9.65	4.65	0.07	ND
TPH	14242.3	8984.5	10901.5	6702.51	5844.43	ND
TAHef (%)	40.94	62.75	54.83	72.22	75.76	ND

TAH total aliphatic hydrocarbons; TVAH total volatile aromatic hydrocarbons; TPH total petroleum hydrocarbons; TAHef TAH remediation Efficiency; ET *Eragrostis tenela,* EI *Eleusine indica,* CD *Cynodon dactylon;* LSD (0.05) least significant difference at 95% confidence limit; ND not determined

Table 2.

Microbial composition of rhizospheric soils of the treatment plants collected and assayed as pooled composite sample respectively

Sample identity	Bacterial counts ($x10^5$ cfu/g)	Bacterial isolates Identified	Fungal counts ($x10^5$ cfu/g)	Fungal isolates Identified
		(Unpolluted soil after 3 months)		
No plant	4.2	*Bacillus substilis, Micrococcus* sp., *Proteus vulgaris*	3.2	*Aspergillus niger, Geotricum* sp., *Penicillium* sp., *Rhizopus* sp.
		(Oil-Polluted soils after 3 months)		
No plant	5.0	*B. substilis, Micrococcus* sp., *P. Vulgaris*	2.4	*Penicillium* sp.
CD	5.6	*B. substilis, P. vulgaris*	4.0	*A. flavus, Rhizopus* sp.
EI	6.0	*B. substilis*	4.4	*A. niger, Fusarium solani*
ET	8.0	*Micrococcus* sp.	3.9	*A. flavus, Penicillium* sp.
EI+CD	6.9	*B. substilis*	6.9	*F. solani, Penicillium* sp.
CD+ET	5.7	*B. substilis*	3.2	*A. niger, Geotricum* sp., *Rhizopus* sp.
EI+ET	4.8	*B. substilis, P. vulgaris*	3.7	*A. niger, Geotricum* sp
All 3 grasses	6.0	*B. substilis*	3.9	*A. niger, F. solani, Penicillium* sp.

ET *Eragrostis tenela,* EI *Eleusine indica,* CD *Cynodon dactylon*

Plant morphological parameters of *Eragrostis tenela* sown in isolation and in combination with the other test grasses have been presented on Table 4. Plant height of *E. tenela* at the nursery, just before transplanting unto treatment bowls was 21.02 cm. This grew by an additional 12 cm alone (29.70 cm). Sown with *E. indica,* plant height was 26.20 cm, compared to 23.87cm, when sown with both *E.indica* and *C. dactylon* ($p>0.05$). There were no significant differences stem width, internode length, number of leaves per culm, leaf blade length and width and peduncle length. Although foliar chlorosis and necrosis were not reported at nursery stage, the test plant however showed significant evidence of necrosis and chlorosis whether sown alone or in combination with other test plants. This observation was similar to that with *Cynodon dactylon* sown alone or in combination (Table 5). For this plant, there was significant increase in plant height ($p<0.05$) when *C. dactylon* was sown alone (45.01 cm) or with *E. indica* (38.96 cm).

Generally, plant morphological parameters of the test plants were better alone than in combinations or two's and three's. This suggests effects of competition. This is even more interesting because the effects of competition was rather in favour of enhanced remediation than when oil-polluted soils were son with single plant species.

Table 3.

Plant morphological parameters of *Eleusine indica* in the present study at 9 weeks after transplanting

	Before transplanting planting	**Alone**	**With *Eragrostis***	**With *Cynodon***	With *Cynodon* and *Eragrostis*	**LSD (0.05)**
Plant height (cm)	41.20	44.60	42.10	43.90	42.07	6.94
Stem width (mm)	5.30	14.00	7.40	10.13	8.54	4.35
Internode length (cm)	2.80	6.90	4.70	5.30	5.96	2.11
Number of leaves per culm	7.32	11.00	9.02	10.31	9.90	3.41
Culm branching (numbers)	1.50	4.00	2.00	3.76	3.57	1.65
Flag leaf blade length (cm)	10.50	23.50	13.32	17.70	16.65	6.43
Flag leaf blade width (mm)	4.32	4.69	4.47	4.67	4.65	1.01
Number of spikes per culm	0	4.00	3.33	4.00	3.56	1.47
Peduncle length (cm)	3.79	4.36	4.36	4.36	4.14	1.73
Length of longest spike (cm)	0	8.00	6.40	7.51	7.34	3.03
Width of longest spike (mm)	0	3.01	3.30	3.43	3.09	0.92
Number of spikelets	0	71.05	53.43	64.53	50.07	18.63

	Before transplanting planting	**Alone**	**With *Eragrostis***	**With *Cynodon***	With *Cynodon* and *Eragrostis*	**LSD (0.05)**
Culm glabrous (laterally flattened)	+	+	+	+	+	NA
Culm colour (white or silver at base and pale green towards the tip)	+	+	+	+	+	NA
Leaves colour (green)	+	+	+	+	+	NA
Leaf blades linear or lanceolate	+	+	+	+	+	NA
Chlorosis	+	+	+	+	+	NA
Necrotic spots	-	+	+	+	+	NA

+ present, - absent; NA not available

Table 4.

Plant morphological parameters of *Eragrostis tenela* in the present study at 9 weeks after transplanting

	Before transplanting planting	**With *Eleusine***	**With *Cynodon***	**Alone**	With *Cynodon* and *Eleusine*	**LSD (0.05)**
Plant height (cm)	21.02	26.20	29.15	29.70	23.87	6.98
Stem width (mm)	1.00	1.00	1.00	1.03	1.06	0.37
Internode length (cm)	2.40	2.42	2.60	2.67	2.43	1.06
Number of leaves per culm	5.00	5.14	5.33	5.33	5.45	0.95
Culm branching (numbers)	3.00	3.00	3.00	3.00	3.07	0.92
Flag leaf blade length (cm)	2.40	3.31	3.59	4.06	4.03	1.67
Flag leaf blade width (mm)	2.28	3.30	3.80	4.33	3.86	2.04
Number of spikes per culm	19.00	21.30	24.00	24.00	21.98	8.64
Peduncle length (cm)	5.00	5.80	6.20	6.20	6.03	2.34
Length of longest spike (cm)	1.80	1.80	2.20	2.20	1.75	1.00
Width of longest spike (mm)	0.30	0.47	0.50	0.50	0.52	0.22
Culm glabrous (laterally flattened)	+	+	+	+	+	NA
Culm colour (white or silver at base and pale green towards the tip)	+	+	+	+	+	NA
Leaves colour (green)	+	+	+	+	+	NA
Leaf blades linear or lanceolate	+	+	+	+	+	NA
Chlorosis	_	+	+	+	+	NA
Necrotic spots	_	+	+	+	+	NA

+ present, - absent; NA Not available

Table 5.

Plant morphological parameters of *Cynodon dactylon* in the present study at 9 weeks after transplanting

	Before transplanting planting	**With *Eragrostis***	**With *Eleusine***	**Alone**	With ***Eleusine*** and ***Eragrostis***	**LSD (0.05)**
Plant height (cm)	28.60	33.00	38.96	45.01	31.54	9.56
Stem width (mm)	1.20	1.80	1.87	2.00	2.00	1.01
Internode length (cm)	2.72	2.90	3.00	3.00	2.38	1.30
Number of leaves per culm	4.00	4.80	5.00	5.00	4.05	2.17
Culm branching (numbers)	4.70	4.72	5.00	5.00	4.63	2.06
Flag leaf blade length (cm)	4.10	4.60	5.50	5.52	4.95	1.87
Flag leaf blade width (mm)	3.52	3.78	4.00	4.00	4.00	1.70
Number of spikes per culm	5.24	5.93	6.73	6.06	5.92	2.01
Peduncle length (cm)	7.40	7.47	7.50	7.50	7.53	1.47
Length of longest spike (cm)	4.10	5.70	6.30	6.32	5.63	2.12
Width of longest spike (mm)	0.32	0.57	1.03	1.00	1.21	0.24
Culm glabrous (laterally flattened)	+	+	+	+	+	NA
Culm colour (white or silver at base and pale green towards the tip	+	+	+	+	+	NA
Leaves colour (green)	+	+	+	+	+	NA
Leaf blades linear or lanceolate	Linear	+	+	+	+	NA
Chlorosis	-	+	+	+	+	NA
Necrotic spots	-	+	+	+	+	NA

+ present, - absent; NA not available

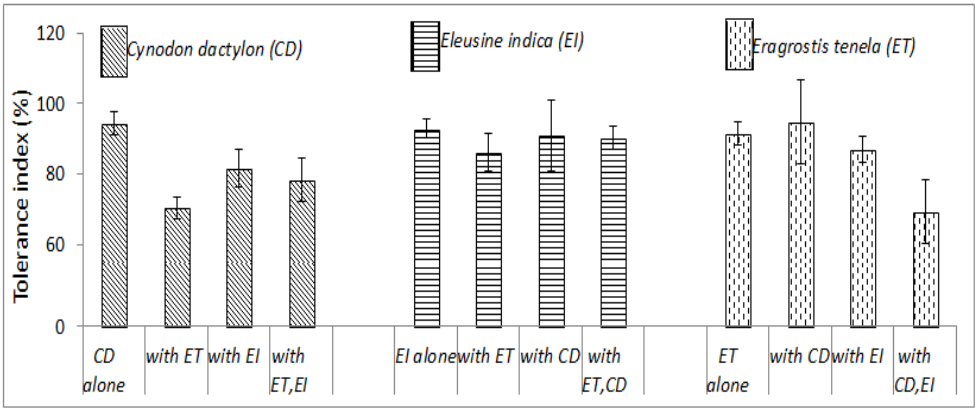

Figure 1. Tolerance index of grasses alone and in combination with other grasses under experimental condition.

Tolerance index for *Cynodon dactylon* alone ubder experimental condition was 94.47%, compared to 92.92% for *E. indica* and 91.58% for *E. tenela* respectively (Fig. 1). The individual grasses showed lower tolerance index in the polluted soils with in association with any other two grasses (i.e. grasses in groups of 3's). in combination of 3's, *E. indica* showed higher tolerance under experimental condition (90.35%).

Conclusions

The interactions among the test plants in the remediation of oil-contaminated soil have been provided in the present study. These plants have therefore demonstrated the ability to survive and provide suitable conditions for rhizobacteria to degrade hydrocarbons whether as single plants in contaminated soils, or in mixed culture.

Acknowledgements. The study was privately funded. The researchers are grateful to Mr. Ben Enebeli, Chief Lab. Technologist at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria for technical and Lab support.

REFERENCES

Abedi-Koupai, J., Ezzatian, R., Vossoughi-Shavari, M., Yaghmae, S., Borghei, M. (2007) The effects of microbial population on phytoremediation of petroleum contaminated soils using tall fescue, *Int. J. Agricult. Biol.,* **9**: 242–246

Anoliefo, G. O., Ikhajiagbe, B., Okonokhua, B. O., Diafe, F. V. (2006) Ecotaxonomic distribution of plant species around auto mechanic workshops in Asaba and Benin City, Identification of oil tolerant species, *African Journal of Biotechnology* **5(19)**: 1757-1762

Anoliefo, G. O., Ikhajiagbe, B., Okonokhua, B. O., Edegbai, B. E., Obasuyi, O. C. (2008) Metal tolerant species distribution and richness in and aroud metal based industries, possible candidates for phytoremediation, *Africa Journal of Environmental Science and Technology* **2 (11)**: 360-370

Anoliefo, G. O., Ikhajiagbe, B. (2011) Plant-microbial interaction in the degradation of crude oil in soil, Synergism in bioremediation, *Nigerian Journal of Life Sciences* **1(1)**: 40-52

Aprill, W., Sims, R. C. (1990) Evaluation of the use of priarie grasses for stimulating polycyclic aromatic hydrocarbon treatment in soil, *Chemosphere* **20**: 253-265

Bordoloi, S., Basumatary, B., Saikia, R., Das, H. C. (2012) *Axonopus copressus* (Sw.) P. Beauv. A native grass species for phytoremediation of hydrocarbon contaminated soil in Assam, India. *Journal of Chemical Technology and Biotechnology, Special Issue, Bioremediation* **87(9):** 1335- 1341

Brooks, M. L. (2000) Competition between alien annual grasses and native annual plants in the Mojave Desert, *American Midland Naturalist* **144:** 92–108

Brown, C. S., Rice, K. J. (2000) The mark of zorro, effects of the exotic annual grass Vulpia myuros on California native perennial grasses, *Restoration Ecology* **8**: 10–17

Cai, Z., Zhou, Q. X., Peng, S. W., Li, K. N. (2010) Promoted biodegradation and microbiological effects of petroleum hydrocarbons by *Impatiens balsamina* L. with strong endurance, *Journal of Hazardous Materials* **177**: 384-389

Carlsen, T. M., Menke, J. W., Pavlik, B. M. (2000) Reducing competitive suppression of a rare annual forb by restoring native California perennial grasslands, *Restoration Ecology* **8**:18–29

Cheesebrough, M. (2001) *District Laboratory Practice in Tropical Countries*, part 2, Cambridge University Press, Cambridge, pp. 355

Daniel, E. S., Musa, J. J., Akos, M. P., Yerima, I. Y., Dada, P. O., Jibril, I., Manta, I. H. (2016) *Assessment of heavy metal pollution in some Nigerian soils: a review*, 37th Annual Conference and Annual General Meeting of the Nigeria Inst. of Agric. Engineers, Minna, Nigeria

Dean, J. R., Xiong, G. (2000) Extraction of organic pollutants from environmental matrices, selection of extraction technique, *Trends in Analytical Chemistry* **19(9)**: 553–561

Dyer, A. R., Rice, K. J. (1999) Effects of competition on resource availability and growth of a California bunchgrass, *Ecology* **80**: 2697–2710

Green, E. K., Galatowitsch, S. M. (2002) Effects of *Phalaris arundinacea* and nitrate-N addition on the establishment of wetland plant communities, *Journal of Applied Ecology* **39**: 134–144

Gunther, T., Dornberger, U., Fritsche, W. (1996) Effects of rye grass on biodegradation of hydrocarbons in soil, *Chemosphere* **33**: 203-215

Ikhajiagbe, B., Anoliefo, G. O. (2011) Impact of substrate amendment on the polyaromatic hydrocarbon contents of a five month old waste engine oil polluted soil, *African Journal of Environmental Science and Technology* **5(10)**: 769-777

Ikhajiagbe, B., Anoliefo, G. O. (2012) Weed biodiversity studies of a waste engine oil-polluted soil exposed at different intervals of natural attenuation and substrate amendment, *Journal of Biological Sciences* **12(5)**: 280 - 286

Ikhajiagbe, B., Anoliefo, G. O., Okoh, H. U., Owenaze, I. (2013) Impact of organic mulching on the enhanced natural attenuation of a petroleum hydrocarbon polluted soil, *Nigerian Society for Experiential Biology Journal* **13(3&4)**: 32 – 42

Ikhajiagbe, B. (2016) Possible adaptive growth responses of *Chromolaena odorata* during heavy metal remediation, *Ife Journal of Science* **18(2)**: 403 – 411

Iyagba, A. G., Offor, U. S. (2013) Phytoextraction of heavy metals by fluted pumpkin (*Telfaria occidentalis* Hooker F.) planted on oil contaminated soil amended with sawdust and siam weed (*Chromolenaodorata*) (L.) R.M King and Robinson) weed, *International Research Journal of Agricultural Sciences and Soil Sciences* **3 (12)**: 422 – 426

McCutcheon, S. C., Schnoor, J. L. (2003) *Phytoremediation: transformation and control of contaminants*, Wiley-Interscience Inc., Hoboken

Merkl, N., Schultze-Kraft, R., Infante, C. (2004) Phytoremediation in the tropics- the effect of crude oil on the growth of tropical plants, *Bioremediation J.* **8**: 177-184

Merkl, N., Schultze-Kraft, R., Infante, C. (2005) Assessment of tropical grasses and legumes for phytoremediation of petroleum contaminated soils, *Water Air Soil Pollut.* **165**: 195 - 209

Onwuka, F., Nwachoko, N., Anosike, E. (2012) Determination of total petroleum hydrocarbon (TPH) and some cations (Na, Ca^{2+} and Mg) in a crude oil polluted soil and possible phytoremediation by Cynodon dactylon L. (Bermuda grass), *J. Environ. Earth Sci.* **2**: 13–17

Qui, X., Leland, T. W., Shah, S. I., Sorensen, D. L., Kendall, E. W. (1997) Field study, grass remediation of for clay soil contaminated with polycyclic aromatic hydrocarbons. *In*: *Phytoremediation of Soil and Water Contaminants*, Kruger, E. L., Anderson, T. A., Coats, J. R. (eds), ACS Symposium Series 664, pp. 186-199

Radha, K. E., Shamsher, K. P., Veerendra, K. B. (2011) Study on Antioxidant activity and strain development of *Bacillus substilis* (MTCC No. 10619), *Journal of Agricultural Technology* **7(6)**: 1693-1703

Reilley, K. A., Banks, M. K., Schwab, A. P. (1996.) Organic chemicals in the rhizosphere, *Journal of Environmental Quality* **25**: 212-219

Shirdam, R, Zand, A. D., Bidhendi, G. N., Mehrdadi, N. (2008) Phytoremediation of hydrocarbon-contaminated soils with emphasis on the effect of petroleum hydrocarbons on the growth of plant species, *Phytoprotection* **89**: 21- 29

White, P. M., Wolf, D. C., Thoma, G. J., Reynolds, C. M. (2006) Phytoremediation of alkylated polycyclic aromatic hydrocarbons in an oil-contaminated soil, *Water Air Soil Pollut.* **169**: 207-220