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Biochemical and physicochemical assessment of the efficacy of some wild-type legumes in the remediation of crude-oil contaminated soils

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ABSTRACT

The efficacy of three wild-type legumes in the remediation of agricultural soils contaminated with 1% (lightly impacted), 3% (moderately impacted), and 5% (heavily impacted) crude-oil was assessed, using soil physicochemical and biochemical properties (soil quality indicators) as evaluation criteria. Results after a 15-month remediation period showed that only L. leucocephala failed to germinate. The level of MC (87%) as well as the activities of Lipases (103%) and ALPs (90%) in the P. pterocarpum-remediated soil samples were significantly ($p>0.05$) elevated, relative to their respective contaminated samples, while the TPH (60%), was significantly ($p>0.05$) reduced. The C. retusa-remediated soils had the level of MC (48%) and the activities of Lipases (59%) and ALPs (73%) significantly ($p>0.05$) elevated, relative to the respective contaminated samples, while the level of TPH (65%) was significantly ($p>0.05$) reduced. The levels of pH, and TOC as well as the activities of the dehydrogenases and ACPs were not significantly ($p<0.05$) different from their corresponding contaminated samples remediated by both legumes. These results indicate that Leucaena leucocephala 'may' not be a good crude-oil remediating leguminous plant, while both Peltophorum pterocarpum and Crotalaria retusa are good crude-oil remediating leguminous plants.

Key Words: Remediation, Wild-type legumes, Crotalaria retusa, Peltophorum pterocarpum, Leucaena leucocephala.

INTRODUCTION

The soil is very important to man human existence for various reasons especially agriculture. However, the soil has been subjected to several abuses including spillage of petroleum (crude oil) and petroleum-by products, dumping of wastes and other contaminating activities (Nwaugo *et al*, 2006, 2007; Osam, 2011; Wellingia *et al*, 1999).

When oil spill occurs on-shore, the soil ecosystem is usually inundated, leading to several conflagrations that may consume several acres of arable land, which is the prime factor in agricultural productivity. Today, environmental managers can choose from a variety of approaches to remediate petroleum-contaminated soil and groundwater. The approach or approaches chosen in such clean-ups had been those orthodox expensive and ineffective conventional practices, (e.g. 'pump-and-treat' and 'dig-and-dump' techniques), which are not environmentally friendly (as they merely transfer the pollutants from one site to another).

An environmentally sound technology (EST) that addresses the inadequacies of these old remediation practices will therefore be pertinent in this era of global economic melt down. Here comes the natural clean-up method, 'phytoremediation' – the technology that utilizes the inherent abilities of living plants for the removal, degradation, or containment of contaminants in soils, sludge, sediments, surface water and ground water. The technology is ecologically friendly, solar-energy driven, and is based on the concept of using "nature to cleanse nature".

Phytoremediation technology has been proved to be a successful method of treating contaminated soils to levels below the maximum permissible level of the contaminants. For instance, Simeonova and Simeonov (2006), successfully phytoremediated a three-kilometer ecological zone contaminated with lead, using *Brassica juncea* plants. The results of their one-planting experiment showed a decrease between 0 and 25.9% of the initial lead concentration at various sample locations.

In their experiment also, Gunther *et al*, (1996) found that soils planted with ryegrass (*Lolium multiflorum*) lost a greater amount of a mixture of hydrocarbons than soils that was unplanted. In their 22-week phytoremediation study, the initial extractable hydrocarbon concentration of 4330mg THC per kg soil decreased to less than 120mg per kg soil (97% reduction) in planted soils, but to only 790mg per kg soil (82% reduction) in unplanted soil.

The examination of the phytoremediation potential of two cold-hardy plants, Arctared red fescue (*Festuca arundinacea)* and annual ryegrass (*Lolium multiflorum*) by Reynolds and Wolf (1999), that were planted together in soils contaminated with crude oil, indicated that contaminated soils planted with two species had significantly lower concentrations of total petroleum hydrocarbon (TPH) compared to unplanted controls. The initial crude oil concentration for planted treatments and unplanted controls was approximately 6200mg TPH per kg soil. After 640 days, crude oil-contaminated soils planted with both species had 1400mg TPH per kg soil (77% reduction), while the unplanted control contained 2500mg TPH per kg soil (60% reduction)

Finally, in a 6-month laboratory study, Pradham *et al*, (1998), identified that alfalfa (*Medicago sativa*), switch grass (*Panicum virgatum*) and little bluestem (*Schizachyrium scoparius*) were capable of reducing the concentration of total PAHs in soil contaminated at a manufactured gas plant (MGP). The initial soil concentration of total PAHs for the three plant treatments and an unplanted control was 184.5±14.0mg total PAHs per kg of soil. After 6 months, the concentration in the unplanted control soil was 135.9±25.5mg/kg while the concentration in planted treatments were much lower (Switch grass, 79.5±3.7mg/kg, alfalfa, 80.2±8.9mg/kg and little bluestem, 97.1±18.7mg/kg).

It is against this background, predicated by the plethora of unsuccessful, environmentally-unfriendly and expensive conventional remediation methods that we were prompted to investigate the effectiveness and efficacy of some wild-type legumes commonly found growing luxuriantly on crude oil impacted soils in the Niger Delta Region of Nigeria, in remediating/reducing the level of petroleum hydrocarbon-contaminated agricultural soils to at least the maximum permissible level, and thus minimize the impact of oil spill on agricultural productivity. This was borne out of the fact that leguminous plants have a lot of advantages over their non-leguminous counterparts because they do not have to compete with microorganisms and other plants for limited supplies of available nitrogen at oil-contaminated soils since they have the ability to fix nitrogen (Frick *et al*, 1999).

Figure 1: YELLOW FLAME TREE (*Peltophorum pterocarpum*)

Figure 2: MIRACLE TREE (*Leucaena leucocephala*)

MATERIALS AND METHODS

1.2. Materials

In addition to the laboratory reagents, the following chemicals, biochemicals, and materials were used for the work: triphenyl tatrazolium chloride (TTC), *p*-nitro phenyl phosphate (PNPP), gas chromatograph (Varian model 3400GC), electrophotometer (B and L spectronic-20), crude oil (obtained from Nigerian Agip Oil Company, NAOC, Ebocha, Rivers State), and over 200 seeds of each of the legumes: Yellow flame tree, *Peltophorum pterocarpum* (figure 1), obtained from

the Convocation arena of the University of Port Harcourt, Nigeria; miracle tree, *Leucaena leucocephala* (figure 2), obtained from Bayelsa State, Nigeria and rattle weed, *Crotalaria retusa* (figure 3), obtained from the International Institute of Tropical Agriculture, IITA. Eneka, Nigeria. These were identified, classified and authenticated as being of high quality by a Professor of Botany in the Department of Plant Anatomy and Physiology, University of Port Harcourt, Nigeria.

Figure 3: RATTLE WEED (*Crotalaria retusa*)

METHODS

1.3.1. Land Mapping/Preparation

Ten widely-spaced plots (measuring 12 x 10 ft each) and labelled E_1, E_2,…E_9, the 10^{th} plot which is the control, - is a non-vegetative geographically virgin area similar to the experimental plots, but unaffected by oil spill and located at a distance of about 2 km from the experimental plots. Preliminary preparation of the seedbeds was undertaken so as to remove any rubbles that would interfere with agronomic practices, e.g. weeds, grasses and little trees were removed to facilitate seedbed preparation. Tilling of the soil was performed to about 8-11cm depth.

1.3.2. Contamination of the plots

This was done as follows:- Plots E_1- E_3 (1-EQ), were uniformly poured 1% by weight of concentration of crude oil at a total quantity of 30 litres per plot as reported by Thoma *et al*, (2002), and modified similarly by the researcher. This was similarly done for plots E_4- E_6 (3-EQ), and E_7- E_9 (5-EQ) but with 3% and 5% by weight of the crude oil respectively. Contaminated samples were collected 7 days after the contamination.

1.3.3. Planting of the wild-type legumes

Planting of the wild-type legumes was done 14 days after contamination using 20 seeds per plot. The target population was to obtain between 10 and 15 plants per m^2, as reported by Simeonova and Simeonov (2006), for *Brassica juncea* planted in lead-contaminated ecological zone.

1.3.4. Sampling Techniques

Triplicate soil samples were collected randomly from three spots at 2 core depths of top surface(0-15cm) and sub-surface(15-30cm), using a long trowel. Post-remediation sampling was 15 months later after removing the legumes. A total of 60 samples, made up of: 6 control samples (2 per spot, i.e. top and sub surface); 18 contaminated samples (6 for each of the plots

contaminated with 1%, 3%, 5% crude oil, and finally 36 post-remediated samples (6 for each of the three plots remediated with *P. pterocarpum*, and *C. retusa*). No soil samples were collected from the 3 plots planted *L. leucocephala* since the plant failed to germinate. The soil samples were wrapped in aluminium foil and labelled accordingly before being sent to the laboratory for the various analyses. Samples for enzymes assays and bacterial load investigations were kept in plastic bags and transported to the laboratory within 2 days of collection in refrigerated coolers to arrest microbial growth.

1.3.5. Determination of soil pH

The pH of the soil samples was determined according to the standard electrometric method as reported by Nwinuka *et al*, (2003).

1.3.6. Determination of soil moisture content

Percentage moisture content was estimated from differential in the weight of soil samples after drying at 110^{o}C for 1 hour and cooling in a desiccator as described by Osuji and Onojake (2004).

1.3.7. Determination of TOC

The percentage total organic carbon (TOC) of the soil samples was determined by the rapid titrimetric method (Walkey and Black, 1934).

1.3.8. Determination of TPH

The determination of total petroleum hydrocarbon (TPH) contents was carried out by the use of gas chromatographic (GC) technique as reported by Osam, (2006).

1.3.9. Determination of soil enzymes' activity

The activity of the soil dehydrogenases was determined, using the triphenyl tetrazolium chloride (TTC) method as described by Casida *et al*, (1964); that of the soil lipases was determined as described by Saisuburamaniya *et al*, (2004); while those of acid and alkaline phosphatases by the use of *p*-nitrophenylphosphate (PNPP) and calcium chloride as described by Tabatabai and Bremear (1969).

1.3.10. Method of data analysis

The data were analyzed using tables, range, means, percentages, graphs (bar charts), standard deviation and hence standard error (SE). Sample mean was calculated for all the three replicate samples, while standard deviation (S.D) was calculated from the sample mean by the standard statistical method for all the variables. The standard deviations were used to calculate the standard errors (±S.E) as reported by Osuji *et al*, (2005). Standard error (±S.E) was estimated at the 95% confidence level by multiplying the standard error with 1.96. Also, all the data obtained were subjected to statistical analysis of variance (ANOVA) technique using computer-aided SPSS statistical programme, and the means separated and compared using Duncan's Multiple Range test (Duncan, 1955) at 5% level of significance.

RESULTS

The seeds of one of the remediating plants, namely, Miracle tree (*Leucaena leucocephala*), failed to germinate in all the three quadrats that they were planted.

The result of the soil pH determined for each of the quadrats is schematically shown in table 1 of the table legend; that of the moisture content analyses in table 2; table 3 is for the percentage TOC, while table 4 is for the TPH. The result of soil dehydrogenases analyzed for each of the soil samples is shown in table 5; the soil lipases in table 6; the soil alkaline phosphatases in table 7, and finally the acid phosphatases in table 8.

DISCUSSION

The figures indicated that the pH of all the soil samples remediated with both legumes increased non-significantly ($p<0.05$), relative to the contaminated samples, while the pH of the contaminated samples dropped non-significantly ($p<0.05$), relative to the control. The pH drop observed in the contaminated soils may result from CO_2 evolution. This had previously been reported by Dalyan *et al*, (1990). The top surface soils were more adversely affected than the sub-surface soils, while the soils remediated with *P. pterocarpum* were non-significantly ($p<0.05$) elevated more than those remediated with *C. retusa* in all the soil samples except in the 5% (5-EQ) remediated sub-surface, where *C. retusa* had a mean pH of 6.81±0.04, as against the mean value of 6.65±0.03 observed for the respective soils remediated with *P. pterocarpum*. . This observation shows that *P. pterocarpum* was slightly more efficient (with 14%) than *C. retusa* (with 12%) in the elevation of their pH.

The moisture content of the soils remediated with *P. pterocarpum* (87%) and *C. retusa* (52%) were significantly ($p>0.05$) higher than those of the contaminated soils and were almost of the same value with all the control samples, except the control top surface soil remediated with *P. pterocarpum*. The decrease in moisture content observed for the contaminated soils may have been due to crude oil accumulation in the pores between soil particles, which might have resulted in reduced oxygen and water permeability through the soil. Soils develop severe and persistent water repellency following contamination with crude oil. The significant ($p>0,05$) elevation of the moisture content by both *P. pterocarpum* and *C. retusa* to the levels close to the control corroborates the observation of Frick *et al*, (1999) who posited that plants that tolerate petroleum hydrocarbons take them up via their roots and may accumulate them to a small degree in their roots and shoots.

Mean % TOC observed in this work for the contaminated soils and those remediated with both legumes were not significantly ($p<0.05$) different, even between the top and sub-surface soils, as well as the control samples. . The reduction of the level of TOC in the remediated soils observed in this work orchestrated by the two legumes in their respective plots clearly shows that the legumes have metabolic and absorption capabilities as well as transport systems that selectively sucked up the contaminants from the growth matrix. Despite the low levels of TOC reduction observed in this work, the finding is in consonance with the similar work of Thoma *et al*, (2002) who observed a similar trend in a soil sample contaminated with 3% by weight weathered crude oil that was phytoremediated with the legume, *Aeschynomene americana*.

The levels of hydrocarbons observed in the remediated soils show that the legumes were very efficient in their rhizosphere degradation since the values were significantly ($p>0.05$) lower than those of the contaminated soil samples. Both *P. pterocarpum* and *C. retusa*-remediated soils had the TPH levels reduced from 184.0 – 74.30mg/kg (60%) and 184.0 – 64.70mg/kg (65%)

respectively, of the contaminated soils. These show that the degradable ability of the two legumes was promising. This can be likened to a similar observation for red fescue and ryegrasses (Reynolds and Wolf, 1999), which significantly reduced TPH from 6200 mg/kg to 1400 mg/kg or 77% after 640 days (21 months) remediation period. Also, the works of Gudin and Syratt (1975), Gunther *et al*, (1996), Schwab *et al*, (1995), similar to the works cited above give evidence of the hydrocarbon degradation ability of leguminous plants in the containment of crude-oil contaminated soils to at least the maximum permissible level.

TABLE 1: Mean (±S.E[a]) pH of remediated soil samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	(cm)	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	7.07 ± 0.023	6.10 ± 0.11	7.04 ± 0.03	6.75 ± 0.04
1-CQ	15 - 30	7.20 ± 0.30	6.12 ± 0.04	7.11 ± 0.03	6.82 ± 0.02
3-CQ	0 - 15	7.07 ± 0.023	5.98 ± 0.04	6.92 ± 0.06	6.80 ± 0.02
3-CQ	15 - 30	7.20 ± 0.30	6.23 ± 0.03	7.08 ± 0	6.87 ± 0.01
5-CQ	0 – 15	7.07 ± 0.023	5.67 ± 0.02	6.73 ± 0.03	6.79 ± 0.06
5-CQ	15 - 30	7.20 ± 0.30	5.91 ± 0.07	6.65 ± 0.03	6.81 ± 0.04

[a]S.E*: Standard error at 95% confidence level*

TABLE 2: Mean (±S.E[a]) MC, (%) of remediated soil samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	(cm)	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	10.2 ± 0.11	4.60 ± 0.15	11.1 ± 0.08	9.40 ± 0.37
1-CQ	15 - 30	11.0 ± 0.05	6.00 ± 0.08	11.8 ± 0.36	9.20 ± 0.39
3-CQ	0 - 15	10.2 ± 0.11	6.40 ± 0.30	12.4 ± 1.57	10.20 ± 0.08
3-CQ	15 - 30	11.0 ± 0.05	7.20 ± 0.30	11.8 ± 1.03	9.80 ± 0.49
5-CQ	0 – 15	10.2 ± 0.11	8.60 ± 0.49	15.5 ± 0.39	11.00 ± 0.08
5-CQ	15 - 30	11.0 ± 0.05	7.80 ± 0.41	11.1 ± 0.20	10.40 ± 0.11

[a]S.E*: Standard error at 95% confidence level*

TABLE 3: Mean (±S.E[a]) TOC, (%) of remediated soil of samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	(cm)	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	0.331 ± 0	1.03 ± 0.018	1.00 ± 0.030	0.86 ± 0.012
1-CQ	15 - 30	0.126 ± 0.004	0.39 ± 0.014	0.26 ± 0.016	0.35 ± 0.016
3-CQ	0 - 15	0.331 ± 0	1.23 ± 0.030	1.21 ± 0.020	1.17 ± 0.08
3-CQ	15 - 30	0.126 ± 0.004	1.09 ± 0.024	0.663 ± 0.005	0.82 ± 0.037
5-CQ	0 – 15	0.331 ± 0	1.70 ± 0.039	1.33 ± 0.008	1.21 ± 0.014
5-CQ	15 - 30	0.126 ± 0.004	1.50 ± 0.035	0.59 ± 0.027	0.86 ± 0.014

[a]S.E*: Standard error at 95% confidence level*

The activities of the lipases in the soil samples remediated with *C. retusa* and *P. pterocarpum* were elevated by 59% and 103% respectively; those of acid phosphatases in the soil samples remediated with *C. retusa* and *P. pterocarpum* by 73% and 90% respectively; those of the dehydrogenases in the soil samples remediated by the two respective legumes by 11% and 16% respectively, while the acid phoshatases by 13% and 11% respectively. The results show that the

dehydrogenases were the least inhibited by the crude oil contamination, while the alkaline phosphatases were the most inhibited in the contaminated samples. The results also revealed the same trend for all the enzymes activities measured in respect of increasing crude oil concentration, with the activities declining as the oil concentration increases before the remediation. These agree with the similar works of Li *et al*, (2005) and Nwaugo *et al*, (2007) who observed that soil pollution reduces soil enzymatic activities. The significantly (p>0.05) elevated enzymatic activities of the alkaline phosphatases and lipases and the non-significantly (p<0.05) elevated activities acid phosphatases and the dehydrogenases of the remediated samples from both quadrats remediated with both legumes show a positive correlation of soil enzymatic activities with bacterial load and corroborated by the position of Frick *et al*, (1999), who reported that soil organisms produced most of the enzymes estimated. This further authenticates the fact that the root systems of legumes have favourable environment that harbour and enhance microbial populations that produce the enzymes. This observation was corroborated with similar works by Atlas and Bartha, (1998) and Nwaugo *et al*, (2007).

TABLE 4: Mean (±S.E[a]) TPH, (mg/Kg) of remediated soil of samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	**(cm)**	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	80.9 ± 0.27	118.30 ± 0.43	63.40 ± 0.15	90.20 ± 0.24
1-CQ	15 - 30	77.20. ± 0.24	101.10 ± 0.08	14.70 ± 0.18	31.60 ± 0.24
3-CQ	0 - 15	80.9 ± 0.27	188.50 ± 0.30	121.00 ± 0.80	142.90 ± 0.18
3-CQ	15 - 30	77.20. ± 0.24	173.30 ± 0.18	46.00 ± 0	35.70 ± 0.35
5-CQ	0 – 15	80.9 ± 0.27	309.10 ± 0.74	133.00 ± 3.50	68.80 ± 0.08
5-CQ	15 - 30	77.20. ± 0.24	216.50 ± 0.22	67.70 ± 0.29	18.70 ± 0.11

[a]S.E*: Standard error at 95% confidence level*

TABLE 5: Mean (±S.E[a]) dehydrogenases activity, (mg/g/6h) of remediated soil of samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	**(cm)**	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	28.60 ± 0.08	29.70 ± 0.41	29.00 ± 1.10	29.80 ± 0.28
1-CQ	15 - 30	20.50 ±0.30	14.80 ± 0.41	16.00 ± 0.30	15.00 ± 0.24
3-CQ	0 - 15	28.60 ± 0.08	16.80 ± 0.23	20.50 ± 0.30	19.50 ± 0.30
3-CQ	15 - 30	20.50 ±0.30	10.40 ± 0.41	15.00 ± 0.24	13.30 ± 0.30
5-CQ	0 – 15	28.60 ± 0.08	11.30 ± 0.08	14.00 ± 0.44	12.90 ± 0.34
5-CQ	15 - 30	20.50 ±0.30	6.20 ± 0.11	9.00 ± 0.71	8.40 ± 0.37

[a]S.E*: Standard error at 95% confidence level*

TABLE 6: Mean (±S.E[a]) lipases activity, (mg/g/30min) of remediated soil of samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	**(cm)**	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	4.73 ± 0.023	5.81 ± 0.020	10.40 ± 0.30	9.10 ± 0.04
1-CQ	15 - 30	2.86 ± 0.024	3.52 ± 0.024	7.00 ± 0.10	5.00 ± 0.05
3-CQ	0 - 15	4.730 ± 0.023	3.84 ± 0.037	3.88 ± 0.033	5.50 ± 0.035
3-CQ	15 - 30	2.86 ± 0.024	2.17 ± 0.030	5.00 ± 0.064	3.64 ± 0.057
5-CQ	0 – 15	4.730 ± 0.023	1.14 ± 0.032	3.05 ± 0.039	2.40 ± 0.043
5-CQ	15 - 30	2.86 ± 0.024	0.80 ± 0.027	2.70 ± 0.049	1.80 ± 0

[a]S.E*: Standard error at 95% confidence level*

TABLE 7: mean (±S.E[a]) ALP activity, (µmol *p*-nitrophenol) of remediated soil of samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	(cm)	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	3.44 ± 0.040	5.17 ± 0.027	8.25 ± 0.057	8.00 ± 0
1-CQ	15 - 30	2.18 ± 0.024	2.21 ± 0.014	5.00 ± 0.033	4.50 ± 0.23
3-CQ	0 - 15	3.44 ± 0.040	3.16 ± 0.011	4.80 ± 0.085	4.20 ± 0.085
3-CQ	15 - 30	2.18 ± 0.024	1.32 ± 0.018	4.00 ± 0	3.00 ± 0.20
5-CQ	0 – 15	3.44 ± 0.040	2.27 ± 0.030	3.84 ± 0.06	3.75 ± 0.043
5-CQ	15 - 30	2.18 ± 0.024	0.63 ± 0.030	2.10 ± 0.018	2.05 ± 0.12

[a]S.E*: Standard error at 95% confidence level*

TABLE 8: Mean (±S.E[a]) ACP activity, (µmol *p*-nitrophenol) of remediated soil of samples

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	(cm)	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.	$\overline{(X)} \pm$ S.E.
1-CQ	0 - 15	3.20 ± 0.08	5.40 ± 0.30	6.00 ± 0.045	6.50 ± 0.029
1-CQ	15 - 30	1.9 ± 0.11	2.90 ± 0.18	3.52 ± 0.020	3.10 ± 0.034
3-CQ	0 - 15	3.20 ± 0.08	3.60 ± 0.29	3.95 ± 0.030	4.00 ± 0.043
3-CQ	15 - 30	1.9 ± 0.11	1.80 ± 0.33	2.00 ± 0.11	2.11 ± 0.020
5-CQ	0 – 15	3.20 ± 0.08	2.50 ± 0.39	2.60 ± 0.037	2.63 ± 0.030
5-CQ	15 - 30	1.9 ± 0.11	0.90 ± 0	0.92 ± 0.040	1.00 ± 0.008

[a]S.E*: Standard error at 95% confidence level*

CONCLUSION

The above results show that *Leucaena leucocephala* 'may' not be good petroleum hydrocarbon-remediating plant since it failed to germinate in the crude oil impacted soils. Out of the eight parameters (or soil quality indicators) used to access the efficacy of *P. pterocarpum and C. retusa*, both legumes elevated the levels of the three that were lowered, (3 significantly at $p>0.05$, and 1 non-significantly at $p<0.05$). Both legumes also reduced the levels of the two parameters that were elevated, (1 significantly at $p>0.05$, 1 non-significantly at $p<0.05$). These imply that both legumes are good phytoremediators of crude-oil contaminated soils.

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