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Physicochemical and biological 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 biological properties (soil quality indicators) as evaluation criteria. Results after a 15-month remediation period showed that only Leucaena leucocephala failed to germinate. The level of MC in the Peltophorum pterocarpum-remediated soil samples was significantly ($p>0.05$) elevated to 87%, relative to the respective contaminated samples, while those of THC (94%), THUB (824%), K^+ (53%), Ca^{2+} (59%) and Mg^{2+} (58%) were significantly ($p>0.05$) reduced; the pH was non-significantly ($p<0.05$) elevated to 14%, whereas the Na^+ (35%) and THB (5%) were non-significantly ($p<0.05$) reduced. The Crotalaria retusa-remediated soils had the level of MC (48%) significantly ($p>0.05$) elevated, while those THC (95%), THUB (712%), K^+ (58%), Na^+ (54%), Ca^{2+} (77%) and Mg^{2+} (52%) were significantly ($p>0.05$) reduced; the pH was non-significantly ($p<0.05$) elevated to 12%, whereas the THB was non-significantly ($p<0.05$) reduced by 12%. These results indicate that L. leucocephala 'may' not be a good crude-oil remediating leguminous plant, while both P. pterocarpum and C. retusa are efficient crude-oil remediating leguminous plants.

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

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 (Osam, 2011; Nwaugo *et al*, 2006, 2007; Wellingia *et al*, 1999).

When oil spills 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.

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).

MATERIALS AND METHODS

1.2.1 Materials

In addition to the laboratory reagents, the following chemicals and biochemicals were used for the work: Forty litres of crude oil (obtained from Nigerian Agip Oil Company, NAOC, Ebocha, Rivers State), over 200 seeds of each of the legumes:

1. Yellow flame tree, *Peltophorum pterocarpum* (figure 1). This was obtained from the Convocation arena of the University of Port Harcourt, Nigeria.

Figure 1: Yellow Flame Tree (*Peltophorum pterocarpum*)

2. Miracle tree, *Leucaena leucocephala* (figure 2). This was obtained from Bayelsa State, Nigeria.

Figure 2: Miracle Tree (*Leucaena leucocephala*)

3. Rattle weed, *Crotalaria retusa* (figure 3). This was obtained from the International Institute of Tropical Agriculture, IITA. Eneka, Nigeria.

Figure 3: Rattle Weed (*Crotalaria retusa*)

These legumes were identified, classified and authenticated as being of high quality by the Department of Plant Anatomy and Physiology, University of Port Harcourt, Nigeria.

METHODS

(i) 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.

(ii) Contamination of the plots 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.

(iii) 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.

(iv) 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.

(ix) 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).

(x) 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).

(xi) Enumeration of bacterial load

The total heterotrophic bacteria (THB) count was performed on nutrient agar (Oxoid), using the spread plate method (Gradi, 1985), while the vapour-phase transfer method was adapted to estimate the population of the total hydrocarbon utilizing bacteria (THUB), as reported by Ebuehi, *et al*, (2005).

(xii) Determination of THC contents of the soil

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

(xiii) Determination of concentration of exchangeable cations

The concentration of the cations: K^{+}, Na^{+}, Mg^{2+} and Ca^{2+} in the soil samples were determined using the flame photometric method as reported by Jackson, (1970).

(ix) 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 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 THC, while tables 4 and 5 show those for the THB and THUB respectively. Finally, tables 6-9 show the results for the exchangeable cations analyzed.

DISCUSSION AND CONCLUSION

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.

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 ($p>0.05$) higher than those of the contaminated soil samples. Both *P. pterocarpum* and *C. retusa* remediated soils with 65% reduced THC of the contaminated soil levels show that the degradable ability of the legumes was promising. This can be likened to a similar observation for red fescue and ryegrasses (Reynolds and Wolf, 1999), which significantly reduced the hydrocarbon content from 6200 mg/kg to 1400 mg/kg or 77% after 640 days remediation period. Also, the works of Gunther *et al*, (1996), and Gudin and Syratt (1975) 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. Finally the strength of *C. retusa* in the containment of soil THC was on the top surface soils (0-15cm), while *P. pterocarpum* was on the sub-surface (15-30cm), buttressing the former as a shallow-rooted legume and the latter as a deep-rooted legume.

The study reveals that the total heterotrophic bacteria (THB) count of both soil samples remediated with planted *C. retusa* and planted *P. Pterocarpum* were not significantly ($p<0.05$) different from those of the contaminated samples, but the total hydrocarbon utilizing bacteria (THUB) counts were significantly ($p>0.05$) different. The soil samples remediated with planted *C. retusa* had the THB counts reduced by 12%, while the *P. pterocarpum*–remediated soils were reduced by 5%. The THUB counts of the *C. retusa*-remediated soil samples were elevated by 712% while that of the *P. pterocarpum*–soils were elevated by 824%. The THB loads in the contaminated soils increased with increasing crude oil concentration, while the THUB counts decreased with increasing crude oil concentration. These agree with the works of Nwaugo *et al*, (2007) and Nwaugo *et al*, (2008) who posited that the less effect on THB could be understood as the group is the sum total of the heterotrophic bacteria (all viable and culturable) present in the soil at that point of contamination, while the high effect on THUB can used as good indicators of anthropogenic pollution of the soil. It also meant that the pollution disturbed the THUB metabolism and proliferation which resulted in low bioload as observed. Unlike the THB which were not much affected in the remediated samples, the THUB were highly affected in the remediated moderately and lightly impacted soils. The THB is a complex group and sum of all the viable bacteria, hence could not be much affected. The highly elevated bioload of the THUB in the remediated samples, with both legumes, *P. pterocarpum* (824%) and *C. retusa* (712%) indicated that the interaction between the roots of the legumes and the micro-organisms (the rhizosphere effect) provided root exudates of carbon, energy, nutrients, enzymes and oxygen to the microbial populations. This plant-induced enhancement of the THUB population according to Atlas and Bartha, (1998) is believed to result in enhanced degradation of the crude oil in the rhizophere. The slighly reduced load of THB in the remediated samples by the legumes, *C. retusa* (12%) and *P. pterocarpum* (5%) indicated low levels of the nitrogen and phosphorus content in the soil occasioned by the containment of these elements, which according to Odokuma and Dickson, (2003b), might have been the limiting nutrient elements. These observations were corroborated by similar works of Martensson (1993), and Oliveira and Pampulha, (2006).

Results of the analyses show that the exchangeable cation with the highest concentration measured in the crude oil contaminated soils was calcium ion (table 7) with a mean concentration range of 2.40±0.018-5.56±0.02meq/100g, followed by sodium ion (table 5) with a mean concentration range of 0.83±0.03–0.95±0.035meq/100g. The least cation measured was potassium ion (table 6) with a mean concentration range of 0.15±0.049-0.33±0.03meq/100g. The result also revealed that the legumes were effective in reducing especially the concentrations of the cations, especially the Ca^{2+} that was mostly impacted. Soil particles carry plant nutrients which exist as ions. The concentrations of the exchangeable cations: Ca^{2+}, Na^{+}, K^{+} and Mg^{2+} increased with increasing crude oil pollution (contamination). Similar trends had been observed by past workers. For instance, Onyeike *et al*, (2000), reported such increases in exchangeable cations of soils from crude oil polluted soil in Ogoni land. Potassium ion (K^{+}) and magnesium ion (Mg^{2+}) concentrations observed in the contaminated and control soil samples were within the range for the low fertility class of Nigerian soils. The high calcium ion (Ca^{2+}) observed in both the control and contaminated soils may be due to anthropogenic origin. The measured amount of all the exchangeable cations in the soils remediated by both legumes were significantly ($p>0.05$) different from those of contaminated soils, implying that both legumes had the capability of reducing the cations introduced into the soil as a result of the simulated crude oil.

In conclusion, *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 2 that were lowered, (1 significantly at $p>0.05$, and 1 non-significantly at $p<0.05$). Both legumes also reduced the levels of the six parameters that were elevated, (5 significantly at $p>0.05$, 1non-significantly at $p<0.05$ by *C. retusa*, and 4 significantly at $p>0.05$, 2 non-significantly at $p<0.05$ by *P. pterocarpum*). These imply that both legumes are good phytoremediators of crude-oil contaminated soils.

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

SAMPLE	DEPTH	CONTROL	CONTAMINATED	REMEDIATED BY	
				P. pterocarpum	*C. retusa*
LOCATION	(cm)	(cm)	$\overline{(X)} \pm$ 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]) THC, (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	91.90 ± 0.11	1534.00 ± 3.70	89.80 ± 0.14	130.00 ± 2.40
1-CQ	15 - 30	77.30 ± 0.08	1224.00 ± 4.60	19.50 ± 0.27	41.90 ± 0.33
3-CQ	0 - 15	91.90 ± 0.11	1770.00 ± 0.80	181.00 ± 5.20	198.00 ± 3.70
3-CQ	15 - 30	77.30 ± 0.08	1594.00 ± 3.70	63.00 ± 3.50	47.00 ± 0.24
5-CQ	0 – 15	91.90 ± 0.11	2965.00 ± 3.00	190.00 ± 2.40	92.90 ± 0.14
5-CQ	15 - 30	77.30 ± 0.08	2291.00 ± 1.40	92.70 ± 0.08	24.80 ± 0.30

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

TABLE 4: Mean (±S.E[a]) thb count (X 10^5cfu/g) 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	48.00 ± 2.00	54.00 ± 3.70	52.00 ± 0.80	51.00 ± 2.00
1-CQ	15 - 30	21.00 ±2.00	27.00 ± 1.10	29.00 ± 5.30	30.00 ± 4.30
3-CQ	0 - 15	48.00 ± 2.00	63.00 ± 3.00	62.00 ± 1.10	60.00 ± 0
3-CQ	15 - 30	21.00 ±2.00	34.00 ± 1.10	32.00 ± 2.40	32.00 ± 2.90
5-CQ	0 – 15	48.00 ± 2.00	230.00 ± 2.00	214.00 ± 2.40	180.00 ± 3.50
5-CQ	15 - 30	21.00 ±2.00	42 00 ± 2.40	40.00 ± 0	41.00 ± 2.00

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

TABLE 5: Mean (±S.E[a]) thub count (X 10^2cfu/g) 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	2160 ± 3.00	370 ± 4.90	2000 ± 25.00	1700 ± 10.00
1-CQ	15 - 30	1200 ± 4.50	170 ± 4.90	1500 ± 30.00	1392 ± 2.40
3-CQ	0 - 15	2160 ± 3.00	295 ± 4.10	1870 ± 6.50	1610 ± 7.90
3-CQ	15 - 30	1200 ± 4.50	130 ± 1.80	1340 ± 4.90	1100 ± 0
5-CQ	0 – 15	2160 ± 3.00	10 ± 0.16	1660 ± 5.70	1570 ± 10.40
5-CQ	15 - 30	1200 ± 4.50	9.25 ± 0.03	718 ± 3.70	620 ± 1.60

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

TABLE 6: Mean (±S.E[a]) Na^+ CONC[b], (meq/100g) 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	0.33 ± 0.04	0.95 ± 0.035	0.40 ± 0.011	0.37 ± 0.008
1-CQ	15 - 30	0.33 ± 0	0.83 ± 0.03	0.38 ± 0	0.34 ± 0.011
3-CQ	0 - 15	0.33 ± 0.04	0.73 ± 0.03	0.66 ± 0.029	0.40 ± 0.018
3-CQ	15 - 30	0.33 ± 0	0.78 ± 0.029	0.59 ± 0.008	0.38 ± 0.052
5-CQ	0 – 15	0.33 ± 0.04	0.88 ± 0.008	0.66 ± 0.011	0.44 ± 0.039
5-CQ	15 - 30	0.33 ± 0	0.92 ± 0.011	0.69 ± 0.014	0.41 ± 0.011

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

TABLE 7: Mean (±S.E[a]) K^+ CONC[b], (meq/100g) 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	0.08 ± 0.023	0.15 ± 0	0.09 ± 0.008	0.07 ± 0.011
1-CQ	15 - 30	0.05 ± 0.011	0.15 ± 0.03	0.07 ± 0.02	0.06 ± 0.008
3-CQ	0 - 15	0.08 ± 0.023	0.15 ± 0.049	0.11 ± 0.014	0.08 ± 0.024
3-CQ	15 - 30	0.05 ± 0.011	0.15 ± 0.011	0.08 ± 0.011	0.07 ± 0
5-CQ	0 – 15	0.08 ± 0.023	0.33 ± 0.03	0.10 ± 0	0.011 ± 0.008
5-CQ	15 - 30	0.05 ± 0.011	0.21 ± 0.024	0.08 ± 0.008	0.08 ± 0.02

[a]S.E*: Standard error at 95% confidence level* ; [b]CONC: *Concentration*

TABLE 8: Mean (±S.E[a]) Ca^{2+} CONC[b], (meq/100g) 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	1.88 ± 0.023	2.87 ± 0.008	1.10 ± 0.011	1.01 ± 0.014
1-CQ	15 - 30	1.57 ± 0.014	2.40 ± 0.018	0.25 ± 0.018	0.41 ± 0.011
3-CQ	0 - 15	1.88 ± 0.023	3.06 ± 0.011	2.22 ± 0.008	1.86 ± 0.024
3-CQ	15 - 30	1.57 ± 0.014	3.04 ± 0.02	0.38 ± 0.023	0.45 ± 0.018
5-CQ	0 – 15	1.88 ± 0.023	5.56 ± 0.02	5.31 ± 0.014	0.75 ± 0
5-CQ	15 - 30	1.57 ± 0.014	3.39 ± 0.02	0.18 ±0.018	0.25 ± 0

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

TABLE 9: Mean (±S.E[a]) Mg^{2+} CONC[b], (meq/100g) 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	0.20 ± 0.023	0.37 ± 0.02	0.19 ± 0.014	0.15 ± 0.011
1-CQ	15 – 30	0.20 ± 0	0.20 ± 0.03	0.08 ± 0	0.09 ± 0.014
3-CQ	0 – 15	0.20 ± 0.023	0.41 ± 0.014	0.19 ± 0.024	0.16 ± 0.008
3-CQ	15 – 30	0.20 ± 0	0.23 ± 0.011	0.07 ± 0	0.11 ± 0.011
5-CQ	0 – 15	0.20 ± 0.023	0.48 ± 0.024	0.23 ± 0.011	0.27 ± 0.011
5-CQ	15 – 30	0.20 ± 0	0.30 ± 0	0.10 ± 0.011	0.16 ± 0

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

REFERENCES

[1] U Dalyan; H Harder; TH Hopner. (1990). Hydrocarbon biodegradation in sediments and soils: A systematic examination of physical and chemical conditions. Part II pH values. Wissensachft and Technik.
[2] DB Duncan; (**1955**). *Biometrics*. **11**:1- 42.
[3] OAT Ebuehi; IB Abibo; PO Shekwolo; KI Sigismund; A Adoki; IC Okoro. (**2005**). *Journal of Applied Sciences and Environmental Management.* **9**(1): 103-106.
[4] CM Frick; RE Farrell; JJ Germid. (**1999**). Assessment of Phytoremediation as an *In-Situ* Technique for cleaning oil-contaminated Sites. (Technical Paper, Petroleum Technology Alliance of Canada, Calgary, AB. P.I.)
[5] PC Gradi. (**1985**). Biodegradation. *Biotechnology and Bio-Engineering*. **27**:660-674.
[6] C Gudin; WJ Syratt. (**1975**). *Environmental Pollution.* **8**: 107 – 112.
[7] T Gunther; U Dornberger; W Fritscz. (**1996**). *Chemosphere*. **33** (2): 203-215.
[8] ML Jackson. (**1970**). Soil Chemical Analysis. New Jersey, Prentice-Hall Inc. P. 498.
[9] AM Martensson. (**1993**). *Bull. Environ. Contam. Toxicol.* **50**: 466-473.
[10] VO Nwaugo; RA Onyeagba; EI Akubugwo; O Ugbogu. (**2008**). *Biokemistri.* **20**(2): 77-84.
[11] VO Nwaugo; RA Onyeagba; N Azu; O Nworie. (**2007**). *Estud. Biol.* **29** (66): 89-97.
[12] VO Nwaugo; RA Onyeagba; N Azu; NC Nwachukwu. (**2006**). *J. Sc. Engr. Tech*. **13**(2):6697–6706.

[13] NM Nwinuka; EB Essien; LC Osuji. (**2003**). Soil Anlysis. In: E. N. Onyeike and J. O. Osuji (Eds). Research Techniques in Biological and Chemical Sciences. Springfield Publishers Ltd. Owerri, Nigeria. Pp. 369-402.
[14] LO Odokuma. AA Dickson. (**2003**). *Journal of Applied Sciences and Environmental Management*. **7** (2): 23-29.
[15] A Oliveira; ME Pampulha. (**2006**). *J. Biosc. Bioeng*. **102**. 157-161.
[16] EN Onyeike; SI Ogbuja; NM Nwinuka. (**2000**). *Environ. Monitoring and Assessment.* **73**: 1991-2125.
[17] MU Osam. (**2011**). Evaluation of the efficacy of selected wild-type legumes in the remediation of crude oil contaminated Agricultural soils. PhD Dissertation, Biochemistry Department, University of Port Harcourt, Nigeria.
[18] MU Osam. (**2006**). Physicochemical Assessment of Omoku Soils Affected by oil spillage. M.Sc Thesis, Biochemistry Department, University of Port Harcourt, Nigeria.
[19] LC Osuji. EJ Egbuson; CM Ojinnaka. (**2005**). *Chemistry and Ecology*. **21** (1): 1-10.
[20] LC Osuji; CM Onojake. (**2004**). *Chemistry and Biodiversity*, **1**: 1708-1715.
[21] SP Pradham; JR Conrad; JR Paterck; VJ Srivastava. (**1998**). *Journal of Soil Contamination* **7** (4): 467-480.
[22] CM Reynolds; DC Wolf. (**1999**). Microbial based strategies for assessing rhizosphere enhanced phytoremediation. Proceedings of the Pytoremediation Technology Seminar. May 31-June 1, 1999 Calgary, AB. Environment Canada: Ottawa. Pp. 125-135.
[23] BG Simeonova; LI Simeonov. (**2006**). *Journal of Environmental Clean-up costs, technologies and techniques*. **16** (2): 113-123.
[24] G Thoma; D Wolf; S Ziegle. (**2002**). Using Plants to Remediate Petroleum-Contaminated Soil-Project. Continuation Annual Report. *http://www.rtdf.org* (Retrieved, 30/07/2008).
[25] B Wellingia; JR Lucy; JN Moore; O Seastone; JE Gamen. (**1999**). *Appl. Environ. Microbiol*. **65**: 1548 – 1555.