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Soil pH, Moisture Content and Some Macro Non-Metallic Elements in Crude Oil Contaminated Soils Remediated By Some Wild-Type Legumes

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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 the soil physicochemical parameters and macro non-metallic elements concentrations as evaluation criteria. Results after a 15-month remediation period showed that only Leucaena leucocephala failed to germinate. The level of moisture content, MC (87%), in the Peltophorum pterocarpum-remediated soil samples was significantly ($p>0.05$) elevated, relative to the respective contaminated samples. The Crotalaria retusa-remediated soils had the level of MC (48%) also significantly ($p>0.05$) elevated, relative to the respective contaminated samples while the levels of nitrogen, N (27%) was non-significantly ($p<0.05$) reduced. The levels of pH in both the P. pterocarpum and C. retusa-remediated soils were non-significantly ($p<0.05$) elevated, while phosphorus, P, non-significantly ($p<0.05$) reduced, by both legumes. These results indicate that miracle tree, Leucaena leucocephala 'may' not be a good remediating legume, while both yellow flame tree, Peltophorum pterocarpum and rattle weed, Crotalaria retusa are good remediating legumes for crude-oil impacted soils.*

KEYWORDS**:** *Crotalaria retusa, Crude oil, Leucaena leucocephala, Peltophorum pterocarpum, Remediation, Wild-type legumes*

I. 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, 2009).

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 meltdown. 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).

II. MATERIALS AND METHODS

2.1. Materials

In addition to the laboratory reagents, the following chemicals and biochemical 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 the International Institute of Tropical Agriculture, IITA. Eneka, Rivers State.

Figure 2: MIRACLE TREE (*Leucaena leucocephala*)

(3) Rattle weed, *Crotalaria retusa* (figure 3). This was obtained from Bayelsa State, Nigeria.

Figure 3: RATTLE WEED (*Crotalaria retusa*)

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

2.2 Methods

2.2.1 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.

2.2.2 Contamination of the plots

The contamination was done as follows:- Plots E_1- E_3 (1-CQ), 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-CQ), and E_7- E_9 (5-CQ) but with 3% and 5% by weight of the crude oil respectively. Contaminated samples were collected 7 days after the contamination.

2.2.3 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.

2.2.4 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.

2.2.5 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).

2.2.6 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).

2.2.7 Determination of the concentrations of soil macro non-metallic elements

The Macro-Kjeldahl method (Kjeldahl, 1883) was adopted to determine total soil nitrogen, while available soil phosphorus was evaluated following the molybdenum blue method using stannous chloride as reported by Wegwu and Onyeike, (2006).

2.2.8 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.

III. 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 ; that for the moisture content analysis in table 2, while tables 3 and 4 show those for the % nitrogen and available phosphorus respectively.

TABLE 1: MEAN (±S.E[a]) pH OF REMEDIATED SOIL SAMPLES

				REMEDIATED BY	
SAMPLE	**DEPTH**	**CONTROL**	**CONTAMINATED**	***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

				REMEDIATED BY	
SAMPLE	**DEPTH**	**CONTROL**	**CONTAMINATED**	***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]) % NITROGEN, N CONC[b], OF REMEDIATED SOIL SAMPLES

				REMEDIATED BY	
SAMPLE	**DEPTH**	**CONTROL**	**CONTAMINATED**	***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	0.115 ± 0.001	0.14 ± 0.020	0.07 ± 0.018	0.09 ± 0.014
1-CQ	15 – 30	0.108 ± 0.002	0.12 ± 0.024	0.06 ± 0.014	0.08 ± 0.024
3-CQ	0 – 15	0.115 ± 0.001	0.18 ± 0.024	0.10 ± 0	0.13 ± 0.018
3-CQ	15 – 30	0.108 ± 0.002	0.17 ± 0.008	0.09 ± 0.008	0.12 ± 0.018
5-CQ	0 – 15	0.115 ± 0.001	0.20 ± 0.008	0.13 ± 0.027	0.15 ± 0.014
5-CQ	15 – 30	0.108 ± 0.002	0.18 ± 0.011	0.11 ± 0.024	0.015 ± 0.011

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

TABLE 4: MEAN (±S.E[a]) PHOSPHORUS, P CONC[b], (ppm) OF REMEDIATED SOIL SAMPLES

				REMEDIATED BY	
SAMPLE	**DEPTH**	**CONTROL**	**CONTAMINATED**	***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	0.44 ± 0.008	0.54 ± 0.030	0.53 ± 0.024	0.52 ± 0.011
1-CQ	15 – 30	0.31 ± 0.014	0.45 ± 0.037	0.50 ± 0.008	0.52 ± 0
3-CQ	0 – 15	0.44 ± 0.008	1.76 ± 0.011	1.10 ± 0.014	1.20 ± 0.029
3-CQ	15 – 30	0.31 ± 0.014	0.88 ± 0.024	0.80 ± 0.008	0.85 ± 0.008
5-CQ	0 – 15	0.44 ± 0.008	3.45 ± 0.018	2.85 ± 0.044	2.90 ± 0.018
5-CQ	15 – 30	0.31 ± 0.014	2.90 ± 0.029	2.00 ± 0.008	2.10 ± 0.014

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

IV. DISCUSSIONS

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) and Osam, *et al.*; (2011a and 2011b) who posited in their earlier works 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.

Result of the macro non-metallic elements measured indicated that available phosphorus and total nitrogen levels were elevated in the contaminated soil samples compared to their controls, and also not significantly (p<0.05) reduced relative to those remediated with both legumes. The observed increases were also greatest in the heavily impacted soils. The soils remediated with planted *P. pterocarpum* had more reduced total nitrogen (44% of the contaminated) than the soils remediated with *C. retusa* (29% of the contaminated). Similarly, *P. pterocarpum* was more effective (9%) than *C. retusa* (6%) in the reduction of available

phosphorus, though the value was insignificant at the 95% level. These show that the legumes were effective in maintaining the soil nitrogen and available phosphorus balance to such a level that bioaccumulation or over reduction leading to deficiency was not feasible. Legumes are plants that have mechanisms in their root systems that provide root exudates (energy, carbon, nutrients, enzymes etc) to microbial populations in the rhizosphere (Cunningham *et al*, 1996). *P. pterocarpum* and *C. retusa* are not exceptions. These exudates induce or enhance microbial populations which result in enhanced degradation of organic contaminants in the rhizosphere. The added nitrogen to the contaminated soil could have been used up by the microbes carrying out the degradation process. Also, the low level of phosphorus in the remediated soils was due to the immobility (reduced availability) of phosphorus; it may not have been sufficiently dissolved in the soil to make it available, while the little that was dissolved may have been rapidly utilized by the existing soil microbial populations.

Several studies serve as examples of the rhizosphere effect in the phytoremediation of organic contaminants. Gunther *et al*, (1996) suggested that plant roots stimulated the microbes, which enhanced the degradation of the hydrocarbon mixture.

V. CONCLUSION

The above results clearly attest to the fact 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 four parameters (or soil quality indicators) used to assess the efficacy of both legumes, they elevated the levels of the two parameters that were lowered, (1 significantly at $p>0.05$, and 1 non-significantly at $p<0.05$) and also non-significantly at $p<0.05$ reduced the levels of the two parameters that were elevated. These imply that both legumes are good phytoremediators of crude-oil contaminated soils.

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