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An Assessment of Some Heavy Metal Elements in Crude Oil Contaminated Soils Remediated By Some Wild-Type Legumes

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ABSTRACT: *The efficacy of the three wild-type legumes, miracle tree (Leucaena leucocephala), yellow flame tree (Peltophorum pterocarpum), and rattle weed (Crotalaria retusa), 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 of heavy metallic elements as evaluation criteria. Results after a 15-month remediation period showed that only L. leucocephala failed to germinate. The levels of all the heavy metals investigated, namely: copper, Cu (49%), lead, Pb (77%), cadmium, Cd (75%), iron, Fe (68%) and zinc, Zn (65%) were significantly ($p<0.05$) reduced, in both the P. pterocarpum and C. retusa-remediated soil samples, relative to their respective contaminated samples. These results indicate that L. leucocephala 'may' not be a good remediating legume, while both P. pterocarpum and C. retusa are good remediating legumes for crude-oil impacted soils.*

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

I. INTRODUCTION

Trace metals are metals occurring at 1000mg/kg or less in the earth's crust [1]. Such metals may be classified as 'heavy' or 'light' with respect to density. Trace 'heavy' metals have densities greater than $5gcm^{-3}$ (e.g. vanadium) whereas 'light' metals have densities less than $5gcm^{-3}$ (e.g. beryllium). Trace heavy metals are in turn classified as nutrient and non-nutrient heavy metals, according to their nutritional and biochemical significance [2]. Cadmium and lead are designated as non-nutrient trace-elements, as they have no known function in humans. These metals are known to be toxic even at very low levels of intake, and present no identified deficiency symptoms. Excessive amounts of trace heavy metals (as well as trace light metals) may occur in the biosphere as a result of normal geological phenomena such as ore formation, weathering of rocks, and leaching or degassing (in the case of mercury). Other activities that could contribute to excessive release of these metals into the environment include burning of fossil fuels, smelting and discharges of industrial, agricultural, and domestic wastes as well as deliberate application of pesticides. Anthropogenic contributions or human activities such as petroleum prospecting and mining as well as oil spillages are also major sources of these metals [3].

The ecological significance of heavy metals, in recent times, is traceable to the growing awareness of their accumulative characteristics and toxicity [4]. These inorganic ions are generally non-biodegradable and undergo an ecobiological cycle. The peculiarity of heavy metals lies in their ability to accumulate unnoticed to toxic levels [5]. This, of course, is distinct from other pollutants such as petroleum hydrocarbons and litter, which may visibly build up in the environment. The toxicological action of cadmium has been attributed to its chemical similarity with zinc, as cadmium may conveniently replace zinc in some enzymes, thus altering their three–dimensional structure and impairing catalytic activity [6].

The soil is very important to 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 [7] [8] [9] [10].

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 [11], 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.

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

II. MATERIALS AND METHODS

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 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 legumes were identified, classified and authenticated as being of high quality by the Department of Plant Anatomy and Physiology, 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 10th 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

Contamination of the plots were done as follow: 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,* [16], 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.

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 [11], 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 concentration of trace heavy metals

The concentration of the trace heavy metals: Cd, Pb, Fe, Cu and Zn in the soil samples was determined by the atomic absorption spectrophotometric (AAS) technique from the acid digests as reported by Osam *et al,* [17].

2.2.6 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*, [18]. 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 [19] 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 trace heavy metals, iron, zinc, cadmium and copper are shown in tables 1, 2, 3, 4 and 5 respectively, below:

TABLE 1: MEAN (±S.E[a]) IRON, Fe CONC[b], (mg/kg) 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-EQ	0 – 15	5576 ± 2.40	6249 ± 3.00	2710 ± 3.00	2289 ± 1.40
1-EQ	15 – 30	4746 ± 5.60	5645 ± 4.90	3116 ± 3.50	3205 ± 4.90
3-EQ	0 – 15	5576 ± 2.40	7041 ± 3.00	2156 ± 6.00	2828 ± 4.90
3-EQ	15 – 30	4746 ± 5.60	6799 ± 0.80	2820 ± 3.90	3031 ± 1.60
5-EQ	0 – 15	5576 ± 2.40	7779 ± 3.00	2096 ± 5.60	1203 ± 2.30
5-EQ	15 – 30	4746 ± 5.60	7697 ± 2.30	2908 ± 2.40	2300 ± 1.40

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

[b] CONC: *Concentration*

TABLE 2: MEAN (±S.E[a]) ZINC, Zn CONC[b], (mg/kg) 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-EQ	0 – 15	5.00 ± 0.079	10.43 ± 0.023	4.43 ± 0.040	6.27 ± 0.023
1-EQ	15 – 30	4.40 ± 0.080	10.20 ± 0	2.14 ± 0.040	2.66 ± 0.049
3-EQ	0 – 15	5.00 ± 0.079	11.50 ± 0.023	6.80 ± 0.008	5.62 ± 0.043
3-EQ	15 – 30	4.40 ± 0.080	10.70 ± 0.040	2.01 ± 0.020	2.25 ± 0.011
5-EQ	0 – 15	5.00 ± 0.079	11.70 ± 0.020	5.22 ± 0.030	4.57 ± 0.024
5-EQ	15 – 30	4.40 ± 0.080	11.67 ± 0.014	2.50 ± 0	2.05 ± 0.049

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

[b] CONC: *Concentration*

TABLE 3: MEAN (±S.E[a]) CADMIUM, Cd CONC[b], (mg/kg) 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-EQ	0 – 15	0.10 ± 0	0.13 ± 0.034	0.018 ± 0.004	0.090 ± 0.020
1-EQ	15 – 30	0.06 ± 0.011	0.12 ± 0.008	0.050 ± 0.011	0.017 ± 0.004
3-EQ	0 – 15	0.10 ± 0	0.20 ± 0.023	0.012 ± 0.011	0.110 ± 0.008
3-EQ	15 – 30	0.06 ± 0.011	0.20 ± 0.030	0.030 ± 0	0.100 ± 0.018
5-EQ	0 – 15	0.10 ± 0	0.33 ± 0.079	0.060 ± 0.030	0.020 ± 0.008
5-EQ	15 – 30	0.06 ± 0.011	0.24 ± 0.020	0.020 ± 0.018	0.050 ± 0

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

[b] CONC: *Concentration*

TABLE 4: MEAN (±S.E[a]) COPPER, Cu CONC[b], (mg/kg) OF REMEDIATED SOIL SAMPLES

				REMEDIATED BY	
SAMPLE	**DEPTH**	**CONTROL**	**CONTAMINATED**	***P. pterocarpum***	***C. retusa***
LOCATION	**(cm)**	$\overline{(X)}$ ± S.E.	$\overline{(X)}$ ± S.E.	$\overline{(X)}$ ± S.E.	$\overline{(X)}$ ± S.E.
1-EQ	0 – 15	2.02 ± 0.023	2.37 ± 0.030	1.77 ± 0.008	1.42 ± 0.024
1-EQ	15 – 30	1.80 ± 0.034	2.21 ± 0.030	1.35 ± 0	1.37 ± 0.024
3-EQ	0 – 15	2.02 ± 0.023	2.60 ± 0.029	1.45 ± 0.034	1.60 ± 0.008
3-EQ	15 – 30	1.80 ± 0.034	2.42 ± 0.023	0.99 ± 0.011	1.58 ± 0.023
5-EQ	0 – 15	2.02 ± 0.023	2.85 ± 0.018	1.08 ± 0.023	1.22 ± 0.020
5-EQ	15 – 30	1.80 ± 0.034	2.60 ± 0	1.07 ± 0.011	0.91 ± 0.014

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

[b] CONC: *Concentration*

TABLE 5: MEAN (±S.E[a]) LEAD, Pb CONC[b], (mg/kg) OF REMEDIATED SOIL SAMPLES

				REMEDIATED BY	
SAMPLE	**DEPTH**	**CONTROL**	**CONTAMINATED**	***P. pterocarpum***	***C. retusa***
LOCATION	**(cm)**	$\overline{(X)}$ ± S.E.	$\overline{(X)}$ ± S.E.	$\overline{(X)}$ ± S.E.	$\overline{(X)}$ ± S.E.
1-EQ	0 – 15	0.44 ± 0.043	1.80 ± 0.008	0.49 ± 0.014	0.52 ± 0.024
1-EQ	15 – 30	0.22 ± 0.008	1.47 ± 0.030	0.46 ± 0.030	0.51 ± 0
3-EQ	0 – 15	0.44 ± 0.043	2.95 ± 0.020	0.52 ± 0	0.77 ± 0.040
3-EQ	15 – 30	0.22 ± 0.008	2.70 ± 0.014	0.50 ± 0.027	0.71 ± 0.018
5-EQ	0 – 15	0.44 ± 0.043	3.35 ±0.023	0.77 ± 0.052	0.90 ± 0.023
5-EQ	15 – 30	0.22 ± 0.008	3.10 ± 0.020	0.72 ± 0.030	0.86 ± 0.011

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

[b] CONC: *Concentration*

IV. DISCUSSIONS

Trace heavy metals such as Fe, Cu, Pb, Mn and Zn are among the 16 essential elements required by plants, though needed in only very small quantities. They may also inhibit microbial metabolism in high amounts. The result of all the heavy metals measured in the contaminated soils shows that the mean range of Fe for the both surface soils was 5645±4.9-7779±3.0mg/kg, while for the soils remediated with *P. pterocarpum* was 316±3.6-2908±2.4mg/kg and for those remediated with *C. retusa* was 1203±2.3-3205±4.9mg/kg. This reveals the outrageously high value of Fe, higher than the acceptable limit of 100mg/kg for both the contaminated and remediated soils. The rest of the metals measured were very low and within the ambit of acceptability. Naturally, soils at various locations have a trace amount of heavy metals even in undisturbed environments. Enhanced concentrations of metals like Fe, Cd, Cu, Pb and Zn in the soils may result to enhanced absorption by plants. Possible bioaccumulation may occur, which may lead to toxic reactions along the food chain [20]. The values obtained for the heavy metals in this study compared favourably with the findings of past workers like Wegwu and Onyeike [14]. However, the high values of Fe measured though significantly (p<0.05) remediated (48% by *P. pterocarpum* and 46% by *C. retusa*), were still far beyond the acceptable limit of 100mg/kg, a second planting of the legumes will be imperative to lower the concentration of Fe in the soils. Several other workers like Nwaugo *et al*, [21] had also measured high Fe concentrations in abandoned mine pits, as well as Akubugwo *et al* [22], who posited that the high Fe content may be due to the reduction of Fe^{3+} to Fe^{2+} as a result of favourable reducing conditions provided by the oil pollution. Such high concentrations of Fe^{2+} in the soil may render the soil toxic for plant growth, hence an overall pollution. The high percentage reduction observed in the remediated oils relative to the contaminated, especially for Fe by both legumes lucidly attests to the ability of the plants in containment of the toxic substances.

Several studies serve as examples of the rhizosphere effect in the phytoremediation of organic contaminants. Gunther *et al*, [23] 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 five heavy metals (or soil quality indicators) used to assess the efficacy of the legumes, *Peltophorum pterocarpum* and *Crotalaria retusa,* both legumes significantly (p<0.05) reduced the levels of the five heavy metals whose concentrations were elevated. These imply that both legumes are good phytoremediators of crude-oil contaminated soils.

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