Available online at www.pelagiaresearchlibrary.com

Pelagia Research Library

European Journal of Experimental Biology, 2013, 3(3):11-17

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Phytoremediation (Series 5): Organic carbon, matter, phosphorus and nitrogen trajectories as indices of assessment in a macrophytic treatment of hydrocarbon degraded soil environment

Edwin-Wosu N. L.

Department of Plant Science and Biotechnology, University of Port Harcourt, Rivers State, Nigeria

---

ABSTRACT

A comparative ecological study was conducted to ascertain the degree of utilization and restoration of nutrient element of crude oil polluted soil by two agro-forestry species (Luecaena leucocephala Lam. De wit. [Lead tree] and Bauhinia monandra Kurz). The study carried out in three phases involves the use of classical analytical methods of Nelson and Somners, Bray No. 1, Micro-Kjeldahl Stewarte methods and SAS statistical package. Result generally show that nitrogen and phosphorus were depleted in the post-pollution, though with the former significantly ($p<0.05$) lower than pre-pollution phase. The organic carbon and matter content had a significant ($p<0.05$) increase in post-pollution than pre-pollution. In phytoapplication process between the species treated soils L. leucocephala soil recorded significant ($p<0.05$) increase in nitrogen and concomitant increase in species content. Bauhinia monandra soil had non-significant ($p<0.05$) increase and decrease in species content than L. leucocephala soil and species respectively for phosphorus content. Carbon and organic matter content recorded reduction between soil and species content with significant ($p<0.05$) difference in L. leucocephala treatment. The trajectories in nitrogen, carbon, organic matter and phosphorus in L. leucocephala treated soil are indicative of their utilization in bioremediation process by L. leucocephala plant. It also shows its enhancing potential in environmental management. The unexpected ineffectivity of B. monandra is attributed to its peculiar non-symbiotic characteristics and the inadequacy of nodulation and low utilization of the nutrient for bioremediation. Based on this L. leucocephala has proved more suitable for bioremediation practice in a polluted terrestrial habitat.

Key words: nutrient elements, phytoremediation, pre-pollution, post-pollution, and post- phytoapplication.

INTRODUCTION

An understanding of terrestrial environmental pollution begins with an understanding of and respect for soil habitat. Through understanding, poor soils can be made good and good ones better still, just as careless and unwise use might still lower the value of a very good soil and perhaps reduce it productivity for a very long time [1]. Soil differ in many ways and its understanding may involve getting to know the best use for many different types, with each being used and managed according to its physico-chemical properties. Among the major elements that are needed in large quantity to stimulate growth are carbon, hydrogen, and oxygen (CHO). Nitrogen and phosphorus are also essential for plant growth in the soil.

Nitrogen and phosphorus nutrient may be lost by various mechanisms, which might include leaching, crop harvest, soil erosion, denitrification, gaseous release, organic and inorganic pollutant release into the environment. Environmental pollution problem are colossal and most significantly it pose serious interference to natural ecological balance within the affected ecosystem. Crude oil pollution adversely affect the soil ecosystem through adsorption to soil particle, provision of excess carbon causing nutrient imbalance and induction of soil nitrogen and phosphorus limitation [2, 3]. Such imbalance causes delay in the natural self rehabilitation of crude oil polluted soils [4]. However, soil may be revived of its lost nutrient element through exogenous sources by agronomic practices, extrinsic and intrinsic fixation of nitrogen, and phosphorus respectively.

The relationship and variation in carbon, nitrogen and phosphorus content of soil are important indices to be considered in a remediation assessment of a polluted soil habitat. Carbon and nitrogen are parts of the component of soil organic matter, which is a by-product of both plant and animal remains. Soil organic matter contains about 60% C and 5%N by weight respectively [1]. The amount present is predicated on the amount present in the plant and animal matter that make up the humus and subsequent rate of humus mineralization. The intrinsic qualities of the soil itself may vary considerably between different soils in the same area according to their physicochemical properties, which thus influence phosphorus fixation based on type and total amount of clay minerals in the soil, presence of hydrous oxides of iron and aluminum, soil reaction and presence of organic matter in the soil [1].

Hence a nutrient imbalanced soil as a result of hydrocarbon pollution takes a long time for natural self remediation; it would then require human intervention through macrophytic application- phytoremediation. Phytoremediation is becoming an increasingly important clean up technology in environmental quality programmes. It is one of the new wave industries in the 21st century, an innovative and established technology geared toward the process of cleaning up the pollution incident of the 20th century using macrophytes. The impact of macrophytes on a polluted soil environment is most often assessed from changes in the physicochemical and biological component of the ecosystem. However, this work is focused on the changes and trajectories associated with organic carbon and matter, nitrogen and phosphorus content in a crude oil remediation process of soil under agro-forestry species.

MATERIALS AND METHODS

Source of Materials

Replicates of microplots of top loam soil (0-15cm depth) were made from the proposed University of Port Harcourt Botanic garden site while two agro-forestry plant species - *Leucaena leucocephala* and *Bauhinia monandra* were obtained from the Rivers State Agricultural Development Programme (ADP), Port Harcourt, and Faculty of Social Science premises, UNIPORT respectively. Crude oil was obtained from Shell Petroleum Development Company (SPDC), Port Harcourt.

Experimental design and Pollution of the Study Site with Crude Oil

The *nested completely randomized design* of Akindele [5] was adopted in which the nested analysis of variance (PROC. ANOVA) procedures [6] was carried out on series of experimental microplots set up in order to obtain data on the nutrient content of the plant species and soil component in different phases of the study. The study sites (micro plots of top loam soil, 0 to 15cm depth) were collected using the Stewarte *et al.* [7] and Song *et al*. [8] sampling methods. The pollutant (crude oil) was applied using a measuring cylinder in doses of 0ml, 25, 50 and 100mls per 14kg of soil in (V/W%) concentrations of 2, 4, and 7% respectively per 78cm^2 surface areas of 10 replicates each and the unpolluted replicates as control.

In each level of pollution, two phytotreatments options (*Leucaena leucocephala* and *Bauhinia monandra*) were performed and replicated 10 times. Differences in post- pollution of the soil were tested using the parameter replicates by treatment interaction and treatment by levels interaction as the error terms. Differences in the post-phytoapplication performance of the species were also estimated using treatment by level interaction and biological parameters as the error terms. Differences in post-phytoapplication soil were estimated using the treatment by level and by physicochemical parameter interaction as the error terms.

Post-Pollution Habitat Reclamation Treatment Using Agro-Forestry Species

Habitat reclamation treatment commenced 7-days after pollution of the different sites using the species. Healthy 7-days old seedlings of the two species were planted into the various levels of these polluted habitats and the control. The growth performance of these seedlings was monitored for a period of 16 weeks (four months) and used as a measure of their level of tolerance in the polluted soil. Pre and post-pollution and post-phytoapplication changes of the soil under the seedling were assessed by means of comparative analysis of the nutrient contents of the plant species and polluted soil. This was used as a measure of efficiency of the two agro-forestry species based on the level of the soil nutrient status. Analysis of total organic carbon and matter, phosphorus and nitrogen was done using classical analytical methods.

Total organic carbon. The Walkley and Black [9] as modified in Nelson and Somners [10] method were employed for both the soil and plant species. This was followed by adapting the principle complete oxidation from the heat of solution and external heating of sulphuric acid (H_2SO_4) and aqueous potassium dichromate ($K_2Cr_2O_7$) mixture. The unused or residual $K_2Cr_2O_7$ (in oxidation) was titrated against ferrous sulphate solution .The used $K_2Cr_2O_7$ (i.e. the difference between added and residual $K_2Cr_2O_7$) gives a measure of organic carbon content of soil and plant samples. This was determined using the designated formular, while Percentage Organic matter (OM) = % Org. C. x 1.729

Phosphorus. The method of Bray and Kuntz [11] as modified in IITA method [12] was adopted and the phosphorus content for soil and plant samples calculated by reference to a calibration curve using Monobasic Potassium Phosphate (KH_2PO_4) solution as standard. Phosphorus content was determined using the formula:

$$(\%)\ P = \frac{(\text{conc.mgg}^{-1})\ \text{x solution vol.(ml)x } 100}{10^{6}\ \text{x sample wt (g)}}$$

$$P\ (\text{mgg}^{-1}) = \%P\ \text{x}10^{4}$$

Total organic nitrogen. The micro-kjeldahl method described by Stewarte *et al*. [7] was used. This method has 3-phases viz: Digestion, Distillation and Titration. Total organic nitrogen was determined using the formula:

$$\%\ N_2 = \frac{\text{titer value x 1.4 x T.V.D x \% factor}}{\text{Sample wt x T.V.D.A. x 1000}}$$

Where
T.V.D = total volume of digest
T.V.D.A = total volume of aliquot (volume of digest analyzed)
% factor =100
1000 = conversion factor of mg/g to %

Data analysis. The remediation performance was estimated using the Statistical Analysis System (SAS) PROC. NLIN procedure [6]. Data were then analysed as a split-split (double-split) plot design with 10 replicates using the Analysis of Variance (PROC ANOVA) procedures [6]. Where significant differences were observed, means were separated according to the procedures of the Duncan's New Multiple Range Test (DNMRT).

RESULTS

The presented results in Tables 1-5 show the nutrient status in terms of nitrogen, phosphorus, organic carbon and matter contents of the soil and foliar in pre-pollution, post-pollution and post-phytoapplication ecological periods in response to the impact of the remediation options (*Luecaena leucocephala* and *Bauhinia monandra*).

Result of nitrogen content (Table 1) shows that the pre-pollution soil had increased nitrogen content significantly *($p<0.05$)* higher than post-pollution. This however declined at the various levels of pollution with non-significant *($p<0.05$)* difference. Though a non-significant *($p<0.05$)* difference was also recorded between the pre-pollution and high level pollution. In post-phytoapplication process (Table 2), within the various species treated soils nitrogen content reduced with increase in pollution significant *($p<0.05$)* lower than control, low (2%) and medium (4%) pollution levels of the *Leucaena leucocephala* treated soil. Similarly, the *B. monandra* treated soil also had reduction within pollution levels with increase in pollution and significantly *($p<0.05$)* lower at all levels than control. Between the species treated soils *L. leucocephala* soil had a higher nitrogen content than *B. monandra* treated soil though significantly *($p<0.05$)* different at low (2%) and medium (4%) pollution levels. Similarly the foliar content recorded a significantly higher content at all pollution levels in *L. leucocephala soil* than in *B.*

monandra soil. Generally, both species treated soils were significantly lower than the pre-pollution and post-pollution phases in nitrogen content.

Table- 1: pre and post-pollution soil nutrient content

Element	Pre-pollution	Post-pollution			Mean	*LSD(p<0.05)*
		Low (2%)	Medium (4%)	High (7%)		
Phosphorus	0.06^{A}	0.05^{A}	0.04^{A}	0.04^{A}	4.75	0.02
Nitrogen	0.21^{A}	0.16^{B}	0.16^{B}	0.18^{AB}	0.18	0.04
Organic carbon	0.85^{C}	1.21^{B}	1.35^{B}	2.20^{A}	1.59	0.40
Organic matter	1.46^{C}	2.09^{B}	2.33^{B}	3.81^{A}	2.74	0.70

Note: Ll - *Leucaena leucocephala*, Bm - *Bauhinia monandra.*

**Means of ten replicates and with the same superscript letter are not significantly different, using the Duncan's New Multiple Range Test (DNMRT).*

Table- 2: post-phytoapplication soil and foliar nitrogen content in a crude oil remediated soil habitat

Pollution Levels	Soil Nitrogen				Species Nitrogen			
	L l. Soil	*B. m. Soil*	*Mean*	*LSD(p<0.05)*	*L. leucocephala*	*B. monandra*	*Mean*	*LSD(p<0.05)*
Control	0.07^{A}	0.06^{A}	0.07	0.02	16.7^{A}	15.3^{A}	16.00	2,50
Low	0.07^{A}	0.05^{B}	0.06	0.01	37.9^{A}	24.5^{B}	31.20	5.20
Medium	0.06^{A}	0.04^{B}	0.05	0.01	16.2^{A}	14.6^{B}	15.40	0.67
High	0.05^{A}	0.04^{B}	0.05	0.02	16.7^{A}	9.8^{B}	13.25	0.95

The phosphorus content (Table 1) had recorded non-significant *(p<0.05)* difference in pre-pollution and post-pollution phases though with a reduction in post-pollution. In post-phytoapplication process (Table 3) within pollution levels both species treated soils recorded a significant *(p<0.05)* reduction at increasing pollution, while between species treated soil *L. leucocephala* soil recorded a lower phosphorus content though non-significantly *(p<0.05)* different from *B. monandra* treated soil. However, *L. leucocephala* foliar phosphorus content increased with increase in pollution level but non-significantly *(p<0.05)* different from *B. monandra* foliar content, which decreased with increase in pollution level. Generally, the phosphorus content of both species treated soil was higher than the content in pre- and post-pollution phases.

Table-3: post-phytoapplication soil and foliar phosphorus content in a crude oil remediated soil habitat

Pollution Levels	Soil phosphorus				Species phosphorus			
	L l. Soil	*B. m. Soil*	*Mean*	*LSD(p<0.05)*	*L. leucocephala*	*B. monandra*	*Mean*	*LSD(p<0.05)*
Control	2.1^{A}	2.2^{A}	2.15	0.20	3.91^{A}	3.02^{A}	3.47	1.01
Low	1.2^{A}	1.7^{A}	1.45	0.70	2.64^{A}	2.61^{A}	2.63	0.12
Medium	0.8^{A}	1.5^{A}	1.15	0.92	2.70^{A}	2.28^{A}	2.50	0.94
High	0.8^{A}	0.9^{A}	0.85	0.40	2.75^{A}	2.15^{A}	2.45	0.75

Table-4: Post-phytoapplication soil and foliar organic carbon content in a crude oil remediated soil habitat

Pollution Levels	Soil organic carbon				Species organic carbon			
	L l. Soil	*B. m. Soil*	*Mean*	*LSD(p<0.05)*	*L. leucocephala*	*B. monandra*	*Mean*	*LSD(p<0.05)*
Control	1.2^{C}	1.8^{C}	1.5	0.7	36.00^{A}	36.00^{A}	36.00	0.01
Low	1.4^{C}	2.2^{B}	1.8	0.5	33.00^{B}	36.00^{A}	34.50	2.80
Medium	1.8^{B}	2.4^{B}	2.1	0.8	27.00^{B}	39.00^{A}	33.00	5.00
High	2.4^{B}	3.6^{A}	3.0	0.5	26.00^{B}	51.00^{A}	38.50	7.54

The result of total organic carbon (Table 1) in pre and post-pollution had recorded increase in carbon content in an increasing pollution state. The post-pollution carbon contents which increases with increase in pollution was significantly *(p<0.05)* higher than the pre-pollution carbon content. In post-phytoapplication process (Table 4) within pollution levels both species treated soils recorded carbon increase at increasing pollution levels and with significant *(p<0.05)* difference higher than control. Between the species treated soil *B. monandra* soil recorded a significant *(p<0.05)* higher content at low (2%) and high (7%) pollution levels. While the foliar content in *L. leucocephala* had reduction in carbon in an increasing pollution level, significantly *(p<0.05)* lower than control, *B. monandra* had increasing carbon content in increasing pollution levels though with non-significant *(p<0.05)* difference at all levels. Generally, both species treated soils recorded higher carbon content than pre- and post-pollution phases.

The total organic carbon content (Table 1) recorded increase with increasing pollution which are significantly *($p<0.05$)* different from pre-pollution and significantly *($p<0.05$)* higher at high level pollution. In post-phytoapplication (Table 5) both species treated soils within pollution levels recorded increase in organic matter content at various levels of pollution increase. However, *L. leucocephala* soil recorded non-significant *($p<0.05$)* difference while *B. monandra* treated soil recorded a significant *($p<0.05$)* difference at high pollution level. Between species treated soil at various level *B. monandra* soil recorded higher organic matter content but only significantly *($p<0.05$)* different at high level pollution. The foliar content was higher in *B. monandra* though non-significantly *($p<0.05$)* different within pollution levels but significantly *($p<0.05$)* higher than *L. leucocephala* foliar content. Generally, the post-phytoapplication treated soil organic matter is higher than the pre and post-pollution soil organic matter content.

Table-5: Post-phytoapplication soil and foliar organic matter content in a crude oil remediated soil habitat

Pollution Levels	**Soil organic matter**				**Species organic matter**			
	L l. Soil	***B. m. Soil***	***Mean***	***LSD(p<0.05)***	***L. leucocephala***	***B. monandra***	***Mean***	***LSD(p<0.05)***
Control	2.10^{B}	3.10^{B}	2.60	2.50	62.24^{A}	62.24^{A}	62.24	0.01
Low	2.42^{B}	3.80^{B}	3.11	2.00	55.10^{B}	62.24^{A}	58.67	5.35
Medium	3.11^{B}	4.20^{B}	3.66	2.50	46.68^{B}	67.43^{A}	57.06	4.50
High	4.20^{B}	6.20^{A}	5.20	1.20	44.10^{B}	88.18^{A}	66.14	5.75

DISCUSSION

In studying the nutrient status of soil environment it becomes pertinent to know the forms in which the nutrient are stored in the soil and the capacity of the soil to make these available to plant as they are needed [1], besides the prevailing conditions that could engender the loss and imbalance of these nutrient elements from soil. The soil is liable to crude oil pollution due to accidental spills of hydrocarbon caused by the inevitable consequence of the exploration, extraction, refining and transportation of petroleum and its products within the terrestrial environment. Though petroleum and its components may eventually be degraded when released into the environment, but prior to such degradation, a considerable environmental damage may have ensued. Also based on the concept of elemental balancing, there may be inequality of needed element due to excess of one element, resulting to an induced deficiency of another and some form of paralysed soil biochemical processes. Oil on soil results in nutrient imbalance due to addition of large amounts of carbon to the soil and reduction in some form of other nutrient in the soil. This becomes an impediment to the occurrence of important soil biochemical process such as organic matter .decomposition, ammonification, nitrification, symbiotic and non-symbiotic nitrogen fixation and geo-chemical cycling of element [13].

There was a considerable difference in nitrogen content between the pre-pollution and post-pollution level which had a significant reduction in nitrogen content as a result of hydrocarbon addition to the soil. This corroborates the assertion that the adverse effects of oil pollution on both microbial population and mineral element of the soil could create condition of nitrogen limitation and toxic nutrient availability to soil [14, 15]. This also could be reaffirmed by the fact that when large amount of carbon rich compound such as petroleum hydrocarbon is added to a soil, the carbon tends to stimulate bacteria and fungi rapidly, which attack the carbon. These organisms need nitrate nitrogen to build up their tissues and thus might temporarily reduce the available nitrogen in the soil [16].

In phytoremediation process the fact that *L. leucocephala* has the potential and efficacy for phytomicrobial symbiotic activity [17], its soil and foliar has higher nitrogen content than *B. monandra* treated soil. This could be due to leaf droppings, adequate aeration and nodulation by the plant roots, thus implicative of its nitrogen fixing potential negating the ill-potential effect of *B. monandra* [17].

Also because of its ability to utilize it for hydrocarbon degradation there was a decline in nitrogen content within the various levels of *L. leucocephala* treated soil and differential increment in comparison with the *B. monandra* treated soil. This might be due to its ‘explanta’ and nodulating potential which could have probably enhanced microsymbiotic association with the plant roots within the rhizosphere [18]. The tolerance and utilization of root nodulating potential of nitrogen fixing plants in the enhancement of bioremediation of crude oil polluted soil has been evaluated. Such growth and nodulation potential of species concerned is also a reflection of their biological performance and capabilities to restore the physicochemical factors of the polluted edaphic environment as well as enhancing hydrocarbon loss [17]. Also, such increment in nitrogen content of the soil could be due to litter droppings, adequate aeration and nodulation by the root, thus its implication for nitrogen fixing unlike the non-symbiotic efficacy of *B. monandra* [18]. The decline in the concentration of this essential nutrient within this period of study in various levels of pollution is considered the driving force behind hydrocarbon degradation.

Dibble and Bartha [19] have noted nitrogen as one of the environmental factors that could accelerate hydrocarbon biodegradation. This conforms to the reason why there was an apparent decline in nitrogen content within the species treated soil. The decrease in species treated soil nitrogen significantly lower than the pre and post-polluted soils could be supported by the observation that disappearance of organic chemical is accelerated in vegetated soils than surrounding non – vegetated bulk soils [20]. This corroborates the fact there could be enhanced microbial activity within the rhizosphere as a result of plant-derived compounds that contain resources limiting to microbial growth and metabolism, upon which an increase in the order of magnitude higher in the soil of root zone than in adjacent unplanted soil [21]. Also the fact that the proportion of hydrocarbon utilizers in the population increases with the introduction of hydrocarbon into an environment as noted by Jones & Greenfield [22] and Ebuehi *et al*. [23], this may accounts for the depletion of nitrogen in polluted soil treated with plant species particularly due to the species potential for microbial habitation.

The decrease in phosphorus as a result of pollution effect in post-pollution phase could be reaffirmed by the earlier observation by Ayalogu *et al*. [15] and Baker & Herson [3] that the effect of crude oil can result to phosphorus depletion and possibly as a result of increase in hydrocarbon utilisers [23]. There was decrease in phosphorus content of *L. leucocephala* treated soil. The slight drop in its values within the period of study may represent the era of utilization by the phytomicrobial associated population. This corroborates Dibble & Bartha [19] and Chiannelli *et al*. [24] in the use of phosphorus by microbial activity for hydrocarbon degradation. There was little or no change in values of this element in the *B. monandra* treated soil. This is suggestive of drastically reduced microbial activities in the soil, hence its inability for nodulation. *Bauhinia monandra* had no nodulation, thus lacks the potential for the desorption of hydrophobic portion of oil from the soil particles. This renders the oil unavailable for the species uptake.

Values of organic carbon and matter showed a considerable difference with increase in their content in post-pollution higher than pre-pollution. The decrease in pre-pollution could be due to lack of exogenous source of carbon from the hydrocarbon [25]. The impact of phytoapplication has reflected a reduction in carbon and organic matter content, when compared to post-pollution though in an increasing order of increased pollution between the species. Despite such reduction the treated soil recorded higher content than that of the controlled phytoapplication due to exogenous source of carbon from the oil. The higher reduction in carbon and organic matter content in *L. leucocephala* treated soil is an indication of more utilization of carbon than in the *B. monandra* treated soil. Though the values increased with increase in pollution for both species, the treated soil of *B. monandra* had a higher carbon level. There was a drop in carbon and organic matter level following increased utilization of crude oil in *L. leucocephala* soil, which may not have been observed in *B. monandra* treated soil.

The slight drop of carbon and organic matter in *L. leucocephala* treated soil population in the rhizosphere following their enhanced root formation and nodulation [17]. The enhanced root and nodulation possibly may have also enhanced desorption of hydrophilic and hydrophobic portion of the locked-up or adsorbed oil films on the soil particles. This makes for their faster dissolution into soil solution for mineralization and species uptake. This could also be reaffirmed by an earlier assertion which have recorded *L. leucocephala* with high level performance in terms of enhanced total petroleum hydrocarbon (TPH) uptake from a species treated soil than the *B. monandra* treated soil [26].

These potentials were lacking in *B. monandra*. This consequently has resulted to an increased organic carbon and matter pools of the species and its soil. This enhanced hydrocarbon degradation, producing carbon, water and carbondioxide in the presence of phosphorus, nitrogen nutrient and other environmental variables reaffirms the assertions by Ladousse & Tramier [27] and Jones & Greenfield [22]. The carbon becomes incorporated into the plant biomass thus depleting the carbon and organic matter content of the soil. The variation in carbon and organic matter pools between the two species corroborates the assertion that a lower carbon generally indicates higher and more rapid mineralization while a higher carbon suggests slower mineralization and greater content of little – altered organic remains [1, 28, 18, 29]. As could be observed it would be pertinent to assert that particular plant species appear to enhance remediation of oil-contaminated soil to some extent than other species. Also such result indicates that the apparent biodegradable capabilities show how natural biological process by anthropogenic response can be used to minimize environmental contamination [26].

REFERENCES

[1] Peter M A, *West African Soil*. London. Oxford University Press. 332pp. **1970**.
[2] Atlas R M, *Microbiology Review* **1981**, 45: 180 – 209.
[3] Baker K H, Herson D, Microbiology and biodegradation. In: Baker K H, Herson D, (Eds.). *Bioremediation*. McGraw Hill. Inc. New York, pp 9 – 60. **1994**.
[4] Kinako P D S, *Environmental Pollution* (Series A) – **1981**, 26: 87 – 91.
[5] Akindele S O, *Basic Experimental Design in Agricultural Research*, Akure, Federal Universty of Technology Akure (FUTA) Press. **1996**.
[6] SAS Institute Inc. SAS for Windows Release 9.1, Canny, United State America, Statistical Analysis Systems Institute Incorporated. **2002**.
[7] Stewarte A, Grimshaw H M, John A P, *Chemical Analysis of Ecological Materials*. Oxford. Blackwell Scientific Publications. Pp. 10 – 270. **1974**.
[8] Song H G, Wang X, Bartha R, *Applied and Environmental Microbiology*, **1990**, 56(3): 652 – 656.
[9] Walkley A, Black, C A, *Soil Science*, **1934**. 37:29-38.
[10] Nelson D W, Sommers L E, *Indian Academy of Science*, **1975**, 84:456- 482.
[11] Bray R H, Kuntz L T, *Soil Science*, **1945**, 59:39- 45.
[12] International Institute of Tropical Agriculture. *Selected Methods for Plant and Soil Analysis* Ibadan, International Institute of Tropical Agriculture (IITA). Manual series No. 1. **1979**.
[13] Odu C T I, *Journal of the Institute of Petroleum* **1972**, 58: 201 – 208.
[14] Adams R S, Ellis R, *American Proceedings*, **1960**, 24: 41 – 44.
[15] Ayalogu E O, Igwilo N H, Ukaigwe N F, Survey of Booba Nabe Soil Status, 18 years after Shell Petroleum Development Company (SPDC) Bomu Well Blowout, 1 -13. **1988**.
[16] Okolo J C, Amadi E N, Odu C T I, *Applied Ecol. Environment. Res.*, **2005**, 3(1): 47 – 53.
[17] Edwin-Wosu N L, Kinako P D S, *Global Journal of Environmental Science*, **2005**, 4(1): 11-18.
[18] Edwin-Wosu N L, *Journal of Environmental Studies*, **2007**, 3 (3): 97 – 110.
[19] Dibble J T, Bartha R, *Applied Environmental Microbiology*, **1997**, 37:729-739.
[20] Steve R, Daniel P, Bioremediation of hazardous waste sites. Phytoremediation approach to implementation US. EPA. Cincinnati Ohio. Dynamic Co-operation Ada OK. **1996**.
[21] Milan C V, William J C, Identification of plant traits that enhance biodegradation of oil. School of Biological Science. Louisiana Tech. University. **2001**.
[22] Jones M A, Greenfield J H, In-situ Comparison of Bioremediation. Methods for a number of 6 residual fuel oil spills in Lee County, Florida. *Proceedings of the 1991 International Oil Spill Conference.* American Petroleum Institute, Washington DC. Pp 533 -540. **1991**.
[23] Ebuehi O A T, Abibo I B, Shekeolo P D, Sigismund K I, Adoki A, Okoro I C, *Remediation of Crude Oil Contaminated Soil by Enhanced Natural Attenuation Technique*. Pp 103 – 106. **2005**.
[24] Chianelli R R, Actel T, Bare R C, George G N, Genowitz M W, Grossman M J, Haith C F, Kaiser F J, Lessard R R, Loiffa K, Mastrachio R L, Minak-Berero R W K, Stiesel E L, Wilkinson J B, Hinton S M, Bioremediation Technology Development and Application to the Alaskan Spill. *Proceedings of the 1991 Oil Spill Conference*. American Petroleum Institute, Washington DC. Pp 549 -556. **1991**.
[25] Edwin-Wosu N L, Phytoremediation Potential of Some Macrophytes on Crude / Waste Oil Polluted Soils in the Rainforest Environment. PhD Thesis, University of Calabar. **2011**.
[26] Edwin-Wosu N L, Albert E, *JASEM*, **2010**, 14(1): 39 – 42.
[27] Ladousse A, Tramier B, Results of 12 years of research in spilled oil bioremediation Inipol EAP 22. In: *Proceedings of the 1991 International Oil Spill Conference*. API. Washington DC. pp 577 – 581. **1991**.
[28] Odokuma L O, Dickson L E, *Global Journal of Environmental Science*, **2003**, 2(1): 29 – 40.
[29] Edwin-Wosu N L, Kinako PDS, *Journal of Nigeria Environmental Society* **2007**, 4 (1): 95 – 99.