Tolerance Potentials of Cocoa (*Theobroma cacao*) in Hydrocarbon Polluted Soils

R. B. Agbor¹*, J. E. Asor², I. U. Bassey³, Chibuzor Obianwa¹, E. O. Ude¹ and A. Ipoh¹

¹Department of Genetics and Biotechnology, University of Calabar, P.M.B. 1115 Calabar, Cross River State, Nigeria.
²Department of Zoology and Environmental Biology, University of Calabar, P.M.B. 1115 Calabar, Cross River State, Nigeria.
³Department of Microbiology, University of Calabar, P.M.B. 1115 Calabar, Cross River State, Nigeria.

Authors’ contributions

This work was carried out in collaboration between all authors. Authors RBA and JEA Designed the study, wrote the protocol and the draft of the manuscript. Authors IUB and CO performed the Statistical analysis. Authors EOU and AI managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BBJ/2015/13863

Editor(s):
(1) Chung-Jen Chiang, Department of Medical Laboratory Science and Biotechnology, China Medical University, Taiwan.
(2) Kuo-Kau Lee, Department of Aquaculture, National Taiwan Ocean University, Taiwan.

Reviewers:
(1) Anonymous, Nigeria.
(2) Anonymous, India.
(3) Kiyoshi Kurosawa, Institute of Tropical Agriculture, Kyushu University, Japan.
(4) Anonymous, Puerto Rico.
(5) Anonymous, Uganda.
(6) Obasi Ajuka Nwogo, Department of Medical Biochemistry, Federal University, Nigeria.

Complete Peer review History: http://www.sciencedomain.org/review-history.php?id=1043&id=11&aid=8906

Received 6th September 2014
Accepted 23rd December 2014
Published 6th May 2015

ABSTRACT

In the present study the tolerance potentials of *T. cacao* in hydrocarbon polluted soil was evaluated. Top soil (0-25 cm depth) was collected from three points, bulked to form composite soil sample. Eight kilograms of the soil sample each were weighed into twenty five (25) perforated bags. The bags were polluted with 0 ml/kg, 50 ml/kg, 100 ml/kg, 150 ml/kg and 200 ml/kg of crude oil respectively with five replicates for each concentration and allowed for 2 weeks before planting. Three seeds of cocoa were sown in each of the polythene bags. Water was applied every three days to keep the soil moist. The results showed that cocoa plants at 8 weeks after planting (WAP) had significantly higher height (P<0.05) than those of 4 weeks after planting (WAP). The plant

*Corresponding author: E-mail: agborreagan@yahoo.com;
1. INTRODUCTION

Phytoremediation is a technique in environmental biotechnology that embraces the use of plants to remove, transfer, stabilize and destroy organic and inorganic contamination from land, groundwater and surface water. Phytoremediation is gaining research interest such that it involves the use of plants which are environmentally friendly in solving the problems of the environment. Phytoremediation offers an aesthetic and low-cost remediation technique to sites with low to moderate contaminant concentration where the pollutants are not located very deep. Crude oil is a raw material for the production of petroleum and other chemicals, and has quite been one of the most important energy sources in the world. It is a natural resource of the industrialized nations because it can generate heat, drive machinery, and fuel vehicle and airplane. Its components are used to manufacture almost all chemical products such as; plastics, detergents, paint and even medicine. However, pollution of soil and water environment by crude oil spill as a result of exploration, production, maintenance, transportation, storage and accidental discharge releases hazardous chemicals to the ecosystem. The presence of the hydrocarbons in terrestrial and aquatic environment affects the plants adversely by creating conditions which make essential nutrients like; nitrogen and oxygen needed for plants growth unavailable to them [1]. Oil contamination causes slow rate of germination in plants, [2] reported that this effect could be due to oil which acts as a physical barrier preventing or reducing access of seed to water and oxygen.

In today’s industrial society, there is no way to avoid the exposure to toxic chemicals and metals because heavy metals are enriched in the environment by human activities of different kind like in developed countries, heavy metal pollution becomes serious due to mining, minerals, smelting and tannery industry [3]. From plants nutritional studies, it is known that plants require a certain amount of trace element, that they respond differently to enhanced or lowered trace element supply, and that in some cases agricultural products may be contaminated with toxic heavy metals. Metal concentrations in contaminated soil result in decreasing soil microbial activities soil fertility and yield losses [4]. Phytoremediation could be easily achieved with higher plants that can accumulate heavy metals at different pollution levels. Ogbo et al. [5] used Paspalum scrobiculatum L. in the phytoremediation of crude oil impacted soil and reported a significant reduction in the hydrocarbon content of the soil. Shahrzad et al. [6] reported on the phytoremediation potential of Trifolium resupinatum in the degradation of hydrocarbons in soil, they found a significant reduction in the total hydrocarbon content of the soil. Differences in metal accumulation may exist between and within plant population. This study examined the tolerance of cocoa plant in hydrocarbon polluted soils through the evaluation of the morphological features of the plant, physicochemical and heavy metal content of the soil.

2. MATERIALS AND METHODS

2.1 Experimental Procedure

This work was carried out at Biological Science Experimental Farm (BSEF), University of Calabar, Calabar. Heavy metals and physicochemical analysis of the soil were carried out in Soil Science Laboratory, University of Calabar. Cocoa pods were obtained from Reagan’s Farm in Boki Local Government Area, Cross River State. Viable seeds were selected from the pods. The crude oil (Bonny light) used for the experiment was collected from the Nigerian Agip Oil Company (NAOC), Port Harcourt, Rivers State. Top soil (0-25 cm depth) was collected from three point around Biological

Keywords: Tolerance; hydrocarbon; cocoa; soil; pollution.
Science Experimental Farm using auger, Bulked to form composite soil sample. Eight kilograms (8 kg) of the soil sample each was weighed into twenty five (25) perforated bags, leaving a space of 2.5 cm from the top to make allowance for addition of water. The perforation of planting bags was at the base to ensure proper drainage and better aeration of soil. The 20 polythene bags were polluted with the following concentrations of crude oil: 0 ml/kg, 50 ml/kg, 100 ml/kg, 150 ml/kg, and 200 ml/kg, respectively and the setup were allowed for 2 weeks before planting.

2.2 Planting of Seeds and Water Application

Three (3) seeds of cocoa were sown in each polythene bag containing the polluted soil and that of the control, application of water was done also to keep the soil moist and to avoid drought. Monitoring of plants was done during the planting period to ensure healthy plants growth and the morphological attributes of the plants were taken. Data were taken four weeks after planting (4WAP) and eight weeks after planting (8WAP).

2.3 Physicochemical Analysis of Soil

The collected soil samples were sieve and air dried. Soil pH was determined using a hand held pH meter in distilled water according Thomas [7]. Soil organic carbon was determined by the chromic acid digestion method of Walkley and Black as reported by Helmke and Sparks [8]. The total (N) Bremner [9] available P was determined by Bray-P method as described by Kuo [10]. Exchangeable soil properties were extracted with neutral normal ammonium acetate buffer according to Helmke and Sparks [8]. Potassium and Sodium were determined using a Flame Photometer.

2.4 Heavy Metal Content of Soil

Two gram (2 g) of each of the sieved soil samples was weighed into a conical flask and digested with 10 ml of 50% hydrochloric acid on a hot plate until 2-3 ml of acid was left. Ten (10) ml de-ionized water was added to the content and demented into 50ml volumetric flask after additional water rinsing and decanted and made up to the mark with de-ionized water. A blank was prepared without soil sample. The extract solution was poured into polythene bottles from where each sample was analyzed for Cd, Cu, Fe, Pd, and Zn using Atomic Absorption Spectrophotometer Model 205.

2.5 Data Collection

The following parameters were taken; days of germination, plants heights, number of leaves, leaf length, leaf width, leaf area, numbers of branches, vine length, petiole length, numbers of vein.

2.6 Statistical Analysis

Data collected were subjected to a two-way analysis of variance (ANOVA), while significant mean were separated using least significant difference (LSD) Test at 5% probability level.

3. RESULTS AND DISCUSSION

3.1 Morphological Features of T. cacao Grown on Hydrocarbon Impacted Soil

A phytoremediation plant is one that can absorb or extract, degrade contaminant, such plant should be able to tolerate high presence or concentrations of contaminant and then grow at a faster rate. This present study has shown that T. cacao is a good material for phytoremediation due to the high growth rate recorded. One main challenges that have hindered the full application of phytoremediation strategies in polluted environment is the stress that is often induce on the rate of seed germination and sprouting of plants. The stress imposed on the seed could be as results of heavy metal contents of the soil that distorted the emergence of the plants. The seed germination of T. cacao delayed may be due to the hydrocarbon contents of the soil (Plate 1). The seed germination was observed thirteen days after planting (13DAP) and sprouting of leaves was observed 16DAP as compared with the control of the plant that germinated 9DAP and sprouting of leaves noticed 12DAP. The result obtained for the plant height of T. cacao examined under four weeks after plant (4WAP) and eight weeks after planting (8WAP) shows that there were significant difference (P<0.05) between the timing variation (Plate 2). The plant height of T. cacao at 8WAP had significantly higher (P<0.05) plant height than that of 4WAP. This implies that the hydrocarbon impacted soil did not significantly affect plant height of T. cacao at 8WAP. However, it was observed that the
different pollution levels did not significantly affects ($p>0.05$) the plant height as compared with the control group. Njoku et al. [11]. Ekpo et al. [12] reported that Glycine max growth performance was significantly improved by the addition of cow dung to crude oil polluted soil. However, the high growth performance of T. cacao on crude oil polluted soil was achieved without nutrient enrichment of the soil. Significant difference ($P<0.05$) were observed in the number of leaves T. cacao at 4WAP and 8 WAP. 8WAP had significantly higher ($p<0.05$) number of leaves than the 4WAP. It was however, observed that 200ml/kg impacted crude soil at 8WAP had significantly higher number of leaves than the control groups and other pollution level at 4WAP and 8WAP. The mean values of the leaf length of T. cacao obtained from the crude oil impacted soil at 8WAP was significantly higher ($P<0.05$) than the leaf length of 4WAP (Table 1). It was observed that the different pollution levels significantly influence the leaf length of T. cacao. However, soil polluted with 50 ml/kg at 8WAP had significantly higher leaf length, followed by control, 50 ml/kg, 100 ml/kg, 150 ml/kg and 200 ml/kg at 8WAP had no significant difference ($p>0.05$) but significantly lower than the control of 8WAP (Table 2). It was observed that the leaf width of T. cacao at 8WAP was significantly higher ($p<0.05$) than that of 4 WAP. The control plant at 8 WAP had significantly higher ($P<0.05$) leaf width than the crude oil impacted soil at 4WAP and 8WAP. The result of the leaf area of T. cacao at (4 WAP) and (8 WAP) shows that 8WAP produces T. Cacao with significantly higher ($P<0.05$) leaf area than 4 WAP and also it control leaf area than 50 mg/kg of crude oil impacted soil. It was also observed that the different pollution level at 4WAP and 100 mg/kg, 150 mg/kg, 200 ml/kg impacted soil at 8 WAP had no significant difference ($p>0.05$). The petiole length of T. cacao at 8WAP was significantly higher ($P<0.05$) than that of 4WAP (Table 1). However, the different pollution level had no significant difference ($P>0.05$) with the control plants.

3.2 Heavy Metal Properties of Crude oil Impacted Soil Grown with Cocoa

It is well known that elements such as Cu, Mo, Ni, Cr, and Zn among others are essential for plant growth at low concentration Taiz and Zeiger [13]. Blaylock and Huang [14] reported that these elements beyond certain threshold concentration become toxic to most plant species. Although, these elements were observed to be significantly high ($P<0.05$) in the soil at varying concentration of crude oil, the T. cacao was observed to be resistant to the effect of the heavy metals. The growth of T. cacao over a period of 8WAP indicates that the plant is also highly tolerant to chromium at a high concentration. Lead is a metal with limited availability for plant uptake due to complexities with solid soil fractions [15], sequential extractions, or a very small fraction of total soil. Lead was observed to be present in a form directly available to the plant. A number of studies have indicated the potential of phytoremediation in reducing the concentration of various contaminants including petroleum hydrocarbon [16]. Interestingly, the growth attributes of the plant remained unaffected even after 8WAP. Heavy metal content of the crude oil polluted soil grown with T. cacao. The result obtained for the heavy metal content of T. cacao soil shows that the iron (Fe) and manganese (Mn) present in the soil at 200 ml crude oil pollution was significantly higher ($p<0.05$) than that of soil polluted with 150 ml followed by control which was found to have significantly low ($P<0.05$) Fe and Mn content. The Nickel (Ni) content of the soil at 50 ml pollution level had no significant difference ($P>0.05$) with the control but significantly higher ($P<0.05$) than soil polluted with 100 ml/kg, followed by 150 ml/kg and 200 ml/kg that had no significant difference ($p<0.05$). The copper (Cu) content of the soil polluted with 50 ml, 100 ml, 150 ml/kg and 200ml/kg had no significant difference ($p<0.05$) in its mean values but significantly higher ($p<0.05$) than the mean of control (Table 3). The zinc (Zn) was observed to be significantly higher ($p<0.05$) in the leaves of soil polluted with 200 ml/kg, followed by soil polluted with 150 ml/kg and 100 ml/kg that had no significant difference ($p<0.05$) in its mean values. This was also followed by soil polluted with 50 ml/kg of crude oil while the control had significantly low ($p<0.05$) Zn content. The result obtained for the cadmium (Cd) content of the soil polluted with 200 ml/kg, 150 ml/kg and 100 ml/kg was significantly higher ($p<0.05$) than that obtained from the control and 50 ml/kg polluted soil with no significant difference ($p<0.05$). The chromium, (Cr) and vanadium (V) content of the soil had no significant difference ($p<0.05$) with the mean of the control. The lead (Pb) content of the soil polluted with 200 ml/kg was significantly higher ($p<0.05$) than that of soil polluted with 150 ml/kg, 100 ml/kg with no significant difference ($p>0.05$) in the mean. This was followed by soil polluted with 50 ml/kg while the control had significantly low ($P<0.05$) lead content. The Cobalt (Co) content of the soil in
control was significantly higher (p <0.05) than that of soil polluted with 50 ml/kg followed by soil polluted with 100 ml/kg, 150 ml/kg, 200 ml/kg which had no significant difference (p >0.05) (Figs. 1-7).

Plate 1. Young *T. cacao* seedlings growing on crude oil polluted soil

Plate 2. Mature *T. cacao* seedlings on hydrocarbon polluted soil

Table 1. Effect of crude oil polluted soils on the growth of *T. cacao*

| Plant parameters | 4 WAP       | 8 WAP       | LSD  |
|------------------|-------------|-------------|------|
| Plant height     | 20.35±0.07  | 22.54±0.1   | 1.65 |
| Leaf length      | 12.81±0.05  | 15.36±0.06  | 1.13 |
| Leaf area        | 49.27±0.04  | 70.62±0.09  | 9.37 |
| Number of leaves | 5.32±0.03   | 6.72±0.04   | 0.37 |
| Petiole length   | 1.64±0.02   | 2.90±0.01   | 0.26 |
| Leaf width       | 5.13±0.03   | 6.04±0.02   | 0.42 |
| No. of vine      | 18.92±0.04  | 23.0±0.06   | 1.49 |
| Vein length      | 2.59±0.02   | 2.94±0.01   | 0.22 |

Means with the same case letter along the horizontal arrays indicates no significant difference (p>0.05), LSD least significant difference, WAP week after planting
Fig. 1. Iron level in crude oil impacted soil grown with cocoa

Fig. 2. Copper level in crude oil impacted soil grown with cocoa
### Table 2. Morphological features of *T. cacao* grown on crude oil polluted soil

| Plant parameters | Control | 50ml/kg | 100ml/kg | 150ml/kg | 200ml/kg | Control/kg | 50ml/kg | 100ml/kg | 150ml/kg | 200ml/kg |
|------------------|---------|---------|----------|----------|----------|------------|---------|----------|----------|----------|
| Plant Height     | 21.16±0.08 | 21.54±0.06 | 20.74±0.05 | 20.72±0.04 | 17.6±0.03 | 22.1±0.02 | 21.56±0.03 | 23.14±0.01 | 23±0.18 | 22.92±0.06 |
| Leaf length      | 13.7±0.04 | 12.66±0.03 | 12.8±0.06 | 12.8±0.04 | 12.08±0.03 | 19.14±0.03 | 16.74±0.02 | 14.54±0.01 | 13.6±0.06 | 12.8±0.08 |
| Leaf area        | 59.6±0.11 | 49.46±0.09 | 49.1±0.06 | 44.32±0.07 | 43.88±0.08 | 103.36±0.07 | 78.06±0.08 | 61.34±0.10 | 56.8±0.09 | 53.52±0.08 |
| No. of leaves    | 4.8±0.05 | 5.2±0.06 | 5.4±0.04 | 5.6±0.06 | 5.6±0.08 | 5.8b±0.04 | 6.2±0.06 | 6.2±0.06 | 7.4±0.08 | 8.0±0.9 |
| Petiole length   | 1.74±0.07 | 1.6±0.08 | 1.66±0.08 | 1.54±0.07 | 1.64±0.06 | 3.76±0.05 | 3.22±0.04 | 2.46±0.06 | 2.52±0.04 | 2.52±0.08 |
| Leaf width       | 5.58±0.08 | 5.14±0.05 | 5.06±0.04 | 5.02±0.07 | 4.86±0.08 | 7.32±0.05 | 6.24±0.06 | 5.38±0.06 | 5.44±0.07 | 5.82±0.08 |
| No. of vine      | 20.4±0.11 | 19±0.10 | 18.8±0.09 | 19±0.12 | 17.4±0.10 | 26.2±0.08 | 24.2±0.09 | 20.8±0.08 | 20.2±0.10 | 23.6±0.06 |
| Vine Length      | 2.88±0.02 | 2.46±0.04 | 2.48±0.02 | 2.48±0.04 | 2.66±0.03 | 3.7±0.05 | 3.12±0.06 | 2.66±0.04 | 2.72±0.02 | 2.5±0.05 |

Means with the same superscript along the horizontal array indicate no significant difference (P≥0.05), WAP week after planting.

### Table 3. Physicochemical properties of soils after treatments using cocoa

| Parameters         | Control | 50 ml | 100 ml | 150 ml | 200 ml | LSD   |
|--------------------|---------|-------|--------|--------|--------|-------|
| Moisture           | 18.6±0.02 | 18.0±0.01 | 16.5±0.02 | 15.8±0.01 | 15.0±0.02 | 1.01  |
| pH                 | 5.5±0.01 | 6.8±0.02 | 7.2±0.03 | 7.4±0.01 | 7.6±0.01 | 0.56  |
| Organic C. (%)     | 1.20±0.02 | 1.6±0.01 | 1.8±0.02 | 1.9±0.02 | 2.2±0.01 | 0.24  |
| Nitrogen (mg/kg)   | 0.20±0.01 | 0.17±0.03 | 0.16±0.01 | 0.14±0.02 | 0.14±0.01 | NS    |
| Phosphorus (Mg/kg) | 3.68±0.08 | 3.48±0.07 | 3.06±0.06 | 28.2±0.08 | 26.1±0.06 | 2.60  |
| Potassium (Cmolkg⁻¹) | 0.26±0.02 | 0.21±0.03 | 0.20±0.02 | 0.18±0.01 | 0.15±0.02 | 0.02  |
| Magnesium (Cmolkg⁻¹) | 0.90±0.04 | 0.84±0.02 | 1.73±0.06 | 1.70±0.08 | 1.64±0.01 | 0.24  |
| Calcium (Cmolkg⁻¹) | 1.36±0.03 | 2.56±0.01 | 6.4±0.03 | 8.0±0.05 | 8.6±0.02 | 0.98  |
| H⁺(Cmolkg⁻¹)       | 0.86±0.04 | 0.09±0.01 | 0.00 | 0.00 | 0.00 | NS    |
| Al³⁺(Cmolkg⁻¹)     | 0.26±0.02 | 0.02±0.00 | 0.01±0.00 | 0.00 | 0.00 | NS    |
| Sodium (Cmolkg⁻¹)  | 0.08±0.01 | 0.10±0.02 | 0.18±0.01 | 0.20±0.02 | 0.24±0.01 | 0.02  |
| ECEC               | 2.6±0.02 | 3.71±0.04 | 8.5±0.02 | 10.08±0.03 | 10.63±0.06 | 1.16  |
| BS                 | 56.92±0.08 | 97.04±0.09 | 99.88±0.06 | 100±0.00 | 100±0.00 | 8.64  |

Means with the same case letter along the horizontal arrays indicate no significant difference (P≥0.05). BS base saturation, ECEC effective cation exchange capacity, LSD least significant difference. NS Not significant.
Fig. 3. Manganese level in crude oil impacted soil grown with cocoa

Fig. 4. Nickel level in crude oil impacted soil grown with cocoa
3.3 Soil Physicochemical Properties in Crude Oil Impacted Soil Grown with Cocoa

The result for soil moisture content shows that soil polluted with 50 ml of crude oil and the control had no significant difference (P>0.05) in their mean values but significantly higher (P<0.05) than the mean value obtained from soil polluted with 100 ml/kg, 150 ml/kg and 200 ml/kg of crude oil. The pH value of polluted soil at different pollution level had no significant difference (P>0.05) but significantly higher (P<0.05) than the control values (Table 3). It was observed from this result that the organic carbon content of the soil polluted with 200 ml/kg of crude oil was significantly higher (p<0.05) than soil polluted with 50ml/kg, 100ml/kg, and 150 ml/kg of crude oil which had no significant difference (P>0.05) but significantly higher (p<0.05) than the control. Stephen and Ijah [17] evaluated the potentials of Glycine max and Sida acute for phytoremediation of waste lubricating oil polluted soil and reported that the pH, moisture, electrical conductivity and phosphorus levels were lower in the S. acuta treatment than G.max treatment. This study revealed that the nitrogen content of the polluted soil and control had no significantly difference (P>0.05). Observation also showed that the soil polluted with 100 ml/kg, 150 ml/kg and 200 ml/kg of crude oil had significantly high phosphorus content (P<0.05) and significantly higher (P<0.05) in soil polluted with 50 ml/kg crude oil and it control group. Stephen et al. [18] reported that no significant difference (p>0.05) exist in the pH, organic carbon and organic matter content while the moisture and phosphorus concentration of the polluted soil was significantly different (p>0.005). The potassium level in the control was significantly higher (P<0.05) than that of soil polluted with 50 ml/kg, 100 ml/kg and 150 ml/kg of crude oil while 200 ml/kg polluted soil had the lowest potassium content in the soil. It was observed that the magnesium content of soil polluted with 200 ml/kg, 150 ml/kg, 100 ml/kg of crude oil had no significantly difference (P>0.05) but significantly higher (P<0.05) than soil polluted with 50 ml/kg and control with no significant difference (P>0.05). The calcium content of the soil polluted with 200 ml/kg and 150 ml/kg of crude oil had no significant difference (P>0.05) but significantly higher (P<0.05) than soil polluted with 100 ml/kg, followed by the soil polluted with 50 ml/kg of crude oil. The control was observed to have the lowest calcium content. The hydrogen and aluminum (H+ and Al3+) and base saturation of the polluted and unpolluted soil were not significantly different (P>0.05). The sodium and effective cation exchange capacity (ECEC) content of the soil polluted with crude oil were significantly higher (P<0.05) than the control.

![Zinc level in crude oil impacted soil grown with cocoa](image-url)
Fig. 6. Chromium level in crude oil impacted soil grown with cocoa

Fig. 7. Lead level in crude oil impacted soil grown with cocoa

4. CONCLUSION

The *T. cacao* exhibited a high tolerance to crude oil polluted soils. However, this plant is worthy for further studies with respect to its use in phytoremediation of crude oil polluted soil because of its availability and abundance in Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Agbor RB, Ekpo IA, Udoﬁa UU, Okpako EC, Ekanem BE. Potentials of cocoa pod husks and plantain peels in the degradation of total petroleum hydrocarbon content of crude oil polluted soil. Arch Appl Sci Res. 2012;4(3):1372-5.

2. Adam GI, Duncan H. Influence of diesel on seed germination. Environ Pollut. 2002;120: 363-70.

3. Wang K, Zhu YM, Zhang ZJ, Wang GH, Shi DC, Christie P. Surface water
phosphorus dynamics in rice fields receiving fertiliser and manure phosphorus. Chemosphere. 2001;42:209-14.

4. De Jing Y, He Z, Yang X. Role of soil rhizobacteria in phytoremediation of heavy metal contaminated soils. J Zhejiang Univ Sci Book. 2007;8:192-207.

5. Ogbo EM, Awertosowo U, Odogu G. Screening of four common weeds for use in phytoremediation of soil contaminated with spent lubricating oil. Afr J Plant Sci. 2009;3(5):102-106.

6. Shahrzad K, Fatemeh M, Shirin K. Phytoremediation of petroleum hydrocarbons by native plants of Damavand regions, Glob J Med Plant Res. 2013;1(1):8-11.

7. Thomas GW. Soil pH and soil acidity. In: D, L, Sparks (Ed.), Method of Soil Analysis. 3. Chemical Methods. SSSA and ASA. Madison. 1996;475-90.

8. Helmke PA, Sparks DL. Lithium, Sodium, Potassium, Cesium and Rubidium. In: D, L, Sparks (Ed.), Methods of Soil Analysis: Part 3. Chemical Methods and Processes. Soil Science Society of America. 1996;5:551-74.

9. Bremner JM. Total Nitrogen. In: Sparks, D. L (ed) Methods of Soil Analysis: Chemical Methods and Processes. Part 3. SSSA, ASA, Madison, Wisconsin, USA. 1996;1123-84.

10. Kuo S. Phosphorus. D. L Sparks Ed. Methods of soil analysis. Part 3 chemical methods and processes. SSSA and ASA. Madison, W. I. 1996;869-920.

11. Njoku KL, Akinola AO, Obol BO. Growth and performance of Glycine max L. grown in crude oil contaminated soil augmented with cow dung. Nat Sci. 2008;6(1):48-56.

12. Ekpo IA, Agbor RB, Okpako EC, Ekanem BE. Effect of crude oil polluted soil on germination and growth of soybean (Glycine max). Ann Biol Res. 2012;3(6):3049-54.

13. Taiz L, Zeiger E. Mineral nutrition and plant physiology. Second (Ed). Sunderland, M. A., Sinaner Associates Inc. 1998.

14. Blaylock MJ, Huang JW. Phytoextraction of metals, In: I. Raskin and B.D. Ensley (Ed.) Phytoremediation of toxic metals: using plants to clean up the environment, John Wiley and Sons, Inc, Toronto, Canada. 2000:303.

15. Riewerts JS, Thornton MN, Farago MN, Farago ME, Ashwore MR. Factors Influencing Metal Bioavailability in Soils; Preliminary Investigations for the development of a critical loads approach for metals. Chem. Spec. Bioavailab. 1998;10:61–75.

16. White PM, Wolf DC, Thoma GT, Reynolds CM. Phytoremediation of akylated polycyclic aromatic hydrocarbon in a crude oil-contaminated soil. Water Air Soil Pollut. 2006;169:207-20.

17. Stephen ET, Ijah NJJ. Comparison of Glycine max and Sida acuta in the phytoremediation of waste lubricating oil polluted soil. Nat Sci. 2011;9(8):190-3.

18. Stephen ET, Yakubu SA, Omebije PE, Edogbo E, Makolo D. Physico-chemical properties of spent lubricating oil polluted soil subjected to phytoremediation. J Environ Earth Sci. 2013;2(1):1-4.