PHYTOREMEDIATION: ASSESSMENT OF *Afzelia africana* GERMINATION AND GROWTH PERFORMANCE IN CRUDE OIL CONTAMINATED SOIL

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Abstract: This study investigated the effect of crude oil on growth of *Afzelia africana* (a nitrogen fixing plant) for potential utilization in remediation of toxicant in the environment. The experiment was carried out in a greenhouse. Seedling emergency, heights and girths, number of leave and nodulation was determined following standard techniques. Standard procedures was also used to ascertain the physical and chemical characteristics (organic matter, pH, nitrogen, phosphorous, potassium, calcium, magnesium and sodium) of the contaminated soil that the plant was sown. Results showed that at varying concentrations: 0, 25, 50, 75 and 100 ml of crude oil the seeding germination rate was 62, 55, 47, 38 and 32%, respectively; the height was 51.50, 42.85, 41.95, 40.80 cm and 34.70 cm, respectively at 16 weeks after planting (WAP); the girths 0.34, 0.26, 0.25, 0.23 and 0.21 mm, respectively, at 16 WAP; the number of nodules were 11, 10, 8, 6 and 4 respectively, and the number of leaves were 8.00, 8.00, 3.00, 3.00 and 1.00, respectively. The results showed a decline in all the growth parameters analyzed as the concentration of crude oil increased. The chemical and physical soil that the plant was sown showed a decrease in all the parameters analyzed. Despite the high concentration of crude oil in the medium, the seed emergency rate was > 25 and it showed positive potentials to withstand adverse effect of crude oil on plant metabolism and physiology. This suggests the potentials of *Afzelia africana* to be used for phytoremediation.

Keywords: Crude oil, soil contamination, nitrogen fixing plant, remediation

Introduction

Soil is an essential natural resource which acts as a central-point for ecosystem dynamics. It plays several ecological roles and it is the platform through which structures are erected and vegetation are cultivated (Izah *et al*., 2017a,b,c; 2018a,b). Several nutrients and biogeochemical cycling also take place in the soil (Izah *et al.*, 2018b) and it offers a good relationship platform for most environment components. According to Kassam (2009), the various environment matrices continuously interact to provide several ecosystem services including food, fuel and fibre which are needed for the sustenance of life. But due to urbanization, industrialization, exploration of natural resources (including crude oil and natural gas) and population growth, the increasing rate of land degradation (viz: desertification, deforestation, bush burning, soil erosion and pollution/ contamination), the decrease in organic matter have increased (Izah *et al.*, 2017d). Thus soil pollution is a major threat to the ecosystem.

In developing nations like Nigeria, some major causes of soil pollution include crude oil spills and its associated products, improper disposal of several waste streams generated from both domestic and industrial activities, use of some pesticides and combustions of hydrocarbons.

Despite the benefits associated to crude oil and its derived products in many producing nations, several adverse environmental effects abound in such countries that have impacted on several components of the ecosystem. Typically the impacts of crude oil arise from excessive flaring of gas into the environment oil spill (Seiyaboh and Izah, 2017a) and poorly managed crude oil waste.

Typically oil spill in several processing including exploration, production, refining, distribution, transportation, marketing (Aigberua *et al.*, 2017; 2016a,b) in many crude oil producing countries like Nigeria. In addition crude oil can spill during transportation due to rupture and corrosion of pipelines, vandalisms, sabotage and illegal bunkering and refining. Several authors have reported that crude oil spillages into aquatic and terrestrial ecosystems often occur during drilling, pipelines burst due to high pressure, corrosion or sabotage of pipes, tank overflows, tank loading, operational failure, leakages and seepages (Adesina, 2000; Osuji and Onojake, 2004; 2006; White *et al*., 2006; Tanee and Kinako, 2008). These activities have considerable adverse effects on the ecosystem including water and sediments, and its associated macroinvetebrates (Gijo *et al*., 2017; 2016a,b; Seiyaboh and Izah, 207b). Crude oil spills were highly reported in soil (Aigberua *et al*., 2017, 2016a,b) in many locations in the Niger Delta region of Nigeria (Izah *et al.*, 2017d). The adverse effects of crude oil pollution on some food crops have been reported by Isirimah *et al*. (1989), Kayode *et al*. (2009a), Kayode and Oyedeji (2012). At high concentrations it could affect plant growth and productivity (Anoliefo and Okoloko, 2000; Kayode *et al*., 2009a). The effect of oil spillage on agricultural soil is of great concern and requires a solution because its tremendous health hazards.

Several bioremediation methods of crude oil polluted soil abound. Some involved the use of surfactants, dispersants, microbes and plants. The use of plants provided a good remediation due to the fact that it utilizes solar energy and aesthetically pleasing to vegetation. Many legumes are able to convert atmospheric nitrogen into nitrogenous compounds useful to the plants. This is achieved by the presence of root nodules containing bacteria of the genus *Rhizobium*. These bacteria have a symbiotic relationship with legumes, fixing free nitrogen for the plants. In return legumes supply the bacteria with a source of fixed carbon produced by photosynthesis. This enables many Legumes to survive and compete effectively in nitrogen poor conditions. *Afzelia africana* is a tree species in the family *Caesalpinaceae* (Keay *et al*., 1989). The tree is widespread with a broad rather open crown and massive branches. It occurs in many African countries. They are prized for their quality wood, their bark which has many medicinal uses, and their nitrogen-rich leaves which enrich the soil (Ejikeme *et al.*, 2010). This study was therefore designed to evaluate germination and growth performance of *Afzelia africana* in crude oil contaminated soils.

Materials and Methods

Study sites

The study was conducted in a Screen House of the Department of Biological Sciences and Central Research Laboratory of the Faculty of Science, Niger Delta University, Wilberforce Island, Nigeria. The resultant soil physical and chemical properties analyzed in this study were conducted at the Central Research Laboratory, Federal University of Technology, Akure, Nigeria.

Source of experimental samples

A loamy-clay soil was collected from 5-year old fallowed plot in the Research and Experimental Farm of Niger Delta University, Wilberforce Island, Nigeria. The crude oil used for the study was obtained from Oporoma Flow Station of Shell Petroleum Development Company. *Afzelia africana* was obtained from National Centre for Genetic and Biotechnology, Ibadan, Nigeria.

Viability test of the seed and germination test of the plant

The seeds were tested for viability following the floating techniques previously described by Anoliefo and Vwioko (1995) and have been applied by Oyedeji *et al.* (2012). Five polythene bags were filled with loamy clay soil to a uniform weight of 3 kg at the Screen House. The soil were contaminated with varying amounts (0, 25, 50, 75 and 100 ml) of crude oil and thoroughly mixed with the soil. Then after, 200 g of well mixed contaminated soil were measured out and dissolved in 1 litre of distilled water for 3 days and the aqueous extracts were filtered and the resultant filtrates were preserved.

Whatman No 1 filter papers were double layered in 20 Petri dishes and were grouped into four for 25, 50, 75 and 100 ml with each concentration having 5 replicates. The control was also established with 5 replicates as well. Ten seeds of the plant were sown in each Petri-dish and moistened daily using the filtrates of the respective concentrations at 07:00 for 10 days. Seed germination counts were noted for 10 days of sowing. Then after, the percentage germination was determined using the formula adopted by Kayode and Oyedeji (2012):

$$\text{Germination test Percentage (Gt \%)} = \frac{\text{Number of seedlings that emerged/dish}}{\text{Total number of seeds sown}} \times 100$$

Growth response in the crude oil-polluted soil

About 3kg of the loamy lay soil were measured into 50 polythene bags and arranged in the Screen House. Then they were grouped into 5 and each group contained 10 bags for the various concentrations of 0, 25, 50, 75 and 100 ml. The bags were watered for two weeks at an interval of 3 days at 07:00 hour. Then after, 3 viable seeds were planted in each of the pots in the various experimental groups and its control two weeks after pollution (2 WAP_o). The resulting seedlings were thinned to one per bag at two weeks after planting (2WAP). Assessment of growth parameters was carried out fortnightly for 16 weeks. The heights and girths were measured with metre rule and Vanier caliper, respectively. The number of nodules and leaves were counted and recorded.

The Relative growth rate (RGR) and growth suppression (GS) were assessed at 16 WAP following the method previously described by Kayode and Tedela (2000).

Analysis of the physical and chemical characteristics of the soil

The physicochemical parameters of the soil were determined following standard procedures. The bulk density, soil organic carbon, organic matter, soil pH were analyzed according to Ibitoye (2006), volume of air in soil, soil water capillarity and porosity according to Akinsanmi (1975), soil nitrogen, calcium and magnesium using the methods of Anderson and Ingram (1996), available phosphorous according to Bray and Kutz (1945) and soil moisture content according to Osuji and Onojake (2004).

Results and Discussion

The germination rate of *Afzelia africana* in crude oil contaminated soil is presented in Fig. 1. At crude oil concentration of 0, 25, 50, 75 and 100 ml the germination rates were 62% (56 COV), 55% (50 COV), 47% (42 COV), 38% (31 COV) and 32% (28 COV), respectively. The results showed that the crude oil reduced the germination of *A. africana* seeds and as the concentration of crude oil increased, the germination of the seeds decreased. This trend was similar to the results obtained by Kayode and Oyedeji (2012) on the effect of crude oil on germination of okra. Typically soil characteristics affect life forms on soil.

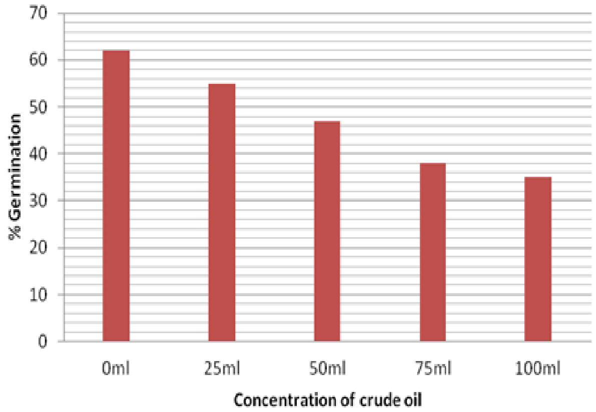

Fig. 1: Percentage germination of *Afzelia africana* in crude oil-polluted soil water extracts

The seedling heights of *A. africana* from crude oil contaminated soil are presented in Table 1. The mean heights was 3.98±1.15, 3.48±2.65, 3.05±1.82, 1.95±2.63 and 1.42±2.71 cm, at the varying concentration of 0, 25, 50, 75 and 100 ml, respectively; at 2 WAP and 51.50±2.95, 42.85±2.57, 41.95±0.61, 40.80±2.02 and 34.70±4.01 cm, respectively at 16 WAP. The results of girths are presented in Table 2. The mean girths were 0.12±0.03, 0.11±0.02, 0.11±0.01, 0.10±0.03, 0.10±0.05 mm respectively, at 2 WAP and 0.34±0.06, 0.26±0.07, 0.25±0.05, 0.23±0.02 and 0.21±0.07 mm, respectively at 16 WAP. Table 3 presents the number of leaves and nodules of *A. africana* cultivated in a crude oil contaminated soil. The numbers of nodules were 11, 10, 8, 6 and 4 nodules, respectively; at varying concentration of the crude oil of 0, 25, 50, 75 and 100 ml while the numbers of leaves were 8.00, 8.00, 3.00, 3.00 and 1.00, respectively.

The rate of germination, seedling heights, girths, number of leaves and nodules decreased as the concentration of crude oil increased. This suggests that crude oil has the tendency to hinder some metabolic processes that take place in the soil and thus affect plant growth and development. These results tend to agree with those of Kayode *et al*. (2009a) on *Vigna unguiculata* and *Zea mays* and Oyedeji *et al*. (2012) on *Abelmoschus esculentus.*

Table 1: Mean height of *Afzelia africana* grown in crude oil-polluted soil

Experimental Time (WAP)	Plant height (cm)/Crude oil concentration (ml)				
	0	**25**	**50**	**75**	**100**
2	3.98±1.15	3.48±2.65	3.05±1.82	1.95±2.63	1.42±2.71
4	8.90±1.85	8.45±1.05	6.85±1.67	5.43±2.07	3.18±1.94
6	17.80±3.02	14.65±1.74	12.17±0.87	8.25±2.82	8.07±2.11
8	26.90±2.45	21.70±2.13	15.44±3.15	14.15±2.71	13.42±2.38
10	34.60±2.20	26.50±1.82	22.51±1.80	22.18±1.41	18.05±1.85
12	40.20±1.75	31.70±2.05	30.15±1.65	29.82±1.68	26.75±2.35
14	45.20±2.60	35.20±1.75	36.60±1.07	34.02±1.80	30.05±3.06
16	51.50±2.95	42.85±2.57	41.95±0.61	40.80±2.02	34.70±4.01
∑X ± SD	229.08±17.97	184.53±15.76	168.72±12.64	156.60±17.14	135.64±20.41
$\Delta H = H_F - H_I$	47.52±1.8	39.37±0.08	38.9±1.21	38.85±0.61	33.28±1.3
RGR	0.18	0.18	0.19	0.22	0.23
GS	0.00	0.168	0.185	0.208	0.326
%GS	0.00	16.80	18.50	20.80	32.60

RGR = Relative growth rate; GS = growth suppression; H_1 = Initial Height; H_2 = Final Height; ΔH = Change in height; $\overline{X}$ = Mean; (±) = Standard deviation

Table 2: Mean girth of *Afzelia africana* grown in crude oil-polluted soil

Experimental Time (WAP)	Plant girths (mm)				
	0	**25**	**50**	**75**	**100**
2	0.12±0.03	0.11±0.02	0.11±0.01	0.10±0.03	0.10±0.05
4	0.14±0.02	0.13±0.03	0.12±0.02	0.12±0.01	0.11±0.04
6	0.17±0.01	0.16±0.02	0.15±0.03	0.14±0.02	0.13±0.04
8	0.21±0.04	0.18±0.04	0.17±0.04	0.15±0.05	0.14±0.03
10	0.26±0.05	0.20±0.01	0.18±0.02	0.17±0.04	0.15±0.05
12	0.29±0.03	0.23±0.02	0.21±0.04	0.19±0.05	0.16±0.06
14	0.32±0.04	0.24±0.01	0.23±0.03	0.21±0.03	0.18±0.06
16	0.34±0.06	0.26±0.07	0.25±0.05	0.23±0.02	0.21±0.07
$\Delta G = G_F - G_I$	0.22±0.03	0.15±0.05	0.14±0.04	0.13±0.01	0.11±0.02
∑X ± SD	1.85±0.28	1.51±0.22	1.42±0.24	1.31±0.25	1.18±0.40

Table 3: Mean number of leaf and nodules of *Afzelia africana* grown in crude oil-polluted soil

Mean No.	0	25	50	75	100	Mean	V	SD
Leaf	11.00	10.00	8.00	6.00	4.00	7.80	8.20	2.86
Nodules	8.00	8.00	3.00	3.00	1.00	4.60	10.30	3.21

V = Variance; **SD =** Standard deviation

Table 4: Physiochemical characteristics of crude oil contaminated soil that *Afzelia africana* was used for remediation

Parameters	Groups	Treatments				
		0	**25**	**50**	**75**	**100**
pH	Contaminated	5.04	4.94	4.75	4.65	4.61
	Control	5.5	5.4	5.3	5.17	5.12
Organic matter, %	Contaminated	1.75	1.7	1.56	1.48	1.42
	Control	2.25	2.05	2.01	1.97	1.85
% N	Contaminated	0.54	0.42	0.34	0.28	0.24
	Control	0.65	0.6	0.51	0.46	0.44
P, mg/kg	Contaminated	7.87	7.8	7.35	7.15	7.02
	Control	12.51	12.25	12.1	12.15	12.01
K, mg/kg	Contaminated	4.08	4.04	3.95	3.68	3.47
	Control	5.5	5.2	5.08	5.01	4.85
Na, mg/kg	Contaminated	2.21	2.15	2.12	2.1	1.9
	Control	3.65	3.5	3.28	3.28	3.28
Ca, mg/kg	Contaminated	19.2	18.45	18.05	18.05	15.8
	Control	26.5	25.45	24.5	23.2	21.7
Mg, mg/kg	Contaminated	2.25	2.05	2.05	1.85	1.85
	Control	3.2	3.14	3.1	3.05	2.85

The physicochemical characteristics of the crude oil contaminated soil used for the study is presented in Table 4. The concentrations of organic matter, nitrogen, phosphorus, potassium, calcium, magnesium, sodium and pH decreased as the concentration of crude oil increased. Thus as the concentration of crude oil increased the acidity of the soil increased. The organic matter is a major parameter used to measure soil fertility. Alteration in organic matter may affects metabolism among soil microbes. In the study the decline in the organic matter tend to suggest that *A. africana* has the potential to remove toxicant for the environment.

The fertilizer determinant parameters (nitrogen, phosphorus and potassium) decreased as the concentration of crude oil increased. The growth of *A. africana* in the crude oil contaminated soil tends to suggest that the plant has the ability to improve soil nitrogen and available phosphorus in the soil. This might be attributed to the tendency of the plant to fix nitrogen necessary to improve microbial populations in the rhizosphere (Cunningham *et al*, 1996). The low level of phosphorus in the remediated soils showed reduced availability. Calcium concentration in the soil was higher compare to magnesium and sodium. The finding of this study showed that *A. africana* is effective in reducing the concentrations of the cations, especially the calcium with higher concentration among the soil exchangeable cations. This trend has been previously observed by Onyeike *et al.* (2000).

Conclusion

In many crude oil-producing nations like Nigeria, oil spill into the environment during drilling, corrosion, rupture, vandalisms of pipeline, operation failure of equipment and bunker activities, is a common occurrence. The crude oil has the tendency to alter the receiving ecosystem, especially the soil physical and chemical properties. The findings of this study suggest that *A. africana* has potential to grow and develop into seedlings in crude oil-contaminated soils. It has the tendency to tolerate the crude oil and subsequently remediate the contaminated soil in its rhizosphere.

Conflict of Interest

Authors have declared that there is no conflict of interest reported in this work

References

Adesina AA. 2000. Environmental geological investigation of hydrocarbon pollution of soil and ground water within the upper continental sequences of the tertiary Niger Delta. Paper presented at a Seminar in the Department of

Geology, University of Benin, Benin-City, Nigeria; 6th March, 2000, pp 1-13.
Aigberua AO, Ekubo A.T, Inengite AK & Izah SC 2016a. Seasonal variation of nutrient composition in an oil spill contaminated soil: A case of Rumuolukwu, Eneka, Port Harcourt, Nigeria. *Biotechnological Research*, 2(4): 179-186.
Aigberua AO, Ekubo AT, Inengite AK & Izah SC 2016b. Seasonal variation of nutrient composition in an oil spill contaminated soil: a case of Rumuolukwu, Eneka, Port Harcourt, Nigeria. *Biotechnological Research*, 2(4): 179-186.
Aigberua AO, Ekubo AT, Inengite AK & Izah SC 2017. Assessment of some selected heavy metals and their pollution indices in an oil spill contaminated soil in the Niger Delta: a case of Rumuolukwu community. *Biotechnological Research*, 3(1): 11- 19.
Akinsanmi O 1975. Certificate Agricultural Science. 1st Ed, Longman Group Ltd.
Anderson JM. & Ingram JSI 1996. Tropical soil biology and fertility. *A Handbook of Soil Methods.* 2nd Ed. CAB International Information Press, Eynsham, 221pp.
Anoliefo GO & Okoloko GE 2000. Comparative toxicity of Forcados blend crude oil and its water soluble fraction on seedlings of *Cucumeropsis manni* Naudin. *Nigerian Journal of Applied Science*, 18:39-49.
Anoliefo GO & Vwioko DE 1995. Effect of spent lubricating oil on the growth of *Capsicum annuum* L. and *Lycopersicon esclentum*, Mill. *Environmental Pollution*, 88: 361-364.
Bray RH & Kurtz LT 1945. Determination of total organic and available forms of phosphorous in the soils. *Soil Science*, 59: 39-45.
Cunningham SD, Anderson, TA, Schwab PA & Hsc FC 1996. Phytoremediation of soils contaminated with organic pollutants. *Advances in Agronomy*, 56: 55-114.
Ejikeme PM, Obasi LN & Egbuonu ACC 2010. Physico-chemical and toxicological studies on *Afzelia africana* seed and oil. *Afr. J. Biotech.*, 9(13): 1959-1963.
Gijo, AH, Hart AI & Seiyaboh EI 2017. The impact of makeshift oil refineries on the physico- chemistry of the sediments of the nun river estuary, Niger Delta, Nigeria. *Sky J. Soil Sci. Environ. Mgt*., 6(1): 19-025.
Gijo AH, Hart AI & Seiyaboh EI 2016a. The Impact of Makeshift Oil Refining Activities on the Physico-Chemical Parameters of the Interstitial Water of the Nun River Estuary, Niger Delta, Nigeria. *Biotechnol. Res.*, 2(4): 193-203.
Gijo AH, Hart AI & Seiyaboh EI 2016b. The impact of makeshift oil refineries on the macro-invertebrates of the nun river estuary, Niger Delta, Nigeria. *Greener J. Biol. Sci.*, 6(6): 112-119.
Ibitoye AA 2006. Laboratory Manual on Basic Soil Analysis. 2nd Ed. Foladave Nig. Ltd, Akure, Nigeria, pp. 1-70.
Isirimah N, Zuofa K & Longanathan P 1989. Effect of crude oil on maize performance and soil chemical properties in the humid forest zone of Nigeria. *Discovery and Innovation*, 1(3): 95-98.
Izah SC, Bassey SE & Ohimain EI 2017a. Geo-accumulation index, enrichment factor and quantification of contamination of heavy metals in soil receiving cassava mill effluents in a rural community in the Niger Delta region of Nigeria. *Molecular Soil Biology*, 8(2): 7-20.
Izah SC, Bassey SE & Ohimain EI 2017b. Assessment of heavy metal in cassava mill effluent contaminated soil in a rural community in the Niger Delta region of Nigeria. EC *Pharmacology and Toxicology*, 4(5): 186-201.
Izah SC, Bassey SE & Ohimain EI 2017c. Assessment of pollution load indices of heavy metals in cassava mill effluents contaminated soil: a case study of small-scale cassava processing mills in a rural community of the Niger Delta region of Nigeria. *Bioscience Methods*, 8(1): 1-17.
Izah SC, Angaye CN, Aigberua AO & Nduka JO 2017d. Uncontrolled bush burning in the Niger Delta region of Nigeria: potential causes and impacts on biodiversity, *Int. J. Molecular Ecol.gy and Conservation*, 7(1): 1-15.
Izah SC, Bassey SE & Ohimain E 2018a. Ecological risk assessment of heavy metals in cassava mill effluents contaminated soil in a rural community in the Niger Delta Region of Nigeria. *Molecular Soil Biology*, 9(1): 1-11.
Izah SC, Bassey SE & Ohimain EI 2018b. Impacts of cassava mill effleunts in Nigeria. *J. Plant and Ani. Ecol.*, 1(1): 14 – 42.
Kassam A 2009. Sustainability of farming in Europe: Is there a role for conservation agriculture? *J. Farm Mgt.*, 13(10): 11-22.
Kayode J & Oyedeji AA 2012. Early Growth Response of Maize (*Zea mays* L.) in spent lubricating oil-polluted soil. *Environtropica Journal*, 8(1-10): 132-138.
Kayode J & Tedela PO 2000. Preliminary investigation on the herbicidal potential of the extracts from maize inflorescence on seeds of three tropical weeds. *Pak. J. Scientific and Ind. Res.*, 47(5): 387-389.
Keay RWJ, Onochie CFA & Stanley OP 1989. Nigerian trees. Oxford University Press, New York.
Onyeike EN, Ogbuja SI & Nwinuka NM 2000. Inorganic ion levels of soils and streams in some areas of Ogoniland, Nigeria as affected by crude oil spillage. *Environ. Monitoring and Assessment*, 73: 1991-2125.
Osuji LC & Onojake CM 2004. Trace heavy metals associated with crude oil: A case study of Ebocha-8 oil-spill-polluted site in Niger Delta, Nigeria. *Chemistry and Biodiversity*, 1: 1708-1715.
Osuji LC & Onojake CM 2006. Field reconnaissance and estimation of petroleum hydrocarbon and heavy metal contents of soils affected by the Ebocha-8 oil spillage in Niger Delta, Nigeria. *J. Envtal. Mgt.*, 79: 133-139.
Oyedeji AA, Adebiyi AO, Omotoyinbo MA & Ogunkunle CO 2012. Effect of crude oil-contaminated soil on the germination and growth performance of *Abelmoschus esculentus*- A widely cultivated vegetable crop in Nigeria. *Am. J. Plant Sci.*, 3: 1451-1454.
Seiyaboh E & Izah SC 2017a. A Review: Impacts of gas flaring on vegetation and water resources in the Niger Delta region of Nigeria. *Int. J. Econ., Energy and Envt.*, 2(4): 48-55.
Seiyaboh EI & Izah SC 2017b. Review of Impact of anthropogenic activities in surface water resources in the Niger Delta region of Nigeria: A case of Bayelsa State. *Int. J. Ecotoxico. and Ecobio.*, 2(2): 61 – 73.
Tanee FBG & Kinako PDS 2008. Comparative studies of biostimulation and phytoremediation in the mitigation of crude oil toxicity in tropical soil. *Journal of Applied Science and Environmental Management*, 12(2):143-147.
White PM, Wolf DC, Thoma GJ & Reynolds CM 2006. Phytoremediation of alkylated polycyclic aromatic hydrocarbons in a crude oil-contaminated soil. *Water, Air and Soil Pollution*, 169(1): 207-220.