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Research Article

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Bioremediation of Crude Oil Polluted Soil: Maize Husk Compost as a Test Bioremediant

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Abstract Crude oil simulated soil was treated with 300g, 450g and 600g concentrations variation of maize husk compost (MHC). The effects of maize husk compost (MHC) treatment on physicochemical properties of crude oil contaminated soil, total petroleum hydrocarbon (TPH) %, biostimulation efficiency (B. E) % were studied. The results indicated that the composting process greatly enhanced the overall rate of total petroleum hydrocarbons mineralization. The greatest reduction of TPH and increase in B. E (%) were obtained in the first compost with 600g of maize husk compost amended soils (82.04% and 88.93%), followed by the 450g of maize husk compost amended soils where TPH (%) reduction and B. E (%) increase were still high (67.52% and 86.55%) respectively. In the compost with 300g of maize husk amended soils, the TPH concentrations decreased and increased in B. E (%) by only (56.96% and 84.06%) respectively. The sequence of TPH reduction (%) and increase in B. E (%) of maize husk compost amended soils followed the same soil physicochemical properties improvement sequence of maize husk compost amended soils. In addition, mineralization time increased with increase in maize husk compost concentration. The composting process played a significant role in the biodegradation of petroleum hydrocarbons and can effectively lead to maximum utilization of maize husk agricultural waste produced by farmers as compost.

Keywords simulated soil, maize husk compost, total petroleum hydrocarbon (TPH), biostimulation efficiency (B. E)

Introduction

Petroleum is the mainstay of Nigeria`s economy since it discovery. However, it is gradually becoming a curse to the people in the oil producing areas of Nigeria due to the devastating effect of oil spillage that is negatively affecting the socioeconomic life of the people. Crude oil spillage is a serious environmental problem that is due to an increasing petroleum exploration, refining and other related industrial activities [1,2]. The contamination of these habitats poses major public health and socio-economic hazards which most often has developed into impetuous protests between some of the oil companies and the surrounding communities [3]. The major constituents of molecules in petroleum oil spills and refined products are biodegradable, and they will gradually diminish from the environment as microorganisms utilize them for their metabolic activities [4]. Agricultural waste is obviously huge in the Niger delta area of Nigeria due to the heavy rainfall average, thick forest belt and large participation of arable farming practices in the region which is apparently contributing to environmental challenges to the society. With the huge agro-waste generated in this region, there is a need to transform the agro-waste to useful purposes. Bioremediation is the latest scientific strategy that has been discovered to be cost effective, possess great potential in removing crude oil from the soil and degrade petroleum hydrocarbons without leaving behind any toxic products to the soil ecosystem. Among the bioremediation strategies, composting is the most cost effective and viable bioremediating strategy due to the surplus availability of its materials. Composting, according to Odokuma [5] is aerobic degradation of solid domestic organic agro-waste into a form that can be used as amendment material for soil. Many agro-waste organic materials have been tested as composting agents in bioremediation of crude oil polluted soil which include water hyacinth [6]; palm oil mill effluent, palm bunch refuse and cassava peels [3, 7-8]; saw dust and waste cotton [9]; cocoa pod husk and plantain peels [10] and unspecified compost [11-12]. However, maize husk compost was discovered to be less utilized in composting strategy in bioremediation. Among cereals, maize is widely grown in southern Nigeria being a staple food crop for the common man. It is important to note that the agro-waste generated by maize is enormously huge and there is need to utilize it for useful purposes devoid of environmental nuisance. In this study, maize husk compost bioremediation potential was put to test as a bioremediant in bioremediation of crude oil polluted soil.

Materials and Methods

Samples Collection: Soil samples were collected at random from an unutilized land at the back of 2-in1 lecture theatre building within the Federal University of Technology, Akure using a hand trowel at a depth of 0-20cm below soil surface, having no pollution history and devoid of petroleum hydrocarbons contamination. Bonny light crude oil with specific gravity of 0.8399 @ 60F/16^{0}C was obtained from Shell Petroleum Development Company (SPDC) flow station, Bonny Island, Rivers state, Nigeria. Maize husk waste was collected at a commercial agricultural farm along Akure-Owo road, Ogbese, Ondo state, Nigeria

Sample Preparation, Artificial pollution and Treatment: Soil was air dried for a period of seven days in a clean well ventilated laboratory and sieved by passing through a 2mm mesh sieve. 1kg of soil was each measured into clean dry experimental pot and moistened with distilled water to ensure proper mixing with the crude oil. Artificial pollution of the soil samples was done by measuring 100ml of crude oil (CO) into the experimental pot containers containing 1kg of soil each. The individual mixtures were thoroughly mixed to achieve a 10% artificial pollution. 10% spiking was adopted to achieve severe pollution because beyond 3% concentration [13], crude oil has been reported to be increasingly deleterious to soil biota [14]. The maize husk waste sample was put in a jute bag for 30days in a damp corner in the Laboratory for proper decomposition. Thereafter, it was crushed to sizes, thoroughly mixed to achieve uniform particle size and stored in neat polythene bags for use. 300g, 450g, and 600g of the maize husk compost (MHC) sample each were added to the experimental pots containing 1kg of 100ml crude oil artificial polluted soil and thoroughly mixed to obtain homogeneity and to allow proper bioremediation for another 90 days with periodic turning for proper aeration and watering. The experimental pots contained natural soil (NS), crude oil polluted soil (COPS) and Maize husk compost amended soil (MHCAS) for 90 days bioremediation study periods. This experimental design was a randomized complete block and it was triplicated.

Soil Physicochemical Analysis: Soil physicochemical characteristics such as soil particle size analysis, pH, K^+, Na^+, Ca^{2+}, Mg^{2+}, cations exchange capacity (CEC), moisture, water holding capacity (WHC), porosity, electrical conductivity, total organic carbon (TOC), organic matter (OM), total nitrogen (N) and phosphorus (P) were determined before pollution, 30 days interval for 90 days after pollution and bioremediation process. Soil pH was determined electrometrically following the procedure outlined by Mylavarapus and Kennelley [15]. Particle size analysis was done using bouyoucos [16] hydrometer method. Soil minerals were determined by the method of Tel [17]. Total organic carbon and matter were determined by the wet dichromate acid oxidation method of Nelson and Sommers [18]. Soil water holding capacity and porosity were determined by the method of Michael [19]. Total Nitrogen was determined using the method of Radojevic and Bashkin [20]. Total Phosphorus was determined by Bray and Kurtz method [21]. Electrical conductivity was carried out as described by Chopra and Kanzer [22]. Soil moisture was determined using the method of Michael [19].

Determination of Total Petroleum Hydrocarbon (TPH): 1g each of the artificially polluted and bioremediated soil samples were dissolved in 10ml of hexane and shaken for ten minutes using a mechanical shaker. The solution was filtered using a Whatman filter paper and the filtrate diluted by taking 1ml of the extract into 50ml of hexane [13]. Procedural blanks and standard solutions were prepared and included to ensure analytical quality control so as to ensure the accuracy and reproducibility of the results. The absorbance of this solution was read at 460 nm with HACH DR/2010 Spectrophotometer using *n*-hexane as blank. Replicate analyses were carried out on the determination of TPH to yield a statistical mean which will be used to determine trueness and also standard deviation of the mean to measure precision [23] [24]. Total petroleum hydrocarbon (TPH) was determined at 15 days interval for 90 days. Percentage of TPH degradation was calculated using the following equation:

$$\% \text{ of } TPH \text{ degradation} = \frac{TPH_{PO} - TPH_{A}}{TPH_{PO}} \times 100$$

Where TPH_{PO} is the crude oil in the untreated soil at zero time and TPH_{A} is the degradation of crude oil in maize husk compost amended soil (MHCAS) at different time.

Biostimulation Efficiency (B. E) %:

Evaluation of crude oil polluted soil and maize husk compost amended soil (MHCAS) Biostimulation Efficiency (B.E) % was calculated at 15 days intervals for 90 days bioremediation period using the following equation [25]:

$$\text{B.E } \% = \frac{TPH_{A}\% - TPH_{P}\%}{TPH_{A}\%} \times 100$$

Where TPH_{A} is the degradation of crude oil in maize husk compost amended soil (MHCAS) at different time and TPH_{P}, the degradation of crude oil in crude oil polluted soil at different time.

Results and Discussion

Nutrients and minerals analysis results of crude oil and maize husk compost samples in this study are shown in Table (1).

Table 1: Results of nutrients and minerals analysis of crude oil (CO) and maize husk compost (MHC)

Parameters	Crude Oil (CO)	maize husk compost (MHC)
pH	4.20 ± 0.000	6.02 ± 0.100
OC %	96.50 ± 0.016	9.58 ± 0.014
OM %	—	16.52
N %	0.11 ± 0.002	0.30 ± 0.001
P %	0.07 ± 0.001	0.27 ± 0.014
K^+ [C mol/kg]	0.05 ± 0.000	0.48 ± 0.005
Na^+ [C mol/kg]	0.06 ± 0.000	0.12 ± 0.001
Ca^{2+} [C mol/kg]	0.10 ± 0.001	0.10 ± 0.002
Mg^{2+} [C mol/kg]	0.07 ± 0.001	0.14 ± 0.001
CEC	0.28	0.84
C/N	877.27	31.93

Results = Mean values ± standard deviation

Table (2) results revealed that crude oil pollution did not change the composition of the Particle size of the soil [26] with sand (65%), clay (45%) and silt (40%) fractions respectively were all in the same range for the natural, artificial crude oil polluted soil and maize husk compost amended soil (MHCAS) soils. A classification of the soil based on the USDA [27] textural class shows that the soil is sandy loam. pH, conductivity, K^+, Na^+, Ca^{2+}, Mg^{2+}, cations exchange capacity (CEC), moisture, WHC and porosity of crude oil polluted soil control (2) were reduced as compared to the natural soil control (1) as shown in Table (2). The observed reduction followed the submissions of Oyedele and Amoo [26]; Osuji and Nwoye [28]; Oyedele et al [29]. The observed reduction in pH and conductivity could be as a result of increase in hydrophobicity of the crude oil polluted soil condition [26] and probably due to release of acidic intermediates that lower the pH. Immobilization of the soil nutrients and minerals by crude oil was the major cause of soil K^+, Na^+, Ca^{2+}, Mg^{2+}, cations exchange capacity (CEC), moisture, WHC, and porosity reduction of crude oil polluted soil (COPS) in Table (2) which made them unavailable to the soil [26, 28]. The added carbon substrate from the crude oil might have led to the increase of organic carbon and organic matter in crude oil polluted soil as compared to the natural soils as revealed in Table (2) [30]. The results in Table (2) revealed the increase in values of pH, conductivity, organic carbon, organic matter, total nitrogen, phosphorus, K^+, Na^+, Ca^{2+}, Mg^{2+}, cations exchange capacity (CEC), moisture, WHC and porosity with increase in concentration of maize husk compost in maize husk compost amended soils as compared to crude oil polluted soil control (2) in the sequence of 300g of MHCTS < 450g of MHCTS < 600g of MHCTS. This is in line with the findings of Romanus [3]. The increase in the soil nutrients and minerals with increase in maize husk compost in maize husk compost amended soil may be due to the

Table 2: Results of physicochemical properties of natural soil (NS), crude oil polluted soil (COPS) and maize husk compost amended soil (MHCAS) for 90 days

Parameters	Natural Soil (control 1) (NS)	Crude Oil Polluted Soil (control 2) (COPS)	300g of MHCTS	450g of MHCTS	600g of MHCTS
Sand (%)	65	65	65	65	65
Clay (%)	45	45	45	45	45
Silt (%)	40	40	40	40	40
Textural class	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
pH	6.02 ±0.050	4.60 ±0.141	6.51 ±0.001	6.73 ±0.100	6.75 ±0.016
Conductivity (μs/cm)	217.24 ±0.002	166.11 ±0.005	234.66 ±0.001	241.91 ±0.000	242.63 ±0.014
Organic carbon (%)	0.69 ±0.000	1.65 ±0.001	0.94 ±0.000	1.56 ±0.002	1.69 ±0.001
Organic matter (%)	1.31 ±0.001	2.89 ±0.005	1.65 ±0.002	2.73 ±0.001	2.96 ±0.000
Nitrogen (%)	0.19 ±0.001	0.10 ±0.000	0.30 ±0.000	0.51 ±0.002	0.67 ±0.000
Phosphorous (%)	22.83 ±0.141	18.68 ±0.100	23.11 ±0.158	23.17 ±0.160	23.21 ±0.100
K^+ [Cmol/kg]	0.27 ±0.001	0.21 ±0.000	0.29 ±0.001	0.34 ±0.002	0.52 ±0.001
Na^+ [Cmol/kg]	0.24 ±0.000	0.20 ±0.002	0.27 ±0.000	0.30 ±0.001	0.36 ±0.005
Ca^{2+} [Cmol/kg]	2.61 ±0.010	1.90 ±0.001	2.82 ±0.002	2.83 ±0.024	3.14 ±0.014
Mg^{2+} [Cmol/kg]	2.33 ±0.002	1.75 ±0.001	2.32 ±0.001	2.46 ±0.002	2.49 ±0.005
CEC	5.45	4.06	5.70	5.93	6.51
C/N	3.63	16.50	3.13	3.06	2.52
Moisture (%)	10.40 ±0.100	10.07 ±0.141	10.59 ±0.158	11.11 ±0.100	11.25 ±0.160
WHC (%)	37.60 ±0.000	36.95 ±0.100	37.51 ±0.100	37.74 ±0.141	37.98 ±0.158
Porosity (%)	46.68 ±0.141	45.79 ±0.160	46.70 ±0.000	46.87 ±0.158	47.11 ±0.100

Results = Mean values ± standard deviation

additional nutrients supplement and energy being supplied by the maize husk compost [3, 31] that microbially mineralized the biostimulated soils that enhanced the improvement of the soil properties [29]. The pH and conductivity increase in maize husk compost amended soils with increase in maize husk compost concentration enhanced more soil microbes to thrive for mineralization of amended soils in that sequence in Table (2). The biohumification increase of maize husk compost increase in the mineralized maize husk compost amended soils might be responsible for the sequential increase of organic carbon and organic matter in order of 300g of MHCAS < 450g of MHCAS < 600g of MHCAS in Table (2). The observed sequential reduction in C/N with increase in maize husk compost loading in maize husk compost amended soils in Table (2) was due to the microbial fixation of nitrogen from atmosphere into the amended soils [29].

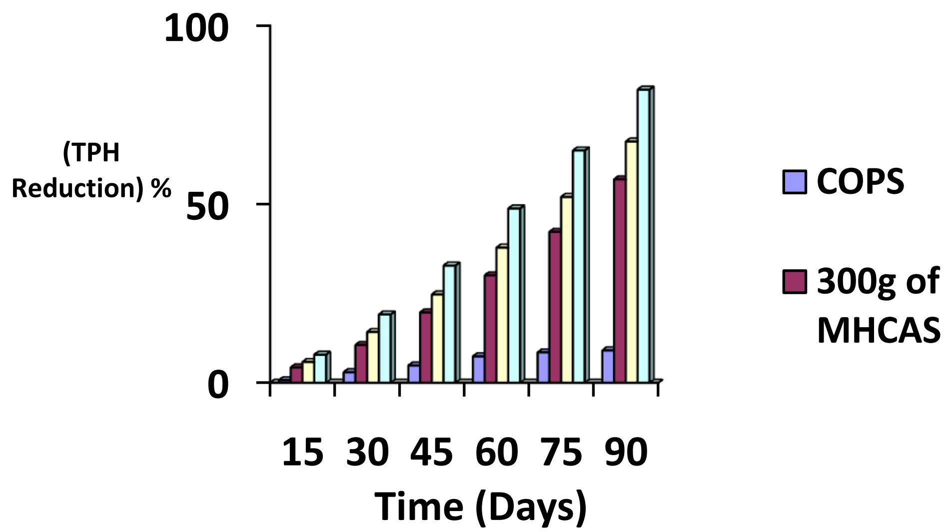

Figure 1: Total Petroleum Hydrocarbons (TPH) % reduction of Maize Husk Compost Amended Soil (MHCAS) for 90 days of Biostimulation Study

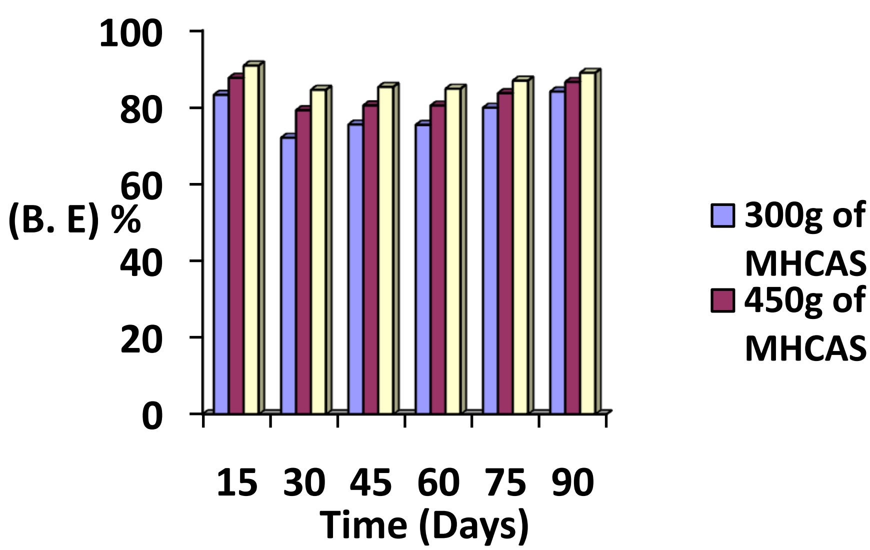

Figure 2: Biostimulation Efficiency (B. E) % of Maize Husk Compost Amended Soil (MHCAS) for 90 days of Biostimulation Study

The increase in moisture, WHC and porosity with increase in maize husk compost in maize husk compost amended soils that was in line with the submissions of Ezeaku [12] may be due to the soil aeration and physiochemical improvement Table (2). There were increases in TPH reduction (%) and biostimulation efficiency (B. E) % with increase in maize husk compost loading in maize husk compost amended soils respectively. The TPH reduction (%) and increased B. E (%) results are in line with the findings of Agarry *et al* [32]; Ahmed *et al* [33]; Rowland *et al* [34]. The increase in the biodegradation factors like time, nutrients, pH, and moisture level with increase in maize husk compost concentration during bioremediation process may be considered to optimize the biodegradation of TPH in maize husk compost amended soils [35]. The highest TPH reduction (%) and increased B. E (%) were observed in 600g of maize husk compost amended soils respectively (82.04% and 88.93%) > 450g of maize husk compost amended soils (67.52% and 86.55%) > 300g of maize husk compost amended soils (56.96% and 84.06%) till the 90^{th} day of the study. The results observations may be due to the cellulose, hemi-cellulose, lignin, phosphorus and nitrogen ratio in the different plant residues like maize husk as well as in the animal dung wastes which enhanced microorganism utilization of crude oil for carbon and energy source [36] for growth, population to degrade crude oil in maize husk compost amended soils [32] [37]. Meanwhile, the total petroleum hydrocarbons TPH (%) reduction level in the crude oil polluted soil (COPS) control (2) system was observed to be 10 % Fig. (1) at the end of 90 days study period. The reduction may be due to the presence of indigenous petrophile microbes in the crude oil contaminated soil that utilized carbon and energy in the crude oil for their metabolism.

Conclusion

This research work was carried out to reveal the effects of 300g, 450g, and 600g concentration variation of the maize husk compost (MHC) samples in experimental pots each containing 1kg of 100ml crude oil artificial polluted soil for 90 days of study period. The remediation process was followed by monitoring the decrease and increase in the TPH of the contaminated soil. There was highest TPH reduction (%) and increased B. E (%) observation in 600g of maize husk compost amended soils respectively (82.04% and 88.93%) > 450g of maize husk compost amended soils (67.52% and 86.55%) > 300g of maize husk compost amended soils (56.96% and 84.06%) for the study period of 90 days. Apart from massive soil nutrient improvement, Maize husk compost (MHC) was a tested organic fertilizer in degrading TPH in crude oil contaminated soil.

Reference

1. Okpokwasili G. C. and Odokuma L. O. Effect of salinity on biodegradation of oil spill dispersants. *Waste Manag.,* 1990,10: 141–146.
2. Odokuma L. O. and Okpokwasili G. C. Seasonal ecology of hydrocarbon-utilizing microbes in the surface waters of a river. *Environ Monit Assess.,* 1993, 27: 175–191.
3. Romanus A. A, Ekundayo A. O., Aigere S. P. and Okwu G. I. Bacterial degradation of petroleum hydrocarbons in crude oil polluted soil amended with cassava peels. *American Jounal of research communication*, 2015, Vol. 3 (7): pp 1-20
4. Prince R.C. Petroleum spill bioremediation in marine environment. *Crit. Rev. Microbiol*., 1993, 19: 217–242.
5. Odokuma L. O. The Genius in the microbe: An indispensable tool for the management of Xenobiotic mediated Environmental flux. *87th Inaugural Lecture, held at Ebitimi Banigo Hall, University of Port Harcourt, Nigeria*, 2012.
6. Offor, U. S and Iyagba, A. G. Biostimulative effects of water hyacinth (Eichhornia Crassipes (mart.) solms) mulch on the germination of okra (abetmochus Esculentus (L.) moench) grown in a crude oil contaminated soil. *European Scientific Jounal*, 2013, Vol. 9, No. 21
7. Otaraku I. J. and Anozie U. Remediation of crude oil polluted soil using oil palm empty bunch. *International Journal of application or innovation in engineering and management*, 2013, Volume 2, issue 8: pp 1- 4
8. Ahamefule H. and Fawole O. Bio-stimulation of a spent Auto-Engine Oil contaminated sandy-loam Utisol Using maize (*Zea mays*) As Test Crop. *International conference on agricultural, Ecological and medical sciences*, 2015: pp 10 – 11

9. Adedokun O. M. and Ataga A. E. Effects of amendments and bioaugumentation of soil polluted with crude oil, automotive gasoline oil, and spent engine oil on the growth of cowpea (vigna ungiculata L. walp). *Scientific Research and Essay*, 2007, Vol, 2 (5): pp. 147 – 149
10. Agbor R. B, Ekpo I. A, Udofia U. U, Okpako E. C and Ekanem E. B. Potentials of cocoa pod husk and plantain peels in the degradation of total petroleum hydrocarbon content of crude oil polluted soil. *Archives of Applied Science Research*, 2012, 4 (3): 1372 – 1375
11. Hichman R., Souabi S., Safi M., Terta M., Arad M., Anouzla A., Hafid M. Soil bioremediation of hydrocarbons and green waste elimination through composting process. *International Journal of Environmental Monitoring and Analysis*, 2014, 2 (6 – 1): pp13 - 22
12. Ezeaku, P. I. and Egbemba, B. O. Yield of maize (manoma spp) affected by automobile oil waste and compost manure. *African Journal of Biotechnology*, 2014, Vol. 13 (11): pp. 1250 – 1256
13. Akpoveta O. V., Egharevba F., Medjor O. W., Osaro K. I. and Enyemike E. D. Microbial Degradation and its Kinetics on Crude Oil Polluted Soil. *Res.J.Chem.Sci*, 2011, Vol. 1(6): pp 8-14
14. Osuji L. C., Egbuson E. J. G. and Ojinnaka C. M. Chemical reclamation of crude-oil- inundated soils from Niger Delta, Nigeria. *Chem. Ecol*., 2005, 21(1): pp 1-10
15. Mylavarapus R. S. and Kennelley E. D. UF/IFAS Extension Soil Testing laboratory (ESTL). *Analytical procedures and Training Manual*, 2002: pp 350
16. Bouyouces G. H. The Hydrometer Method for the Determination of Soil Particle Size. *Agron, J.*, 1951, 43: pp 434- 438
17. Tel D. A. Soil and plant analysis. *IITA*, *Department of Land Resource Science University of Fuclph, Guclph, Ontario, Canada*, 1984: pp 117-190.
18. Nelson D. W. and Sommers L. E. Total carbon, Organic matter, In: Page, A.L et al. (Eds.), Methods of soil Analysis Part 2. *Agronomy Monograph 9, 2nd Edn., American Society for Agronomy and Soil Science Society of America. Madison, Wisconsin*, 1982: pp 539 – 579
19. Micheal A. M. Irrigation theory and practice. *Pub. Bikas publishing house PUT Ltd, New Delhi*, 1978
20. Radojevic M. and Bashkin V. N. Practical environmental analysis. *Royal society of chemistry, Cambridge U.K*, 1999
21. Bray R. H. and Kurtz L. T. Determination of total organic and available forms of phosphorus in soils. *Soil Sci.*, 1943, 59: pp 39-45
22. Chopra G. and Kanzar C. Analytical Agricultural chemistry. *2nd edition, Prentice-Hall, India*, 1988
23. Stanton R. E. Rapid methods of Trace Analysis for Geochemical Applications. *London: Edward Arnold Ltd*, 1966
24. Valcarcel M. Principles of analytical Chemisry. *New York: Springer-Verlag Berlin Heidelberg*, 2000.
25. Zahed M. A, Aziz H. A, Isa M. H, Mohajeri L., Mohajeri S. Kinetic modeling and half life study on bioremediation of crude oil dispersed by Corexit 9500. *J Hazard Mater*, 2011, 185: pp 1027-1031.
26. Oyedele A. O. and Amoo, I. A. Remediation Of Crude Oil Polluted Soil Using Cow Dung Manure In Relations To The Growth Of Maize (*Zea mays L.*). *Canadian Open Agricultural and Soil Science Journal*, 2014, Vol. 1, No. 1:pp. 1-16
27. United State Department for Agriculture (USDA). *National Resources Conservation Service,* http://websoilsurvey.nrcs.usda.gov, 2002: pp 2-29
28. Osuji L. C. and Nwoye I. An appraisal of the impact of petroleum hydrocarbons on soil fertility: the Owaza experience. *Afri J of Agric Res*., 2007,2 (B): pp 318-324
29. Oyedele A. O., Oloye M., Daramola B. and Ayoko O. Evaluation of Organic Manures Effects on Crude Oil Polluted Soil. *Canadian Open Soil Science and Environmental Management Journal*, 2015, Vol. 1, No. 1: pp. 1-13
30. Okolo J. C., Amadi E. N. and Odu C. T. I. Effects of Soil treatments containing poultry manure on crude oil degradation in sandy loam soil. *Applied Ecology and Environmental Research*, 2005, 3(1): pp 47-53.

31. Okoh A. I. Biodegradation alternative in the cleanup of petroleum hydrocarbon pollutants. *Biotechnol. Mol. Biol. Rev*., 2006, 1: pp 38-50.
32. Agarry S. E. and Jimoda, L. A. Application of Carbon – Nitrogen supplementation from plant and Animal sources in-situ soil Bioremediation of diesel oil: Experimental Analysis and Kinetic Modeling. *International Journal of Environment and Bioenergy*, 2013, 6 (2): pp 146 – 165
33. Ahmed A. B., Nadia A. A. and Kuther H. M. Ex-situ bioremediation of soil contaminated with crude oil by use of actinomycetes consortia for process bioaugumentation. *European Journal of Experimental Biology*, 2015, Vol. 5, No. 5: pp 24 – 30
34. Rowland U. O., Yusuf O. L M. and Ify L. N. Bioremediation of crude oil contaminated soil using organic and inorganic fertilizer. *J. Pet. Environ Biotechnol*, 2015, Volume 6. Issue 1; pp 1–6
35. Ubani O., Atagana, H. I. and Thantsha, M. S. Biological degradation of oil sludge: A review of the current state of development. *African Journal of Biotechnology*, 2013, Vol. 12(47): pp. 6544-6567
36. Ibekwe V. I., Ubochi, K. C., and Ezeji, E. U. Effect of organic nutrient on microbial utilization of hydrocarbons on crude oil contamination soil. *Africa Journal of biotechnology,* 2006, Vol. 5 (10): pp. 983- 986.
37. Molina B. L., Rodriguez V. R., Hernandez V. M., Vega J, C., Zapata P. O., Mendoza C. A. Albores, A., *Appll. Soil Ecol*. 2004, 27: pp 165-175.