IN-SITU BIOREMEDIATION OF CRUDE OIL POLLUTED SOIL USING PSEUDOMONAS *SP.*

***Woyengibunugha Ere**[1] and **Domoyi Castro Mathhew**[2]
[1]*Department of Agricultural and Environmental Engineering, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.*
[2]*Department of Agricultural and Environmental Engineering, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.*
Email: *[1]woyinse1234@gmail.com
[2]domoyicastromathhew@gmail.com

Abstract

This paper investigated the effectiveness of pseudomonas sp. in the degradation of crude oil contaminated soil for a period of 60 days. The crude oil contaminated soil was supplemented with different concentration of the pseudomonas sp. throughout the period of study. Microbiological and physicochemical parameters including Total Petroleum Hydrocarbon (TPH) content were monitored from the baseline to the 60 days. Results showed significant decreases in the physiochemical parameters during the study period. The percentage loss of (TPH) at the end of the investigation was 90.10%. The hydrocarbon utilizing bacterial isolate were pseudomonas sp., Flovobacterium sp. This study showed that pseudomonas is an effective nutrient source for biodegradation.

Keywords: *Bioremediation, crude oil, pseudomonas.*

I. INTRODUCTION

Contaminant of soil environment by hydrocarbons (mostly petroleum hydrocarbons) is becoming prevalent across the globe. This is probably due to heavy dependence on petroleum as a major source of energy throughout the world, rapid industrialization, population growth and complete disregard for the environmental health. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with a range of certainty of 200,000 metric tons per year (Kvenolden and Cooper, 2003). Release of hydrocarbons into the environment whether accidentally or due to human activities is main cause of water and soil pollution (Kvenvolden and Cooper, 2003). These hydrocarbon pollutants usually cause disruptions of natural equilibrium between the living species and their neutral environment. Hydrocarbons components have been known to belong to this family of carcinogens and neurotoxic organic pollutants (Das and Chandran, 2010). Bioremediation, a process involving the use of microorganism can be adopted to reduce, eliminate or transform contaminants in soils, sediment, air and/ or water (Sihag and Pathak, 2014). The technology involves the manipulation of microbial metabolic processes and enzymatic actions to degrade compounds of concerned polluted sites. Due to the ubiquity of hydrocarbon -degradation, microorganisms in the soil, bioremediation of crude oil contaminated soil is considered of greater economic value in terms of the cost and effectiveness.

Bioremediation approach was applied in this study; substrate degradation with microbial growth rate was determined using the international standard methods (Lee Huffman & Mano, 2000; Maxam, Rila, Dott & Eisentraeger, 2002). Bioremediation techniques allow the evaluation of substrate degradation, as well as microbial growth rate. It employs pseudomonas *sp.* which is like many bacteria, osmotically sensitive growing rapidly to feed on substrate using carbon as energy source (Backhaus & Howard 2000; Adams & Stauber 2004). This paper investigated the effectiveness of bioremediation of crude oil contaminated soil using pseudomonas *sp.*

II. MATERIALS AND METHODS

A. Study Area

The soil used for this study was collected from Igbedi community in Kolokuma and Opokuma Local Government Area of Bayelsa State. Igbedi in Niger Delta region of Nigeria, farming and fishing are the predominant occupation of the people. There have been various cases of crude oil pollution in this area as reported in the Igbedi environmental assessment report by united Nations Environmental Programme (UNEP, 2010).

B. Samples Collection

Soil samples from crude oil contaminated sites were collected at about 0-15cm depth, using soil augers at four different locations within the community. The four samples were lumped together

in a plastic bag and transported to the Soil Science Laboratory in Rivers State University, Nkpolu, Port Harcourt, for further analysis to be conducted on the soil characteristics.

C. Experimental Design

Three treatment options with varying concentration of pseudomonas sp. in polluted soil and a control were set up. The different treatment options were coded as VD-0 to VD3. Cell VD-0 was the control volume, i.e. did not receive any treatment, whereas cells VD-1, VD-2, VD-3 were marked to receive 1000g, 800g and 700g of pseudomonas *sp*. respectively during the remediation period. Biodegradation under a controlled environmental condition was monitored for 60 days of study. The experimental design is as shown in table 1.

D. Microbial Sampling

The soil samples were later transported to the Department of Microbiology in Rivers State University, Nkpolu, Port Harcourt for the purpose of isolation, identification and characterization of a possible microorganism presence in the soil.

E. Physicochemical Analysis of Soil

The pH of the soil samples was measured in 1:1 (soil: water) ratio using winlab digital pH meter. Moisture content and total organic carbon contents were determined following the methods of Walkey and Black (2013). Soil Nitrogen and Phosphate were determined quantitatively following the methods of America Public Health Association (1985). In determining the Total Petroleum Hydrocarbon (TPH) of the soil, 5g of contaminated soil was weighed in a strip beam balance and put in a conical flask and 10ml of toluene (hydrocarbon solvent) was added to the 5g contaminated soil and then stirred vigorously. The solution was then filtered using a filter paper via funnel into test tube and the residue was thrown away. The filter paper was then tested using a spectonic ZID spectrophotometer at 420nm wavelength. This wavelength guaranteed the maximum absorption of hydrocarbons. These filtrates ere then transferred in different test tubes one after the other and absorbent reading was taken in the process. A blank sample (toluene alone) was first tested for its absorbent and then the machine was adjusted to the zero mark before other readings were taken. A chart of absorbent again TPH (mg/kg) was then used to read the total. The total petroleum hydrocarbon was calculated with reference to Odu et al. (1985) using the standard curve, and multiplication by the appropriate dilution factor.

Table 1: Experimental Design

CELLS	DESCRIPTION
VD- 0	2Kg of polluted soil
VD-1	2Kg of polluted soil + 1000g of pseudomonas sp.
VD-2	2Kg of polluted soil + 800g of pseudomonas sp.
VD-3	2Kg of polluted soil + 700g of pseudomonas sp.

F. Enumeration of Total Hydrocarbon Utilizing Bacteria and Fungi

Vapour-phase method was adopted to estimate the population of Total Hydrocarbon Utilizing Bacteria (THUB) on a modified Mineral Salt Agar (MSA) with the following composition: Nacl = 10.0g, $MgSO_4$ = 0.44g, KH_2PO4 = 1.23G, Agar = 15.1g in one litre of distilled water as described by Chikere and Okpokwasili (2011). A sterile filter paper was saturated with crude oil and placed inside the cover of each petri dish, kept in an inverted position, the plates containing 0.1ml of aliquots of serially diluted soil samples were incubated at 35-37^{0}c for 5-7days. The crude oil served as the only source of carbon and energy for the growing culture. After incubation, the colonies were counted and the mean counts were recorded. The same procedure used for the enumeration of hydrocarbon utilizing bacteria was adopted for the enumeration of hydrocarbon utilizing fungi with addition of 0.1ml lactic acid for the inhibition of the growth of hydrocarbon utilizing bacteria.

III. Results

Baseline microbiological and physiochemical properties of the crude oil contaminated soil and properties of pseudomonas sp. are shown in Table 2. The baseline microbiological parameters were higher in the crude oil contaminated soil than in pseudomonas *sp.* except for hydrocarbon utilizing fungi. The baseline physicochemical parameters where higher in pseudomonas *sp.* than in the contaminated soil except for TPH which was only present in the contaminated soil and total organic carbon.

IV. Discussion

The amount of hydrocarbon utilizers in the pseudomonas *sp.* and the contaminated soil were considerably high and adequate for bioremediation. The pH of pseudomonas has been documented by other researchers to fall within the range of 5.0-8.0 (Fig.1).

Table 2. Baseline properties of contaminated soil and pseudomonas compost

Parameters	Contaminated soil	Pseudomonas Sp..C
Total Heterotrophic Bacteria (Cfu/g)	1.94 ×104	1.43 ×105
Total Heterotrophic fungal (Cfu/g)	1.23 × 106	7.3 × 105
Hydrocarbon Utilizing Bacterial	8.2 ×105	6.6 × 105
Hydrocarbon Utilizing Fungi	5.4 × 105	9.3 × 105
Total Petroleum Hydrocarbon (mg/kg)	13286.3	
pH	5.6	6.7
Moisture (%)	11.4	56.7
Total Nitrogen (%)	4.0	21.0
Total Phosphorus (%)	0.71	4.45
Total Organic carbon (%)	11.8	7.56

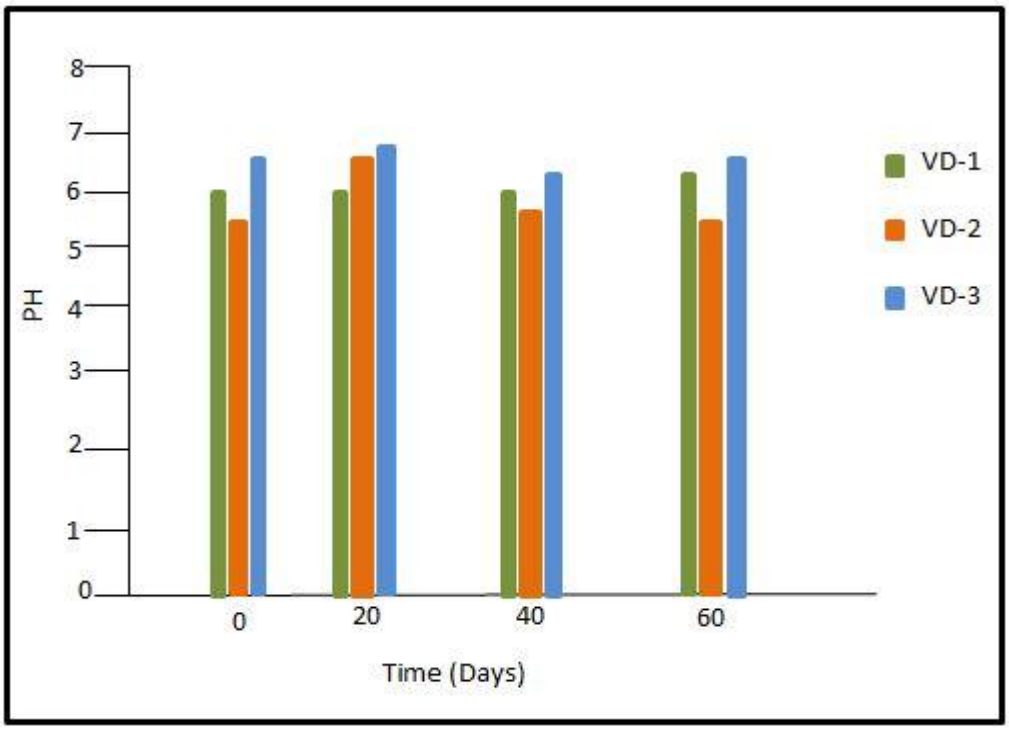

Fig.1: Change in pH values of the treatment options during remediation study

The pH value of the various treatment set up were slightly acidic and alkaline. This may be attributed to the enhancement or enrichment of the nutrient levels (by supplementing with various amounts of pseudomonas sp. of the soil). The Total Organic Carbon (TOC) content of the contaminated soil prior to amendment was 11.80% at the end of 60 days, the TOC present in the sample was 7.30% for VD-1, 7.40% forVD-2, 8.51% for VD-3 experiments respectively, as shown in Fig2.

There were slight decreases in Total Organic Carbon (TOC) concentration in the various codes. The loss in TOC has been correlated with biomass increase in microbial systems. Ibiene et al. (2011) and Adenipakum and Ogunjobi (2011), however reported slight increases in TOC in their bioremediation studies. The total nitrogen content of the contaminated soil prior to amendment was 4.0% as shown in table 2. After amendment of the experimental soil samples with various concentration of pseudomonas *sp.*, the total available soil nitrogen at day 60 of study period was 7.56% for VD-1, 9.67% for VD-2, 11.67% for VD-3 experiments respectively, as shown in fig 3.

Also, the total phosphorus content of the contaminated soil after amendment with various concentration of pseudomonas *sp.* for VD-1, VD-2 and VD-3 after 60 days of the study increased to 3.96 mg/kg, 6.21 mg/kg and 9.16 mg/kg respectively (fig.4) when compared with the baseline of 0.71 mg/kg.

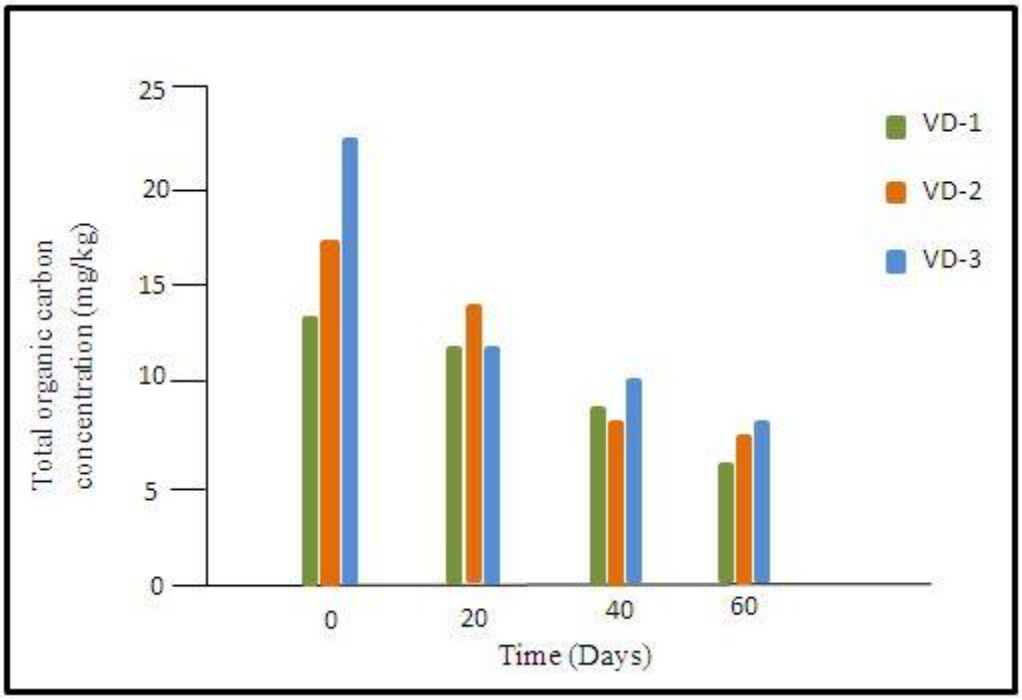

Fig. 2: Changes in Total Organic Carbon (TOC) concentration of the treatment options during remediation study

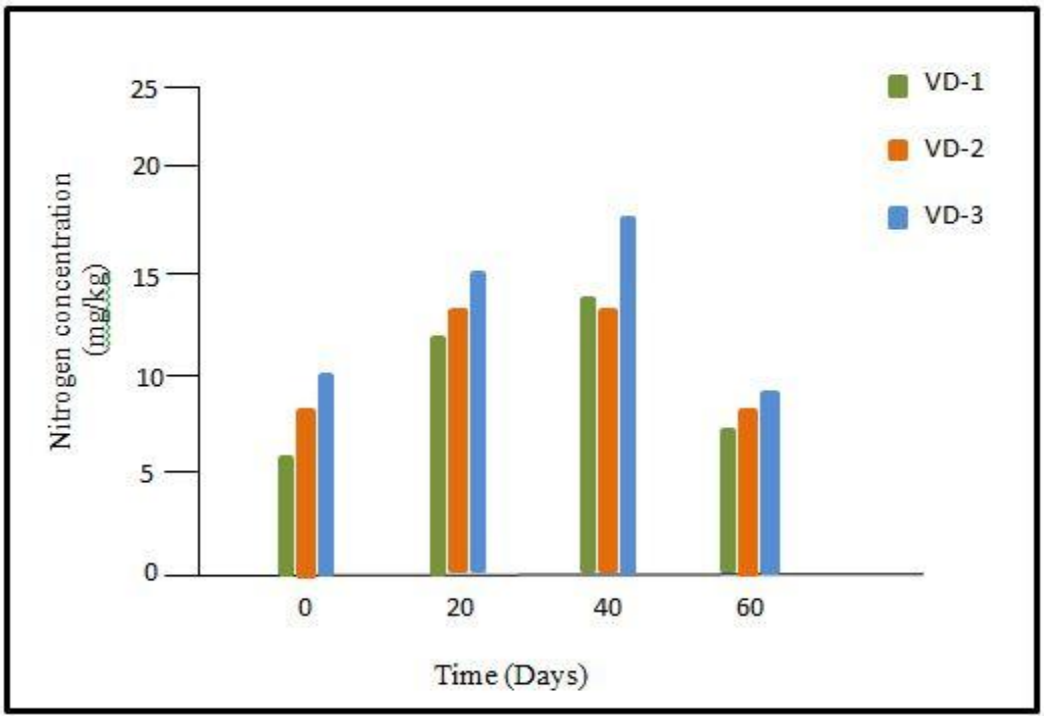

Fig.3: Changes in Nitrogen concentration of the treatment options during remediation study

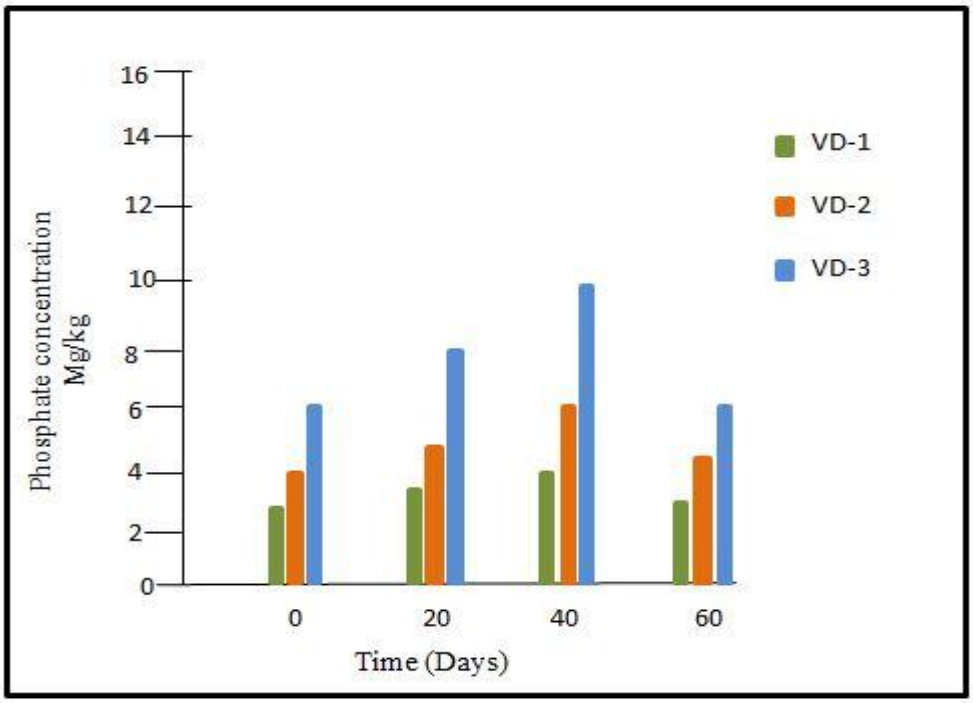

Fig.4: Changes in Phosphorus concentration of the treatment options during remediation study

The result for Total Petroleum Hydrocarbon (TPH) shown in Fig.5 revealed a total percentage loss of 90.10% VD-3 after 60 days of study, a significant decrease with respect to time when compared to the baseline concentration. Decrease in TPH indicates the effectiveness of pseudomonas *sp.* as a very useful bioremediating organic substance. Stanley et al. (2017) and Adenipekum and Ogunjobi (2011) in their study also reported a very high percentage loss of about 90% of TPH.

The hydrocarbon utilizing bacteria isolated were both Gram positive and negative bacteria. Okerntugba et al. (2015) reported that gram negative bacteria have a dominant population in crude oil contaminated soil. Bioremediation of crude oil by pseudomonas *sp*. yielded favorable results when compared with myco-remediation. Pseudomonas *sp.* provides great capacity to remediate polluted soil when compared to other members of pleurotus family previously and commonly used in bioremediation.

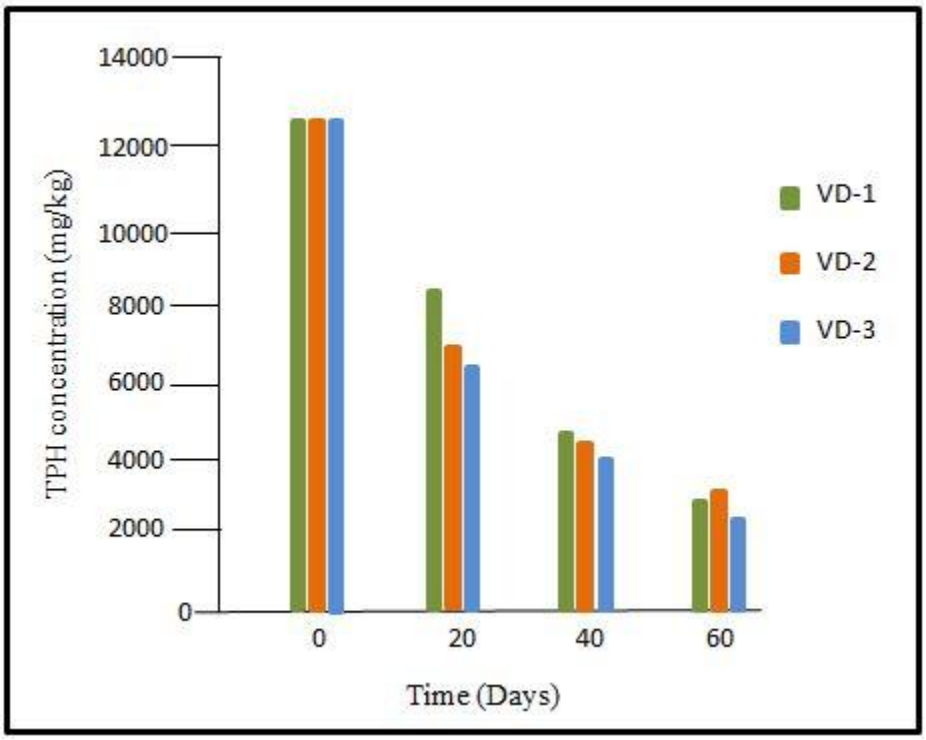

Fig.5: Changes in Total Petroleum Hydrocarbon carbon (TPH) Concentration of the treatment options during remediation study

V. Conclusion

This research has shown the effectiveness of pseudomonas *sp.* in bioremediation. The result of the total petroleum hydrocarbon of 1212 mg/kg and a percentage loss of 92.10% after 60 days of study shows a significant decrease with time when compared with the initial TPH concentration of the contaminated soil of 13286.3 mg/kg. Therefore future work should focus on how bioremediation of crude oil contaminated sites with optimum combination of pseudomonas *sp.* and other organic wastes.

References

[1]. Stanley H.O, Maeba S.N, Gbenekanu D.K and Ugboma (2018). Crude oil Degradation using sp.ent mushroom compost (SMC) of pleurotus Florida. Asian Journal 2(1): 1-7, 2018.

[2]. Kvenvoiden, F.& Cooper, T. (2003). Bioremediation potential of white Rot fungi in the reclamation of crude oil polluted soil, journal of environmental Engineering, 129, 366-376.

[3]. Das N& Chandran P (2011). Microbial Degradation of petroleum hydrocarbon contaminants: An overview. Biotechnology research International Hindawi Access to research publkishers, Egypt 1-13.

[4]. Sihag. S, Pathak H (2014). Factor affecting the rate of biodegradation of PAHS. International Journal of pure and Applied Bioscience 2014; 2(3): 185-202.

[5]. lee C. C, Huffman. G L, & Mao. Y.L. (2000). regulatory framwork for the thermal treatment of various waste stream. journal of hazard mater 76:13-12.

[6]. Maxam, G; Halin, S. Dott, W& Einstentraeger, a. (2002). Assesment of the influence of use on ecotoxico logical characteristics of synthetic ester lubricants Ecotoxicol. 11; 349-355.

[7]. Adams, M.S.& Stauber, J.L (2004). Development of a whole-sediment toxicology test using a benthic marine micro alga. Environ toxicol. Chem. 8:1957 – 1968.

[8]. Backhaus & Howard (2000). Bioremediation of hydrocarbon contaminated soil. Chemosp.here 56 (8), 803-881.

[9]. United Nations Environment Programme (UNEP), Environment al Assessment of Ogoniland report; 2010.

[10]. Walkey A, & Black I.A (2013). An examination of the Deegtjareff method for determining organic carbon in soils: Effect of variations in digestion conditions and of inorganic soil constituents. Soil science 2013; 63:251-263.

[11]. American Public Health Association (APHA): Standard methods for the examination of water and waste WATER 16TH EDITION, American public health association. Washington, D.C.;1985.

[12]. Ibiene AA. Orji FA, Ezidi CO, Ngwobia, CL.Bioremediation of hydrocarbon contaminated soil in the Niger Delta using sp.ent mushroom compost and other organic wastes. Nigerian Journal of Agricultural Food and Environmental. 2011; 7(3): 1-7.

[13]. adenipekum C,O & Ogunjobi (2011). Bioremediation of cutting fluids contaminated soil by pleurotus tuber region. The Environmentalist. 2011; 32:11-18.

[14]. Stanley H.O, Offorbuike OM, Stanley CN. Bioremediation of crude oil contaminated soil using pleurotus pulmonarius, a whiote rot fungus. IOSR Journal of environment science, Toxicology and food Technology. 2017; 11(4): 122-128.

[15].Chikere CB, Okpokwasili GS. (2011). Monitoring of microbial hydrocarbon remediation in the soil. African journal of biotechnology. 2011: 1(3) 117 -138.

[16]. Odu C.T: Esuruoso O.F; Ninoboshi L.C; (1985). Environmental study of the Nigerian Agip Oil Company operation areas. In proceedings of the soil and fresh water vegetation conference, Milan (Italy); 1985.