Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 https://dx.doi.org/10.4314/sa.v20i1.10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

APPLICATION OF NITROGEN FIXING BACTERIA AND POULTRY DROPPINGS FOR ENHANCED BIOREMEDIATION OF CRUDE OIL POLLUTED-SOIL

*** Wokem, V.C and Momoh, E. D.**

Department of Microbiology, Faculty of Science
University of Port Harcourt, P.M.B 5323, Port Harcourt, Nigeria
*E-mail: chineduvin@yahoo.com Phone: (+234)08130306131

Received: 17-02-2021
Accepted: 16-03-2021

ABSTRACT

The exploration, production and refining of crude oil has led to severe environmental degradation in the oil producing communities of the Niger Delta region of Nigeria. Enhanced bioremediation of tropical rainforest soil artificially polluted with crude oil, bioaugmented with nitrogen fixing bacteria (NFB) and biostimulated with poultry droppings was carried out *ex situ*. Soil sample was collected at 15cm depth from tropical rainforest soil of the University of Port Harcourt, Nigeria. The NFB was isolated from roots of leguminous plant *Arachis hypogea*, identified as *Nitrobacter* species. Bioaugmentation by application of NFB served as option A, option B (biostimulation by application of poultry droppings), option C (No amendment) served as the control. Bioremediation was monitored for 28 days for interval of 14 days, and determined using the percentage ratio of total petroleum hydrocarbon (TPH) losses for each period to TPH at initial day (day zero). Results of total culturable heterotrophic bacterial (TCHB) counts showed that highest range in option B (1.9×10^4 - 2.4×10^9Cfu/g) than in option A (7.8×10^6-2.29×10^7Cfu/g) and C (6.75×10^6-2.6×10^7Cfu/g) respectively. Similarly, hydrocarbon utilizing bacterial (HUB) counts had higher range in option B (1.20×10^5-1.9×10^7Cfu/g) than in option A (8.30×10^4 - 2.30×10^5Cfu/g) and option C control (4.3×10^4 -1.69×10^5 Cfu/g) respectively. Changes in physicochemical parameters during the study showed reductions in nitrate, phosphate and TPH in all the options expect pH which showed slight increase in option C (6.20-6.24). Characterization and identification for bacteria revealed the following HUB genera *Pseudomonas*, *Citrobacter*, *Bacillus*, *Corynebacterium*, *Micrococcus*, *Klebsiella*, *Staphylocuccus* and *Nitrobacter*). The percentage losses in TPH from gas chromatography (GC) results showed the following; option A (44.24%) option B (61.08%) and option C - control (27.28%) respectively. The results from this study showed that option B, the application of poultry droppings as biostimulant was more efficient than the application of NFB in enhanced bioremediation of crude oil polluted soil, hence the use of poultry droppings which is available as organic waste, eco-friendly and cost-effective is recommended as biostimulant for enhanced bioremediation in environmental cleanup of crude oil impacted-sites of the Niger Delta region of Nigeria.

Key Words: Bioremediation, Crude oil polluted-soil, Biostimulation, Bioaugumentation, Poultry droppings, Nitrogen fixing bacteria.

INTRODUCTION

Crude oil and its derivatives are essentially hydrocarbons and their release to the environment through spillage, illegal bunkering and refining activities contaminates the soil and aquatic ecosystems with hydrocarbon pollutants (Ekpo *et al.*, 2012; Akpahwe and Solomon, 2012; Ekpo and Nwaankpa, 2005). Apart

from the economic and aesthetic losses to the land caused by crude oil spills, plants and animals in terrestrial environment are equally affected. Crude oil toxicity due to the presence of polycyclic aromatic hydrocarbons (PAHs) has greater environmental and public health implications as it can pass on to human population through the food chain (Hall *et al*, 2006, Tiido *et al;* 2006; Ordinioha and Sawyer, 2010; Ordinioha and Brisibe, 2013). The ingestion, contact and inhalation of high concentration of hydrocarbon containing compounds may result in acute renal failure, hepatoxicity, chemotoxicity, infertility and cancer (Nessel, 1999; Ordinioha and Brisitre, 2013).

Bioremediation is the use of biological processes and agents especially microbial, to degrade environmental pollutants into less toxic forms (Vidali, 2001; Dawson *et al;* 2007, Margesin and Schinner, 2001) In bioremediation, it is only when environmental conditions permit microbial growth and activity would application of bioremediation technologies be effective. Thus, manipulation of environmental parameters to achieve fast growth rate and optimal activities is necessary (Mukred *et al.,* 2008; Gomez and Sartaji, 2014). Bioaugmentation as a technique of bioremediation is the addition of large population of selected microorganisms grown in the laboratory removed from the contaminated site back to the media (Vadali, 2001.) Genes could be introduced into native species using other genetic vectors such as plasmids (Crisafi *et al.,* 2016). Lack of essential nutrients (nitrogen, phosphorus and potassium) is one of the major factors that affect biodegradation of hydrocarbons by microorganism in soil (Abioye *et al.,* 2012). Thus, the application of organic or inorganic nitrogen rich nutrients (biostimulation) is an effective technique to enhance the biodremediation of contaminated sites (Hollender *et al,* 2003, Semple *et al,* 2006, Walworth *et al,* 2007; Tyagi *et al.,* 2011) The ultimate aim of enhanced bioremediation technologies is to have a safe, ecologically friendly and cost-effective means which will reduce the pollutants to a level referred to ; "As low as reasonable practicable (ALARP)" (MADEP, 2002, API, 2001; Environmental Agency, 2003; Polland *et al.,* 2005, US-EPA,1999; El-Fantoussi and Agathos, 2005; Dawson *et al.,* 2007) cite references in chronological order.

The present study evaluated the potential of nitrogen fixing bacteria isolated from leguiminous plant (*Arachis hypogea*) as bioaugmentation agent to enhance indigenous microbes to degrade the pollutants and organic waste (poultry droppings) as biostimulants to provide favourable nutrients which could improve degrading abilities of hydrocarbon utilizing bacteria in the crude oil polluted soil, for enhanced bioremediation (Andreolli *et al.,* 2015, Nwankwegu *et al.,* 2016).

MATERIALS AND METHODS

Sample Collection: The soil sample used was collected randomly with soil augar at the depth of 0-15cm (topsoil) from the tropical rain forest agricultural farm land of the University of Port Harcourt. The composite samples were mixed together for homogenicity and collected into sterile black polythelene bag then transported to the laboratory for analysis. The soil sample for isolation of nitrogen-fixing bacteria was collected from the soil of rhizosphere regions of groundnut plants (*Arachis hypogea*) obtained from the Agricultural

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 *https://dx.doi.org/10.4314/sa.v20i1.10*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

Farm Land (Department of Crop Science) of the University of Port Harcourt, Nigeria. The soil sample was collected into sterile black polyethylene bag and taken to the Department of Microbiology Research Laboratory.

The poultry droppings were obtained from God's Favour Poultry Farm in Rumuokoro community of Obio/Akpor Local Government Area of Rivers State, Nigeria. It was transported to the Research Laboratory in sterile polyethylene bag and oven dried for 60mins at temperature of 125^0C to reduce its pathogenic effect (Semple *et al.*, 2001).

Reagents and Media

All the reagents employed in this study were of analytical grade and were products of Sigma Chemical Company St. Louis, Missouri, USA and BDH Chemical, Ltd, Poole, England. All microbiological media used were products of Oxoid and Difco Laboratory, England. Nutrient Agar (NA), MacConkey's agar, Plate Count agar (PCA), Bushnell Haas Agar (BHA) and Mannitol Ashhy free Nitrogen agar. Bonny light crude oil used for pollution of the soil sample and isolation of hydrocarbon utilizing bacteria (HUB) was obtained from Shell Petroleum Development Company (SPDC), Port Harcourt, Nigeria.

Experiment Setup

Five hundred grams (500g) of soil sample were weighed and placed in three set of plastic containers labeled A, B and C, each having volume of about 1,500cm^3. Each container was polluted with 50ml of Bonny light crude oil and thoroughly mixed with wooden spatula. Option A, was inoculated with 50ml broth culture of isolated nitrogen fixing bacteria (NFB). Option B was addition of 50g of oven dried and grinded poultry droppings while in Option C, there was no amendment which served as the control (Table 1). The set up were allowed to stand at room temperature (28 ± 2^0C). The content of each set up were tilled three times a week for proper aeration. Periodic sampling for microbiological and physicochemical analysis from each set was carried out at day 0, 14 and 28 respectively.

Table 1: Bioremediation experimental set up for crude oil polluted-soil

Experimental setup option	Description of remediation treatment
A	500g CPS + 50ml NFB
B	500g CPS + 50g POD
C	500g CPS (Control)

CPS – Crude oil polluted soil, NFB – Nitrogen fixing bacteria broth, POD – Poultry Dropping

Enumeration of Bacterial Populations

The total culturable heterotrophic bacterial (TCHB) counts of the crude oil polluted soil sample, the unpolluted soil sample and the amended soil samples were carried out using spread plate method on plate count agar (PCA) (APHA, 1998; Sylvia *et al.*, 2005; Chikere *et al.*, 2009). A serial ten-fold dilution was prepared using one gram (1g) of each soil sample, to obtain up to 10^{-5} dilution. Aliquote portions (0.1ml) of 10^{-5} dilutions were inoculated onto PCA plates in triplicates and spread with sterile bent

glass rod. Plates were then incubated at 37°C for 24h. Plates were examined for colony formation and enumeration after incubation period. Counts were expressed in cfu/g.

The HUB counts were carried out by inoculating 0.1ml of 10^{-5} dilution on Bushnel Haas agar (BHA) plates using the spread plate method (APHA, 1998; Hamanura *et al.,* 2006). Sterile filter papers (Whatman No. 1) saturated with filtered crude oil were aseptically placed on the inside cover of each plate kept in an inverted position and incubated at 37^0C for 48h. The crude oil served as a sole source of carbon and energy by the vapour phase transfer method (Hammaura *et al.,* 2006). Plates were then examined for colony formation and enumeration after incubation period. Counts were expressed in cfu/g.

Isolation and Identification of HUB

Discrete colonies of culturable hydrocarbon utilizing bacteria on BHA plates were purified by sub-culturing repeatedly on nutrient agar (NA) plates by streaking and incubated at 28 ± 2^0C for 24h. Discrete colonies were further sub-cultured onto NA slants in Bijoux bottles and stored in the refrigerator at 4^0C. Identification was based on cultural, microscopic morphology, use of selective media and biochemical characteristics with reference to determination schemes of Bergey and Holt (1994) and Cheesbrough (2006).

Isolation of Nitrogen Fixing Bacteria (NFB)

The isolation of NFB from the soil of rhizosphere region of *Arachis hypogea* was carried out using Ashby's Mannitol Free Nitrogen Agar (AMFNA). The medium was composed of 40g $MgSo_47H_2O$, 5g$CaCo_3$, 20g, $Nacl_2$, 2.5gK_2SO_4, 0.2g, K_2HPO_4 and 20g of agar all dissolved in one litre of distilled water. The pH was adjusted to 7.4 using pH meter and autoclaved at 121^0C for 15min. After autoclaving, the medium was dispensed into sterile petri dishes for solid agar medium (Hamza *et al.,* 2017).

The soil sample from the rhizosphere region of the plant (*Arachis hypogea*) was grounded with sterile motar and pistle to liberate the adhering microorganisms. One gram of the grounded soil was weighted into a test tube containing 9ml of distilled water and homogenized by vortexing, serial dilutions out up to 10^{-5}. Aliquots of 0.1ml of appropriate dilutions (10^{-5}) were inoculated onto the MAFNA plates in triplicates by spread plate method (APHA, 1998). The plates were incubated at 28^0C for 48h.

Identification of NFB

Discrete colonies from the MAFNA plates were sub-cultured repeated by streaking on NA plates and incubated at 28^0C for 24h. (Pervim *et al.,* 2017; Gyorgy *et al.,* 2010; Hamza *et al.,* 2017). Pure cultures were further sub-cultured onto stant NA in Bijou bottles and preserved in refrigerator at 4^0C. Identification of the isolates were carried out by observing colonial and cell morphology, as well as different biochemical tests which included indole motility, spore staining, catalase, utilization, citrate utilization, methyl red-Voges Proskuer and starch hydrolysis with reference to Bergey and Holt, (1994); Cheesbrough, (2006); Sadik *et al.,* (2016).

Physicochemical Analysis

The physicochemical parameters analyzed in the study were pH, Nitrate, phosphate and total petroleum hydrocarbon (TPH). The

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 *https://dx.doi.org/10.4314/sa.v20i1.10*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

parameters were analyzed in the unpolluted soil sample, polluted soil sample and amended soil samples during the bioremediation period. All the parameters were determined using standard laboratory procedures adopted from Stewart *et al*. (1994) and ASTM-E 1943 (1998) test methods. The pH was determined using Jenway digital pH meter (model 3015 USA).

Determination of percentage losses in total petroleum hydrocarbon (TPH) in soil polluted with crude oil.

Soil samples for gas chromatographic analysis were extracted with methylene chloride. Aliquot of each extract was injected into a gas chromatograph (GC) (HP 5890, Hewlett Packward, Avohdale, PA, USA) equipped with flame ionization detector (FID). The extractible TPH was quantified from the calibration graph (ASTM – E1943, 1998). Results were expressed in parts per million (PPM). The percentage losses in TPH of the soil samples (treated and untreated) during study period were determined by obtaining the differences in the TPH values of GC results of day 0 and that of day 28 after the bioremediation period. Biodegradation or percentage loss in TPH was calculated using the formula.

$$\% \text{ loss TPH (Biodegradation)} = \frac{\text{TPHi} - \text{TPHii}}{\text{TPHi}} \times 100$$

Where, TPHi = is the initial total petroleum hydrocarbon of day 0. TPHii = the total petroleum hydrocarbon at day 28 of the amended and unamended soil samples.

RESULTS

Table 1: Total culturable heterotrophic bacteria (TCHB) counts during bioremediation of crude oil polluted soil amended with different treatment options

Days	Treatment options		
	Option A (CPS + NFB) (cfu/g)	**Option B (CPS + POD) (cfu/g)**	**Option C (CPS (Control) (cfu/g)**
0	7.8×10^6	1.98×10^7	6.75×10^6
14	1.58×10^7	1.90×10^9	1.28×10^7
28	2.29×10^7	2.40×10^9	2.08×10^7

Key: CPS = Crude oil polluted soil, NFB = Nitrogen fixing bacteria, POD = Poultry dropping.

Table 2: Changes in hydrocarbon utilizing bacteria (HUB) counts during bioremediation of crude oil polluted soil amended with different treatment options.

Days	Treatment options		
	Option A (CPS + NFB) (Cfu/g)	**Option B (CPS + POD) (Cfu/g)**	**Option C (CPS Control) (Cfu/g)**
0	8.30×10^4	1.20×10^5	4.3×10^4
14	1.60×10^5	1.10×10^7	1.3×10^5
28	2.30×10^5	1.90×10^7	1.69×10^5

Key: CPS = Crude oil polluted soil, NFB = Nitrogen fixing bacteria, POD = Poultry droppings.

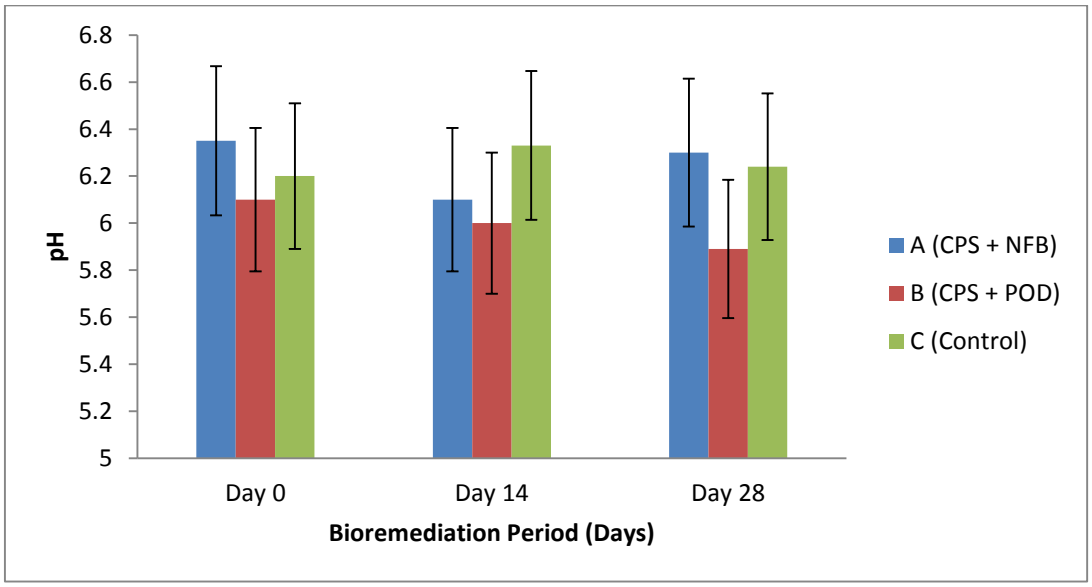


Fig. 1: Changes in pH values in the various treatment options during the bioremediation period (please use replicates of readings to show the standard deviation (SD) and error bars for statistical inferences)

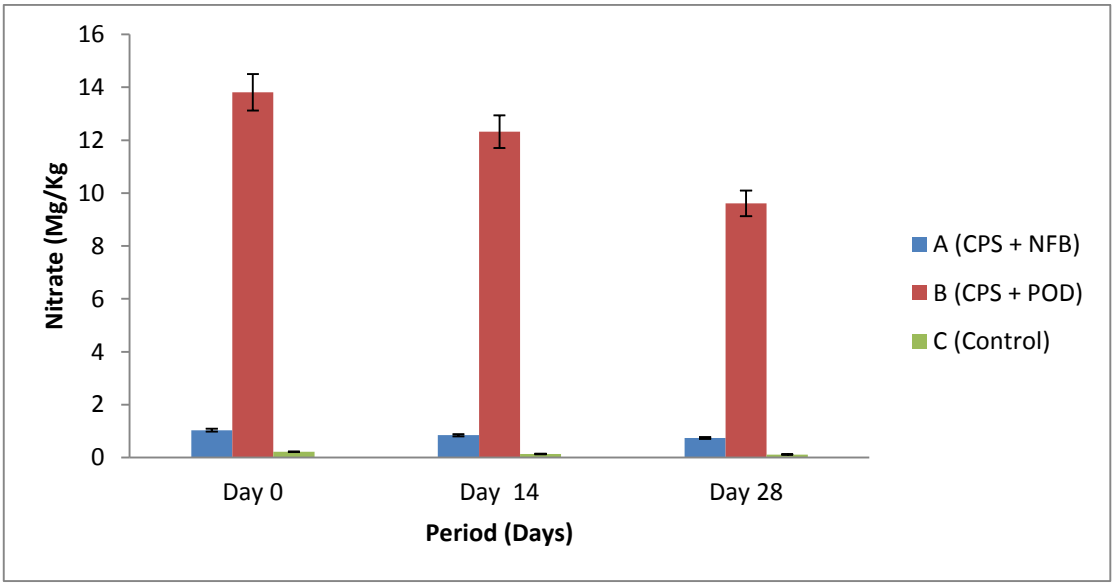


Fig. 2: Changes in Nitrate values in the various treatment options during the bioremediation period (show SD) and error bars in all Figs)

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 *https://dx.doi.org/10.4314/sa.v20i1.10*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

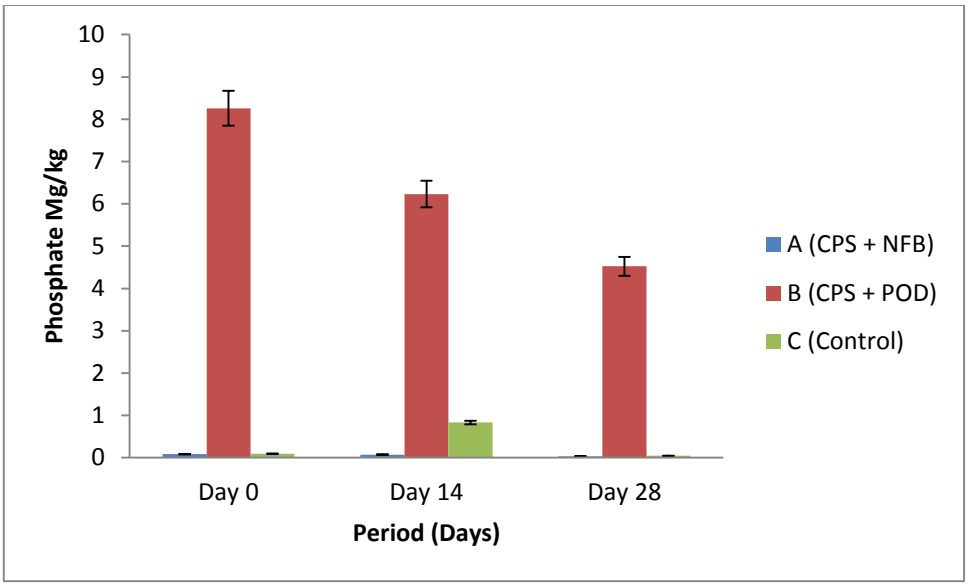


Fig. 3: Changes in phosphate values in the various treatment options during the bioremediation period

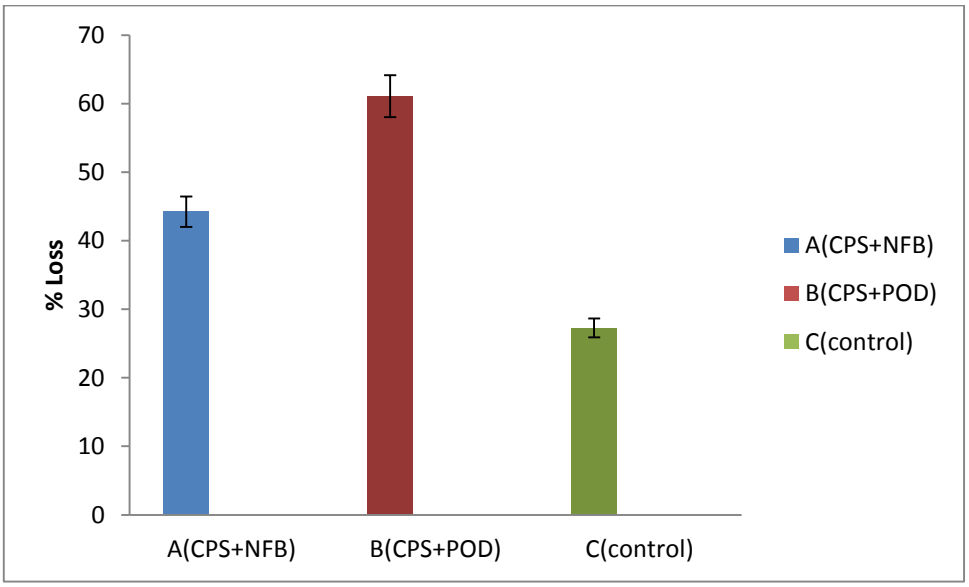


Fig. 4: Percentage losses in TPH of different treatment options during the bioremediation of crude oil polluted soil at day 28

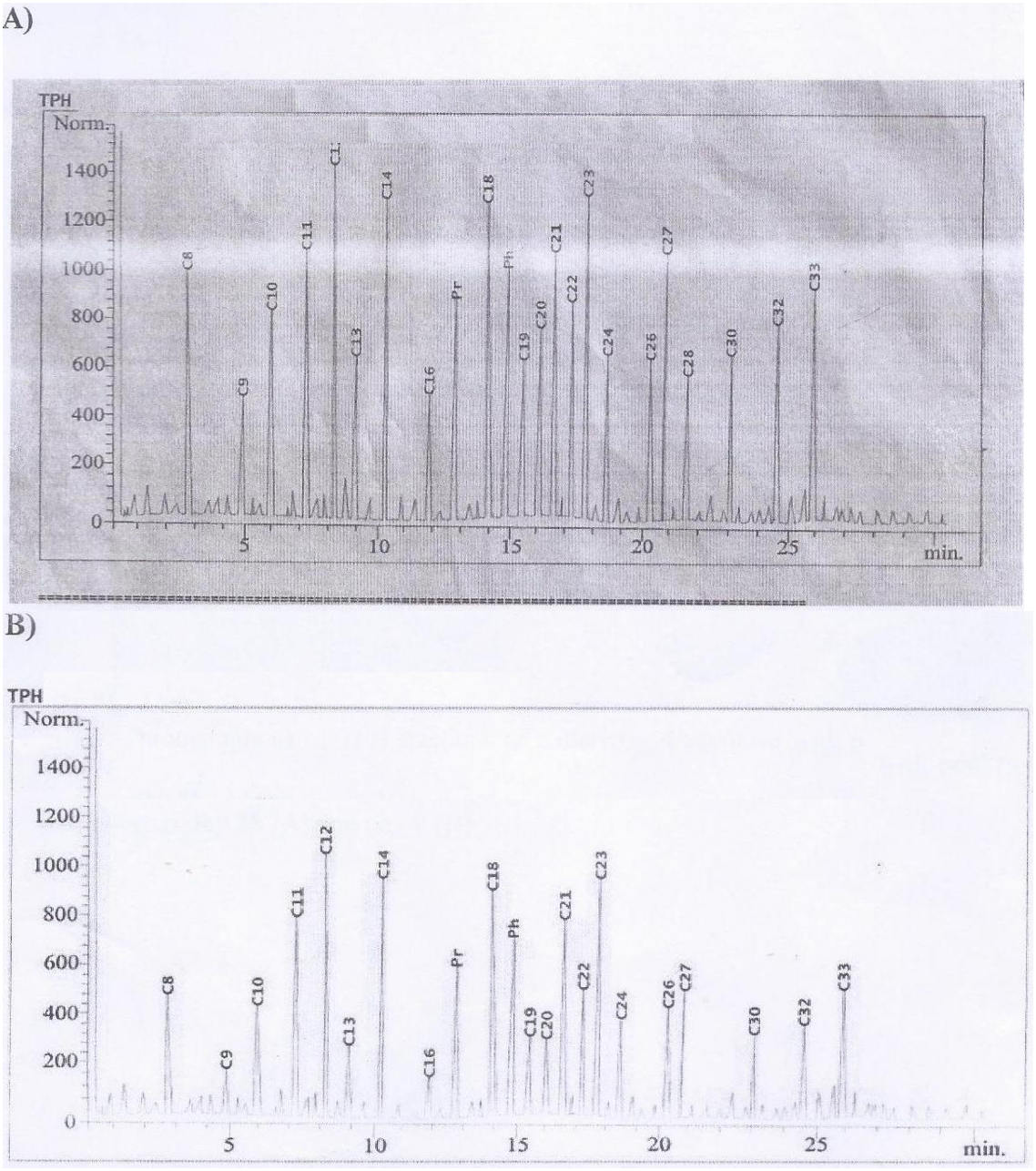
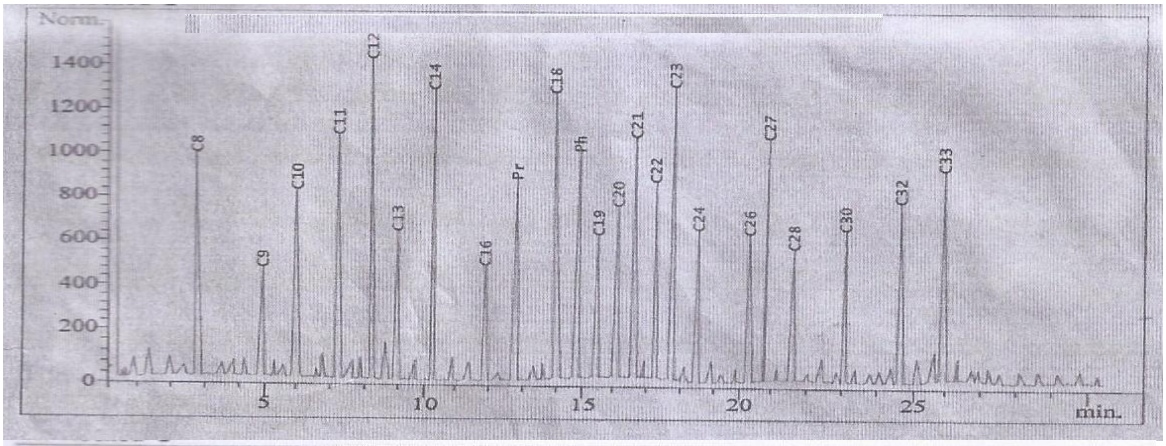


Fig 5: Chromatograms of TPH fractions of polluted soil sample amended with NFB A at day 0 and B at day 28.

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 *https://dx.doi.org/10.4314/sa.v20i1.10*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

A



B

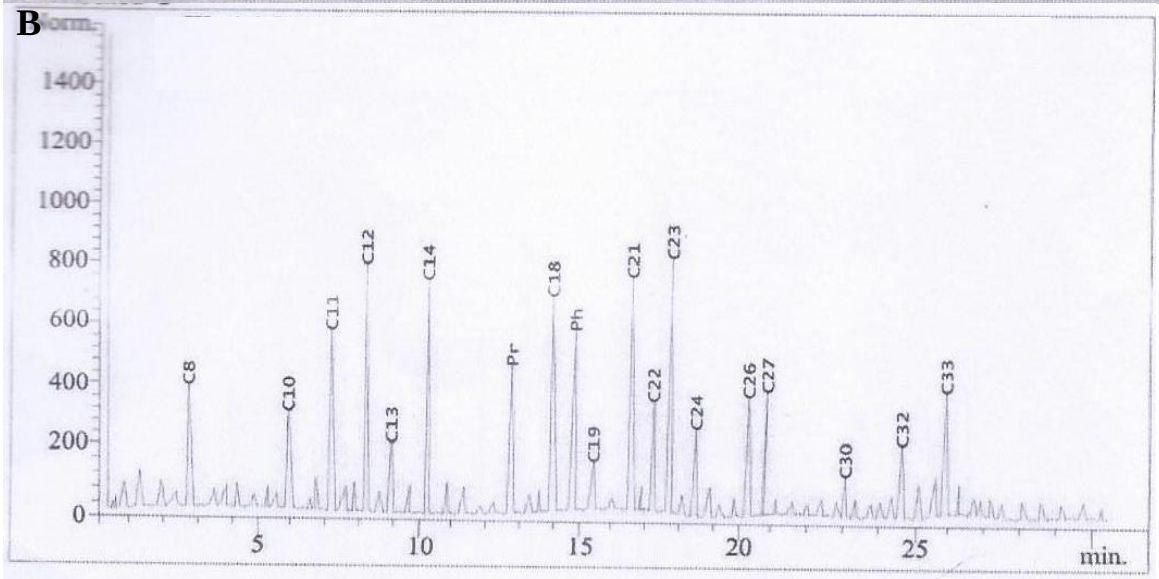


Fig 6: Chromatograms of TPH fractions of polluted soil sample amended with poultry droppings A at day 0 and B at day 28.

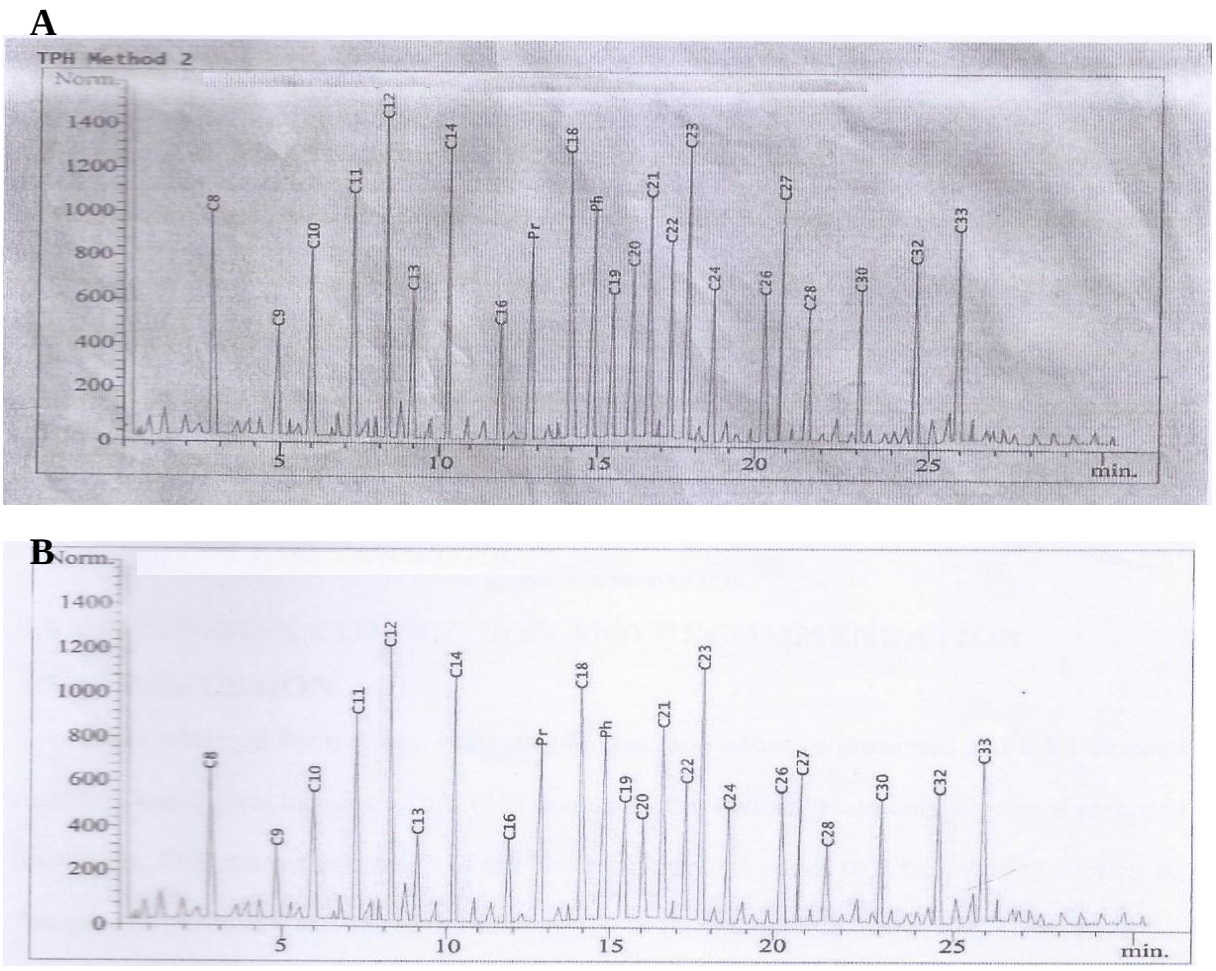


Figure 7: Chromatograms of TPH fractions of polluted soil without amendment A at day 0 and B at day 28

DISCUSSION

Results of changes in the populations of total culturable heterotrophic bacterial (TCHB) counts and hydrocarbon utilizing bacteria (HUB) counts in the various bioremediation treatment options are presented in Tables 1 and 2 respectively. The results reveal that option B (amendment with poultry droppings) had higher TCHB and HUB counts than option A (amendment with nitrogen fixing bacteria) and option C (no treatment: control) throughout the bioremediation period. The highest counts of TCHB and HUB recorded in option B ($1.98x10^7$ – $2.40x10^9$cfu/g) and ($1.20x10^5$ – $1.90x10^7$) is attributable to the addition of poultry droppings as biostimulant which provided the necessary nutrients for proliferation of microorganisms in the polluted soil. Similar results have been obtained in previous studies in use of biostimulants to enhance bioremediation of hydrocarbon impacted soil (Ekpo and Udofia, 2008; Abioye *et al.*, 2009; Agarry *et al.*, 2010 Onuoha, 2013; Omoni *et al.*, 2015; Wokem and Madufuro, 2020,). The reason for the higher counts of bacteria in the amendment with poultry droppings than in options A and C (Control) may be the result of the presence of appreciable quantities of nitrogen and phosphorus in the organic waste. Poultry droppings or chicken droppings also known

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 *https://dx.doi.org/10.4314/sa.v20i1.10*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

as chicken manure are the faeces of chickens. They are used as organic fertilizer, especially for soil low in nitrogen. Of all animal manures poultry droppings has the highest amount of nitrogen, phosphorus and potassium (Omoni *et al.*, 2015). On the other hand, in option A (Bioaugmentation with NFB), which recorded less TCHB and HUB than option B could be that the addition of the NFB culture being extraneous (non indigenous) may have been limited to compete well enough with the indigenous species to develop and sustain the required population level adapted to proliferate in the polluted soil, hence lower microbial populations in option A (Vidali, 2001; Crisafi *et al,* 2016). The unamended polluted soil sample option C recorded the least microbial populations in TCHB and HUB counts range. TCHB ($6.75x10^6$ – $2.08x10^7$cfu/g), HUB ($4.3x10^4$ – $1.6x10^5$cfu/g). The lower microbial populations in option C could be as a result of non-amendment of the polluted soil. The crude oil may have posed toxic effect to the indigenous microorganisms and the lack of organic nutrients to enhance microbial activities. The immediate effect of the presence of crude oil in the soil is a depression in the population of soil microorganisms (Okpokwasili and Odokuma, 1996; Wokoma, 2002).

The hydrocarbon utilizing bacterial isolates identified in the crude oil polluted soil amended with NFB and poultry droppings included the following genera; *Micrococcus* sp., *Pseudomonas* sp., *Citrobacter* sp., *Bacillus* sp., Corynebacterium sp., *Klebsiella* sp and *Staphylococcus* sp. The nitrogen fixing bacteria identified was *Nitrobacter* sp by use of the selective medium Ashby's mannitol free nitrogen agar. The HUB isolates identified in the present study has been observed in similar studies (Solomon *et al.,* 2018; Omoni *et al.,* 2015; John and Okpokwasili 2012). Indigenous microorganisms are well adapted to their environment. The introduction of bacteria that has been previously exposed to the presence of crude oil (petroleum products) produces greater removal efficiency (Cooney *et al.,* 1985; Maleszak *et al.,* 2004). The present study showed that the isolates had the advantage of being well-adapted to the crude oil polluted soil, being biostimulated by organic waste in option B, leading to higher percentage (61.08%) biodegradation of oil contaminants than in option A (44.24%) which was treated with non-indigenous bacteria (NFB) and was not stimulated with organic waste and option C (27.28%) being control that was not amended or treated. Results obtained from the physicochemical parameters in the present study showed different degree of reductions in NO_3, and PO_4 in the various treatment options, exception of pH which showed slight increases and decreases during the period of bioremediation (Fig 1). The pH of the various treatment options is a function of the chemical composition of the soil, the treatment technique and microbial activities. The pH changes during the monitoring period may be due to reduction in acidic compound production and/or proton secretions by microorganisms resulting in production of slightly alkaline compounds (Odokuma and Ibe, 2003). The decreases in pH for the treatment options mostly in option B, could be due to production of acidic or alkaline metabolites which are secreted by the microorganism. The presence of these metabolites changed pH of each medium (Delyan *et al.,* 1990; Mayo and Noiks, 1996). Changes in nitrate

and phosphate showed the same trend of reduction from day 0 to day 28 in the treated soil samples and the untreated sample (control) (Figs. 2-3). These reductions in nitrate and phosphate values during the bioremediation period in the various treatment options, indicates that the microorganisms were utilizing the metallic salts of the anions as sources of nitrogen and phosphorus (Odokuma and Akpokodje, 2004; Chikere *et al.,* 2001; Bento *et al.,* 2005; Ogugbue *et al.,* 2017).

The results of percentage losses in TPH of the treatment options are represented in Fig 4. The results showed that option B (treatment with poultry droppings) has the highest % losses in TPH (61.08%) than option A (44.24%) and the control option C (27.28%). The higher % losses in TPH recorded in bioremediation option B, suggest that biostimulation (application of poultry droppings) enhanced the bioremediation of hydrocarbon polluted soil more than bioaugmentation (application of NFB) option A, and option C, where there was no treatment (natural attenuation). These observations from the present study are in agreement with literature and reports in previous studies regarding the use of biostimulation/bioaugmentation in bioremediation of hydrocarbon polluted-environments (Mukred *et al.,* 2008; Ayotamuno *et al.,* 2011; Mona *et al.,* 2015; Macaulay, 2015; Crisafi *et al.,* 2016; Wokem and Madufuro, 2020). The higher rate of TPH reduction reported in this study with the application of poultry droppings could be due to bioavailability of the nutrients in the organic waste to the indigenous bacterial species in the crude oil polluted soil (Chukwudozie, 2013; Omoni *et al.,* 2015; Solomon *et al.,* 2018). The probable minimal reduction in TPH in the untreated soil sample (option C) could be attributable to natural attenuation processes of volatilization, evaporation, spreading, photo-oxidation and biodegradation by the indigenous bacterial species which were not enhanced by application of any nutrient or augmented with bacterial species. This process is slow and takes long period for the microorganism to degrade the hydrocarbon. The loss of TPH due to natural attenuation at various time interval have been reported (Abu and Dike, 2008; Agarry and Ogunleye, 2012; Bento *et al.,* 2005).

The unamended soil sample (optional C) had TPH reduced from 18,394.2623ppm at day 0 to 13,375.2613ppm at day 28, representing 27.28%. The crude oil polluted soil treated with poultry droppings (option B), the TPH reduced from 18,200.5435ppm to 7,084.0535ppm at day 28 indicating 61.08% while that of polluted soil treated with NFB (option A), the TPH was reduced from 17,884.2623ppm to 9,970.6523ppm representing 44.24% respectively (Fig. 4).

Figs. 5-7 show the chromatograms obtained for the treated crude oil polluted soil and the untreated polluted soil sample (control) during the period of bioremediation. A indicates the chromatograms at day 0 while B indicates chromatogram at day 28 respectively in each treatment options.

The chromatograms of the crude oil polluted soil treated with NFB and poultry droppings as well as the untreated polluted soil sample shows that $C_1 - C_7$ were non-existent in the polluted soil samples while heavier fractions ($C_8 - C_{35}$) predominated. However the two treatments (options A and B) were able to reduce the $C_{18} - C_{35}$ fractions appreciably though at different rates with option B indicating greater reductions

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 *https://dx.doi.org/10.4314/sa.v20i1.10*
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

CONCLUSION

The results obtained from the present study indicated that application of poultry droppings as biostimulating agent enhanced the bioremediation of crude oil polluted soil more efficiently than application of nitrogen fixing bacteria (bioaugumentation technique). Hence the use of poultry droppings as an organic waste or manure in the bioremediation of crude oil polluted sites is recommended since it is readily available, ecofriendly and cost-effective. The technology can be transferred into pilot-scale study for *in-situ* bioremediation clean-up of hydrocarbon impacted-land of the Niger Delta region of Nigeria thereby improving agricultural productivity in these crude oil devasted environments.

REFERENCES

Abioye, O.P., Abdul-Aziz, A.R and Agamuthu, P (2009). Stimulated biodegradation of used lubricating oil in soil using organic wastes, *Malays J. Sci.*, 28 (2): 127-133.

Abioye, O.P., Agamuthu, P and Abdul-Aziz, A.R (2012). Biodegradation of used lubricating oil by microbes isolated from pristine soil environment. *Malays. J. Sci.*, 31 (1): 1-7.

Abu, G. O and Dike, P.O (2008). A study of natural attenuation processes involved in a microcosm model of a crude oil impacted wet land sediment in the Niger Delta. *Bioresour. Technol.*, 99:4761-4767.

Agarry, S.E and Ogunleye, O (2012). Box enhanced design application to study enhanced bioremediation of soil artificially contaminated with spent engine oil using biostimulation strategy. *Int. J. Ener. Environ. Engine.*, 12:3-31.

Akpahwe, L and Solomon, L (2012). Crude oil theft and its environmental consequences: The way forward. Lead paper presented at the 22nd Annual General Meeting/Conference of the Nigerian Environmental Society, Yenagoa, Bayelsa, 6th – 8th Dec., 2012.

American Petroleum Institute –API (2001). *Risk-based methodologies for evaluating petroleum hydrocarbon impacts at oil and natural gas E & P sites.* Regulatory and scientific affairs Department, API publication No. 4709, Washington D.C.

American Public Health Association – APHA (1998). Standard methods for examination of water and waste water 20th Ed., Washington DC: American Works Association Water Pollution Control Federation.

American Society for Testing Materials – ASTM –E1943 (1998). *Standard Guide for remediation of soil by natural attenuation at petroleum release sites.* West Conshocken, Pennsylvania.

Andreolli, M., Lampis, S., Brignoli, P and Vallini, G (2015). Bioaugmentation and biostimulation as strategies for bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: *A comparative study J. Environ. Manag.*, 153:121-131.

Ayotamuno, M.J., Okparanma, R.N and Amadi, F (2011). Enhanced remediation of an oily sludge with saline water. *Afri. J. Environ. Sci. Technol.*, 5 (4): 262-267.

Bento, F. M., Camargo, A.A.O., Okeke, B.C and Frankenhorger, W. T (2005). Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresour. Technol.*, 69: 1049-1055.

Bergy, D. H and Holt, J.G (1994). *Bergey's Manuel of Determinative Bacteriology* 9th ed. The Williams and Wikins Company, Baltmiore.

Cheesbrough, M (2006). *District Laboratory practice in Tropical countries,* part 2, London, Cambridge University Press, P. 58-100.

Chikere, C.B., Okpkwasili, G.C and Chikere, B.O (2009). Bacterial diversity in a tropical crude oil polluted soil undergoing bioremediation. *Afri. J. Biotechnol.,* 8:2535-2540.

Chukwudozie, S. O (2013). Stimulated biodegradation of spent lubricating motor oil in soil amended with animal droppings. *J. Nat. Sci. Res.,* 3 (12): 2224 – 3186.

Cooney, J.J (1984). The fate of petroleum pollutants in fresh water ecosystems: In *Petroleum Microbiology* Atlas, R.M ed., Macmillam, New York, USA.

Crisafi, F., Genovase, M., Smedite, F., Russo, D., Cataliforna, M and Yakimov, M (2016). Bioremediation techniques for polluted sea-water sampled after an oil-spill in Taranto gulf (Italy): A comparison of biostimulation, bioaugmentation and use of washing agent in microcosom studies. *Marine Pollut. Bullet,* 106(1-2):119.

Dawson, J.J., Godsiffe, E.J., Thompson, I.P., Ralebiso-Senior, T.K., Killham, K.S and Paton, G.I (2007). Application of biological indicators to assess recovery of hydrocarbon impacted soil. *Boil. & Biochem*., 39:164-177.

Delyan, U.H., Harder, M and Hopner, T. H (1990). Hydrocarbon biodegradation in sediments and soils. Systematic examination of physical and chemical conditions part II pH values. *Wisseichaft Technik. Sci. Technol*., 4: 337 – 342.

Ekpo, I.A., Agbor, R.B., Okpako, E.C and Ekanem, F.B (2012). Effect of crude oil polluted soil on germination and growth of soyebean (*Glycine max*). *Annals of Bio. Res.* 3(6): 3049 – 3054.

Ekpo, M.A and Nwaankpa, I.L (2005). Effect of crude oil on microorganisms and growth of ginger (*Zingiber officianale*) in the tropics. *J. Sust. Tropic. Agric. Resour.,* 16:67-71.

Ekpo, M.A and Udofia, U.S (2008). Rate of biodegradation of crude oil by microorganisms isolated from oil sludge. *Afric. J. Biotechnol.,* 7: 4495 – 4499.

El-Fantroussi, S and Agathos, S.N (2005). Is bioaugmentation a feasible strategy for pollutant removal and site remediation? *Curr. Opin. Microbiol.,* 8:268-275.

Environmental Agency –EA (2003). Principles of evaluating the human health risks from petroleum hydrocarbons in soils. A consultation paper, research report, 5-080/TRI. *Environmental Agency,* Bristol.

Gyorgy, E., Mara, G., Mathe, I., Lasio, E., Marialigeti, K., Albert, B., Oancea, F and Lanyi, S (2010). Characterisation and diversity of the nitrogen fixing microbiota from a specific grassland habitat in the civic mountains. *Romanian biotechnol. Lett.,* 15 (4): 5474-5481.

Hall, A., Hugunin, J.K., Deaville, R., Law, R.V., Auchin, C.R and Jepson, P.D (2006). The risk of infection from polychroninated biphenyl exposure in the harbour porpoise *(Phocena phocoena).* A case control approach. *Envt. Health Perspect.,* 704-711.

Hamanura, N., Olson, S.H., Ward, D.M and Inskeep, W.P (2006). Microbial population dynamics associated with crude oil biodegradation in diverse soils. *Appl. Environ. Microbiol.,* 72: 6316 – 6324.

Hamza, T.A., Husssum, Z., Mitku, R., Ayalew, P and Belayneh, T (2017). Isolation and characterization of nitrogen fixing bacteria from Rhizosphere soil collected from Shell Mele Agricultural Centre, South Ethiopia; *J. Agric. Sci. Food Technol.* 3 (7): 117-124.

Hollender, J., Althoff, K., Mundt, M and Doth, W (2003). "Assessing the microbial activity of soil samples, its nutrient limitation and toxic effects of contaminants using a simple

Scientia Africana, Vol. 20 (No. 1), April, 2021. Pp 109-124 https://dx.doi.org/10.4314/sa.v20i1.10
© Faculty of Science, University of Port Harcourt, Printed in Nigeria *ISSN 1118 – 1931*

respiration test." *Chemosphere,* 53 (3): 269-275.

John, R.C and Okpowasili, G.C (2012). Crude oil-degradation and plasmid profile of nitrifying bacteria isolated from oil-impacted mangrove sediment in the Niger Delta of Nigeria. *Butt. Environ. Cont. Toxicol*., 88 (16): 1020-1026.

Macaulay, B.M (2015). Understanding the behaviour of oil-degrading microorganisms to enhance the microbial remediation of spilled petroleum. *Appl. Ecology Environ. Resour.,* 13 (1):247-262.

Margesin, R and Schinner, F (2001). Biodegradation and bioremediation of hydrocarbons in extreme environments. *Appl. Mirobiol. Biotechnol.,* 56: 650-663.

Massachusetts Department of Environmental Protection MDEP (2002). *Characterizing risks possed by petroleum contaminated sites*. Implementing the VPH/EPH/APH approach policy WSC-02-411. MADEP Executive Office of Environmental Affairs, Common Wealth of Massachusetts.

Mayo, A.W and Noike, T (1996). Effects of temperature and pH on growth of heterotrophic bacteria in waste stabilization ponds. *Water Res.,* 30 (2): 447-455.

Mona, S.Z., Mohammed, M.N.A and Hossan, H.N.A (2015). Bioremediation of petroleum contaminants in aquatic environments. *Life Sci. J.,* 12 (5): 127-139.

Mukred, A.M., Hamid, A.A., Hamzah, A and Yusoff, W.M.W (2008). Development of three bacteria consortium for the bioremediation of crude petroleum-oil in contaminated water. *Online J. Biologic Scis.,* 8 (4): 1608-4217.

Nessel, C.S.A (1992). Comprehensive evaluation of the carcinogenic potential of middle distillate fuels. *Drug and Chem. Toxicol.,* 22:165-180.

Nwankwegu, A.S., Onwosi., C.O., Orji, M.U., Anaukwu, C.G., Okafor, U.C., Azi, F and Manins, P.E (2016). Reclammation of DPK hydrocarbon polluted agricultural soil using a selected bulking agent. *J. Environ. Manag.,* 172:,36-42

Odokuma, L. O and Akpokodje, E. G (2004). Biodegradation of stimulated landfill leachate in tropical of depth 6.5m. J. Appl. Sci. Technol., 4 (1): 6-14.

Odokuma, L.O and Ibe, C. O (2003). Bioremediation of paper and polyethylene using immobilized and free bacterial cells. *Nig. J. Microbiol.,* 17 (1): 17-25.

Omoni, V.T., Aguoru, C.U., Edoh, E.O and Makinde, O (2015). Biostimulation of hydrocarbon utilizing bacteria in soil contaminated with spent engine oil using banana and plantain agro-wastes. *J. Soil Sci. Environ. Manage.,* 6 (8): 225-233.

Onuoha, S.C (2013). Stimulated biodegradation of spent lubricating motor oil in soil amended with animal droppings. *J. Nat. Sci. Res.,* 3 (12): 105 – 116.

Ordinioha, B.A and Sawyer, W (2010). Acute health effects of crude oil spill in a rural community in Bayelsa State, Nigeria. *Nig. Med. J.,* 19:140-144.

Ordinioha, B and Brisibe, S (2013). The human health implications of crude oil spills in the Niger Delta, Nigeria: An interpretation of published studies. *Nig. Med. J.,* 54:10.

Pervin, S., Jannat, B., Sanjee, S and Farzana, T (2017). Characterization of Rhizobia from root nodule and rhizosphere of *Lablab purpureus* and *Vigna sinensis* in Bangladesh. *Turkish. J. Agric. Food Sci. Technol.,* 5 (1): 14-17.

Pollard, S.J.T., Duarte-Davidson, R., Askari, K and Stuff, E (2005). Managing the risks from petroleum hydrocarbon contaminated sites: Achievement and future research

directions. *Land Cont. Recl.,* 13 (2): 115-122.

Sadik, A.S., Noof, A.E and Sonya, H.M (2016). Isolation and characterization of free-Nitrogen fixer bacterial strains (*Azotobacter* sp) and their phages from maize Rhizosphere soil at TAIF Park. *J. Biotechnol.,* 13 (1): 31-37.

Semple, K. T., Reid, B.T and Femo, T.R (2001). Impact of composting strategies on the treatment of soils contaminated with organic pollutants. *Environ. Pollut.,* 112: 269-283.

Semple, K.T., Dew, M.M., Doick, K.J and Rhodes, A. H (2006). Can microbial mineralization be used to estimate microbial availability of organic contaminants in soil? *Environ. Pollut.,* 140 (1): 164-172.

Solomon, L., Ogugbue, C.J and Mbakwem-Aniebo, C (2018). Exploring the potentials of Nipa palm (*Nypa fruticans*) ash and rabbit droppings for enhanced *Ex-situ* Bioremediation of crude oil contaminated soil. *Asian J. Biotechnol. Biores. Technol.,* 3 (2):1-7.

Stewart, E.A., Maxcrimshaw, H., Parkinson, J.A and Quarmby, C (1974). *Chemical analysis of biological materials.* Blackwell scientific publication, Oxford, London.

Tiido, T.A., Rignell-Hudbom, B.A.G., Joson, Y.L., Giwereman, H.S., Penderson, B., Wojtyniak, J.K., Ludwieki., V., Lesovoy, V and Zvyezday, B (2006). Impact of PCB and P-DDE contaminants on human sperm Y:X chromosome ratio: Studies on three European populations and the International population in Greenland. *Environ. Health Perspect,* 718-724.

Tyagi, M., Yafonseca, M and Carvalho, C (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation process. *Biodegrad*., 22.

United States Environmental Protection Agency, U.S EPA (1999). Use of monitored natural attenuation at superfund, RCRA corrective action and underground storage tank sites, OSWER Directive 9200, Washington DC, USA., 4-17.

Vidali, M (2001). Bioremediation an overview. *Pure and Appl. Chem.,* 73 (7): 116-172.

Walworth, J., Pond, A., Snape, I., Rayner, J., Ferguson, S and Harvey, P (2007). "Nitrogen requirements for maximizing petroleum bioremediation in a sub-antarctic soil". *Cold Regions Sci. Technol.,* 48 (8):84-91.

Wokem, V.C and Madufuro, C (2020). Application of cow dung and sawdust as biostimulants for enhanced bioremediation of diesel contaminated soil. *J. Appl. Sci. Environ. Manage.,* 24 (1): 49-57.