Journal of Science Research

Enhanced bioremediation of crude oil contaminated soil in Oleh, Delta State, Nigeria

Ifukor C. I., Akinsete* S. J., Lateef S. A.
Department of Environmental Health Sciences, Faculty of Public Health,
College of Medicine, University of Ibadan, Oyo, State, Nigeria
*Correspondence Author: Email: s.j.akinsete@gmail.com. Tel.: +234 8134539776

Abstract

Crude oil pollution of soils is an ongoing challenge in the Niger Delta region of Nigeria, resulting in decreased farmlands with the overall threat to food and nutrition security, as well as human health. Thus, bioremediation suited for organic pollutants should assume priority in addressing this enormous challenge. Therefore, this study investigated the efficacy of Remediation-By-Enhanced-Natural-Attenuation (RENA) method in remediating crude oil contaminated soil in Oleh, Delta State, Nigeria. Remediation-by-enhanced-natural-attenuation was performed on a crude oil contaminated plot. Soil samples (0-20 cm) were collected at pre-remediation, one and three weeks remediation periods to determine Total Petroleum Hydrocarbon (TPH), Polycyclic Aromatic Hydrocarbon (PAH), microbial counts, nutrients and heavy metals. Pre-remediation TPH concentration (mg/kg) of 274.84±0.81 significantly reduced to 195±1.6 after three weeks. Similarly, there was a gradual decrease (36%) in PAH concentrations during the remediation period. Naphthalene and related compounds (possible carcinogens) accounted for over 50% of PAH in pre-remediation soil. The concentrations of Ni, Cr, Cd, and Zn decreased significantly at the end of the study. Hydrocarbon utilising bacteria peaked at 2.38±0.06 (cfu/g×10^2) in week one but reduced to 1.12±0.07 (cfu/g×10^2) in week three. Total fungi peaked (1.75±0.04 cfu/g ×10^4) at week three remediation period while the hydrocarbon utilising fungi peaked (1.16±0.06 cfu/g×10^2) at week one remediation period. The study demonstrated that remediation-by-enhanced-natural-attenuation significantly increased indigenous hydrocarbon utilising species, with a significant reduction in TPH, PAH and heavy metals concentrations after 3-week remediation period. Our study further confirms the practicability of RENA in reclaiming crude oil contaminated soil in Nigeria.

Keywords: Remediation-by-enhanced-natural-attenuation; soil; total petroleum hydrocarbon; polycyclic aromatic hydrocarbon; microbial counts

Introduction

The apparent concern for the soil compartment of the environment where crude oil contamination exists has not only recently come to the fore but has assumed a position of priority more recently in response to attainment of food and nutrition security and climate change, which are crucial sustainable development goals [1]. Furthermore, in Nigeria, there is a recent re-focus on agriculture consequent upon the current recession experienced in the country. Although, this presents the opportunities for improving socioeconomic status and livelihoods, one important subject matter that must be tackled is the question on how clean and safe our soils are for food production and ultimately human health. This is all important owing to large releases of hydrocarbonbased fuels into the terrestrial and aquatic environments which has significant environmental, ecological and health consequences [2, 3]. Crude oil spillage in Nigeria is one of the greatest environmental challenges and can be attributed to operational procedures, equipment failure (accidental rupture of disasters [2, 4]. Crude oil contamination contributes to the increasing global climate change, hinders plant growth and development, and threatens food security in the soil environment, thereby making remediation a necessity.

© *Journal of Science Research, 2018*

Although, there is a wide range of remediation technologies, bioremediation has been found to be especially suited for organic pollutants. These Bioremediation techniques are noninvasive, cost-effective, and environmentally friendly compared to their counterparts (physical and chemical methods of remediation) [5, 6, 7], and are also sustainable and have high public acceptance. According to [7], bioremediation is defined as a process, which relies on biological mechanisms to reduce (degrade, detoxify, mineralize or transform) concentration of pollutants to an innocuous state using two major approaches (biostimulation and bioaugmentation).

Remediation by Enhanced Natural Attenuation (RENA) is an integrated biological and physicochemical process used together to accelerate the natural processes of petroleum hydrocarbon attenuation or reduction by systematically tilling the contaminated soil with the addition of nutrients [phosphorous and nitrogen] and appropriate environmental conditions [3, 8]. The main goal of RENA is to enhance soil nutrient capacity that promotes the growth of indigenous hydrocarbon degrading bacteria and fungi population which in turn reverse the negative impacts of crude oil contamination [9, 10]. As a bioremediation technology, the demonstration of its efficacy and safety and the communication of same is important for its adoption [11]. Although several studies have also reported the efficacy of RENA to contribute to the enhancement of crop performance grown in contaminated soils [12, 13] and contaminant reduction in crude polluted soil [14], few field investigations have reported the impact of RENA on heavy metal concentrations in contaminated soil. Some studies [15] have reported high levels of heavy metal (e.g., nickel and vanadium) concentrations in Nigerian crude oil blends as against environmental standards of the World Health Organization. Therefore, this study was carried out to evaluate the efficacy of RENA in the bioremediation of crude oil contaminated soil.

Materials and methodology

Study area and soil sampling

The field experiment was carried out at Oleh (latitude 5° 25′ N and longitude 6° 8′ E) headquarters of the Isoko South Local Government Area, Delta State, southern Nigeria. Remediation-by-enhanced-natural-attenuation processes adopted were tilling, fertilisation (NPK 15:15:15) at 12.5 tons/ha and windrowing [9, 16] on a 3m by 2m crude oil contaminated plot, of the blowout region within the Nigerian Petroleum Development Company (NPDC) Oleh/Olomoro flow station for three weeks. An uncontaminated nearby farmland was also sampled to serve as control. Soil samples were collected at 0 – 20 cm depth at pre-remediation, one and three week's remediation periods to determine total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbon (PAH), microbial counts (total heterotrophic bacteria, hydrocarbon utilising bacteria, total fungi and hydrocarbon utilising fungi), nutrients and heavy metals. Sub-samples for total petroleum hydrocarbon and polycyclic aromatic hydrocarbon determination were stored at 4°C in the refrigerator until analysed. Soil samples were air-dried, sieved (2 mm) and stored in air-tight cellophane bags until physicochemical and heavy metal analyses. All analyses were performed on the 2mm sieved samples except for organic carbon and heavy metals which were determined on finely ground samples.

Determination of physicochemical properties

Physicochemical properties of the soil were determined as follows: soil pH in 1:2 soil - water suspension using a glass electrode pH meter, organic carbon was determined by the wet (acid–dichromate) combustion method [17], while total nitrogen and phosphorus were determined using standard methods on a UV spectrophotometer at the wavelength of 470nm. Total metals (Zn, Fe, Cr, Pb, V, Ni, and Cd) in soil were determined after di-acid digestion with nitric–hydrochloric acids using atomic absorption spectrophotometer.

Determination of Total Petroleum Hydrocarbon and polycyclic aromatic hydrocarbon

Total Petroleum Hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) concentrations were determined by modified US EPA 8270 on gas chromatograph (GC-MS) using a mixture of n-Hexane and dichloromethane in the ratio of 3:1 as the extracting solution and quantification was by method of internal standard (d_8 Naphthalene).

Microbiological analysis

Isolation and enumeration of bacteria and fungi

The total heterotrophic bacterial and total fungi counts were performed by inoculating 0.1 ml of serial diluentsonto nutrient agar (Oxoid) and Sabouraud Dextrose agar (Oxoid), respectively, using the pour plate method. The plates were incubated at 28 ± 2°C for 48 hours. The number of colonies were counted and recorded as colony forming unit per gram (cfu/g). For the hydrocarbon utilising bacteria (HUB) and fungi (HUF) counts, a mineral salt medium (10.00 g/L NaCl, 0.42 g/L

$MgSO_4.7H_2O$, 0.29 g/L KCl, 0.83 g/L KH_2PO_4, 1.25 g/L $NaHPO_4$, 0.42 g/L $NaNO_3$ and 20.00 g/L agar) was used and plates were counted after incubation at 28°C for 7 days using the vapour-phase transfer method as described by Ebuehi et al.,[9].

Data analysis

The statistical analysis was performed using SPSS version 17 software. One-way analysis of variance (ANOVA) was used to determine differences among means and level of significance was tested at $P < 0.05$.

Results and discussion

Physicochemical properties and heavy metals constituents of soil during remediation process

The physicochemical properties of soil (Table 1) showed some variations during the remediation by enhanced natural attenuation (RENA) process. The initial soil pH at the pre-remediation period in this study was very acidic (Table 1), which was similar to the range reported by Amadi et al.,[18] and Mmom and Deckor[12] for crude oil impacted sites in their respective studies. However, the pH significantly increased (Table 1) to near neutral range over the 3-week remediation period which can be attributed to amendment with NPK fertiliser during the RENA process. Also, the increase in soil pH during the remediation period reached optimal levels necessary for plant and microbial activities. Similarly, concentrations of total organic carbon (TOC), nitrogen (N) and phosphorus (P) varied during the remediation process (Table 1). While TOC and P decreased during the remediation periods, N increased after one week but decreased to about half the concentration at the end of week three (Table 1). The initial high TOC concentration in the contaminated soil is indicative of the presence of organic contaminants as reported in another study [19]. Thus, hydrocarbon source accounted for the increase in pre-remediation total organic carbon level in this study as observed in other studies [20, 21] that reported over eightfold increase in total organic carbon content in crude oil contaminated soil when compared to control. However, the observed increase in N can be attributed to addition of NPK fertiliser while the utilization of the nutrient for growth and reproduction accounts for the decrease. According to Boufadel et al.,[22], addition of supplemental nutrients (N and P) in their proper concentrations enhances the efficacy of hydrocarbon degrading microbes.

There was an observed significant increase in nickel concentration in pre-remediated soil when compared to the remediation periods and control soil (Table 1). This suggests that crude oil contamination is associated with elevated nickel concentrations which supports the work of Dickson and Udoessien [15] which reported that Nigerian crude oil could be a source of heavy metals particularly nickel and vanadium. However, in this study concentration of vanadium was below detectable limits. Nickel concentration reduced after one week of remediation and below detection limits after the 3-week remediation period.

Table 1. Physicochemical parameters and heavy metals in crude oil contaminated soil during remediation process

Parameters	Remediation Period			Control soil
	Pre-Remediation	7 days	21 days	
pH	4.83 ± 0.16b	6.06 ± 0.19a	6.50 ± 0.24a	4.80 ± 0.01b
Total organic carbon (mg/kg)	4410 ± 0.02a	30 ± 0.00d	190 ± 0.01c	1200 ± 0.02b
Nitrogen (mg/kg)	4.77 ± 0.12d	17.70 ± 0.57b	9.95 ± 0.74c	43.04 ± 0.19a
Phosphorus (mg/kg)	9.08 ± 0.14b	1.80 ± 0.02d	3.76 ± 0.06c	15.35 ± 0.26a
Cadmium (mg/kg)	6.01 ± 1.40	0.00	0.00	0.00
Chromium (mg/kg)	121.54 ± 0.14a	51.00 ± 1.60d	68.15 ± 0.14b	58.54 ± 0.24c
Lead (mg/kg)	7.39 ± 0.04	0.00	0.00	0.00
Nickel (mg/kg)	110.18 ± 1.33a	8.53 ± 0.67b	0.00	8.13 ± 0.85b
Iron (mg/kg)	1122.50 ± 1.89d	1403.50 ± 1.29c	2516 ± 2.04a	1478 ± 1.71b
Zinc (mg/kg)	11.31 ± 0.05a	2.25 ± 1.89d	9.65 ± 1.02b	5.81 ± 0.02c

Means followed by lowercase letters are significantly different across rows; Mean ± standard deviation (n = 4)

Furthermore, lead (Pb) and cadmium (Cd) concentrations decreased below detection limits after weeks one and three of remediation, respectively. Conversely, iron (Fe) concentration significantly increased during the remediation periods relative to the pre-remediation concentration. The increase in pH from 4.8 (pre-remediation) to 6.5 (3-week remediation period), and improved aerobic conditions by windrowing increased microbial activities which has been reported to favour the dissolution and availability of iron in soil [23]. These findings are also in agreement with other studies [24, 25] that identified similar heavy metals in crude oil polluted sites in Nigeria. Also, Wegwu et al., [14], reported a significantly higher concentration of heavy metals in crude oil polluted soils when compared to the remediated and control soils.

Total petroleum hydrocarbon and polycyclic aromatic hydrocarbons

The total petroleum hydrocarbon (TPH) and polycyclic aromatic hydrocarbon (PAH) concentrations in the contaminated soil were significantly reduced after one week and 3-week remediation periods (Table 2). The TPH concentrations decreased by 26% and 29% at the end of weeks one and three, respectively. While PAH decreased by 11% and 36% at the end of weeks one and three, respectively, thus indicating the effectiveness of RENA. These observations corroborate the findings of Onifade et al., [10] and Wegwu et al., [14], where RENA successfully remediated crude oil spill contaminated farmland. Their studies revealed attainment of > 90% removal of total petroleum hydrocarbon after 18 and 8 weeks remediation periods, respectively. They also reported a reduction in PAH using RENA and suggested that the marked reduction in the levels of PAHs for remediated samples may be attributed to the physical and chemical processes including dilution, dispersion and volatilization during excavation and tilling of the soils, as well as microbial degradation. Although the percentage TPH loss in this study was less than those earlier reported [10, 14, 20], the values were similar to those reported by Mmom and Deekor [12]. The reason for these differences could be the short duration (three weeks) of RENA employed in this study compared to those previously mentioned.

Table 2. Petroleum hydrocarbon concentrations of remediated crude oil contaminated soil

Parameters (mg/kg)	Remediation Period			Removal efficiency (%)		Control soil
	Pre-Remediation	7 days	21 days	7 days	21 days	
TPH	274.84 ±0.81a	203.32 ±0.95b	195 ±1.48c	26.0	29.0	0.00
PAH	0.28 ±0.01a	0.25 ±0.01b	0.18 ±0.02c	10.7	35.7	0.00

TPH –Total petroleum hydrocarbon; PAH – Polycyclic aromatic hydrocarbon; Means followed by lowercase letters are significantly different across rows; Mean ± standard deviation (n = 4)

Constituents of polycyclic aromatic hydrocarbon

Gas chromatography revealed that the constituent PAHs identified in the crude oil contaminated soil in this study included: naphthalene, 2-methyl naphthalene, 1-methyl naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene and benz(a)anthracene at pre-remediation, after one and three weeks remediation periods (Fig.1). Although benz(a)anthracene was not identified in the pre-remediation phase of the study, the identified constituent PAHs are similar to those identified in crude oil contaminated soils in the earlier studies [26, 27].

One interesting observation in this study revealed a consistent reduction in the percentage of naphthalene, 2-methyl naphthalene and 1-methyl naphthalene (Fig.1) which constituted bulk of the PAHs over the remediation period. On the other hand, acenaphthalene, acenaphthene, fluorene, phenanthrene and anthracene and benz (a) anthracene increased as remediation progressed. We suggest these compounds were not degraded by the indigenous microbial species hence a build-up occurred. The main concern attributed to PAHs in the environment is their role as carcinogens [28]. Some of the PAHs reported in this study, specifically, naphthalene and its related compounds (1-methyl naphthalene and 2-methly naphthalene) have been implicated as carcinogens. The International Agency for Research on Cancer (IARC) classified naphthalene as a possible human and animal carcinogen (group 2B substance) based on limited evidence in human and less than sufficient evidence in animal studies [29]. Benz(a)anthracene has been classified as Group 2a (probably carcinogenic to humans) substance [30], while anthracene, flourene, and phenanthrene are classified as group 3 (carcinogenicity not classifiable) substances [29].

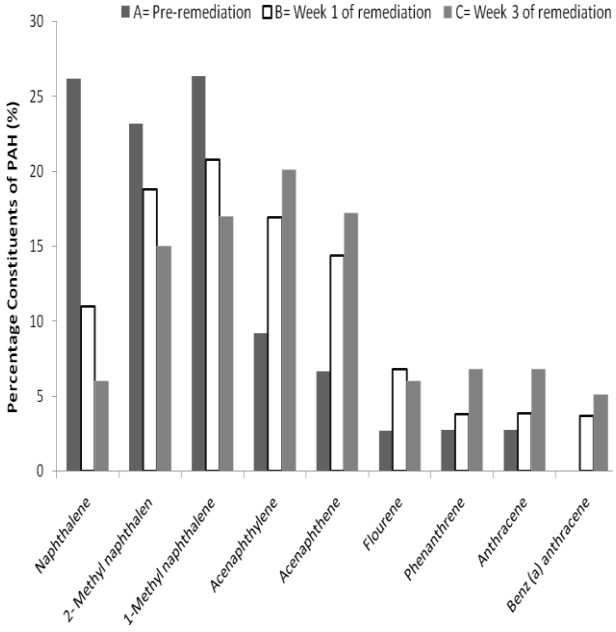

Figure 1. Constituents of Polycyclic aromatic hydrocarbon (PAH)

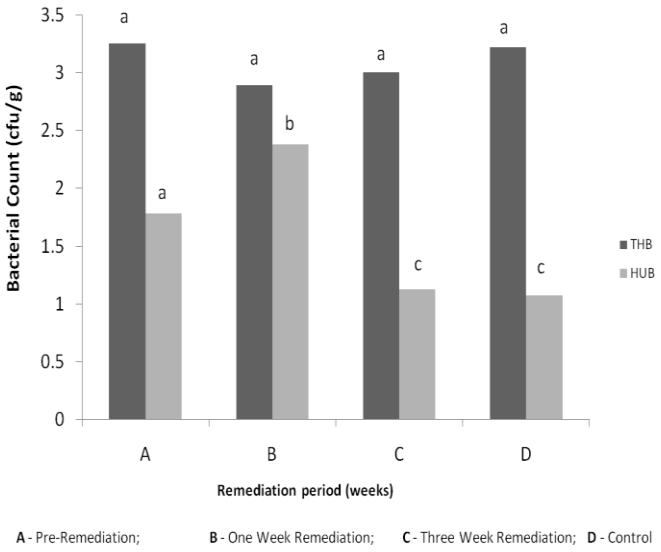

Figure 2. Change intotal heterotrophic bacteria (THB; $x10^4$cfu/g) and total hydrocarbon utilizing bacteria (HUB; $x10^2$cfu/g) during remediation

Effect of remediation by enhanced natural attenuation on microbial profile

The results revealed there was no significant difference in the total heterotrophic bacteria (THB) counts during the pre-remediation and remediation periods (Fig. 2). However, the total hydrocarbon utilizing bacteria (HUB) increased from 1.78 x 10^2cfu/g to 2.38 x 10^2cfu/g by the end of one week remediation period but reduced significantly to 1.12 x 10^2cfu/g at the end of the third week of remediation period (Fig.2). Although, the TF counts increased during the remediation period, they were not significantly different from the pre-remediation and one week remediation periods. However, the TF count was similar to the control soil by the end of the remediation period (Fig. 3). On the other hand, no hydrocarbon utilizing fungi (HUF) isolate was detected at the pre-remediation period. However, by the end of one week RENA process, HUF count (1.16 x 10^2cfu/g) was isolated and this gradually decreased to 1.0 x 10^2cfu/g at the end of the three week remediation (Fig. 3). The observed reduction in HUB at the end of the third week of the study period was due to depletion in limiting nutrient (N : P), which in turn reduced microbial activities (growth) in the soil by the end of the study [3]. This explanation is further supported by Onyenjoro [31] who developed a model establishing the dependency and efficacy of the growth of HUBs on the availability of substrates and the effective control of other environmental factors such as exposure to high temperatures and controlled moisture content of the soil.

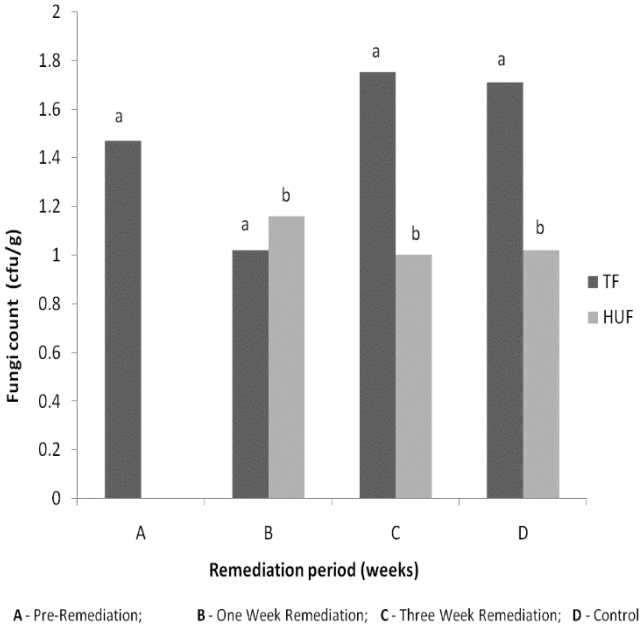

Figure 3. Change in Total Fungi (TF; $x10^4$cfu/g) and Hydrocarbon utilizing Fungi (HUF; $x10^2$cfu/g)

Conclusion

Evidences from this study have shown that crude oil was associated with the observed increase in TPH, PAH, some heavy metals and crude oil associated total organic carbon, while remediation by enhanced natural attenuation (RENA) demonstrated the ability to clean up crude oil contaminated soil. This ability however is said to be dependent on certain controlled factors such as soil pH, nutrient

availability and amendment, microbial consistency and the extent of soil contamination by the crude oil. Interestingly, the study revealed a consistent reduction in the percentage of naphthalene, and related compounds which constituted the bulk of the PAHs as the remediation progressed. Generally, there was a significant increase in indigenous hydrocarbon utilising species, during the 3 weeks of remediation process. Our study further confirms the practicability of RENA in reclaiming crude oil contaminated soil in Nigeria. This is crucial in attaining the sustainable development goals 2 and 3 which are addressing food and nutrition security and human health.

References

[1] UN 2016. The sustainable development goals report. New York

[2] Adelana, S.O., Adeosun,T.A., Adesina, A.O. and Ojuroye, M.O. 2011. Environmental pollution and remediation: challenges and management of Oil Spillage in the Nigerian coastal areas. *American Journal of Scientific And Industrial Research,* 2(6): 834-845

[3] Nwogu, T. P., Azubuike, C. C., and Ogugbue, C. J. 2015. Enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with *Capra aegagrus hircus* (goat) manure. *Biotechnology Research International* Volume 2015: 1-7.

[4] Yakubu, M. B. 2007. Biological approach to oil spills remediation in the soil. *Academic Journals of Biotechnology*, 6 (24): 2735-2739 Available online at http://www.academicjournals.org/AJB.

[5] Dados, A., Omirou, M., Demetriou, K., Papastephanou, C., and Ioannides, I.M. 2015. Rapid remediation of soil heavily contaminated with hydrocarbons: a comparison of different approaches. *Annals of Microbiology*, 65(1): 241–251.

[6] Silva-Castro, G. A., Uad, I., Rodr´ıguez-Calvo, A., Gonz´alez-L´opez, J. and Calvo, C. 2015. Response of autochthonous microbiota of diesel polluted soils to land-farming treatments. *Environmental Research*, 137: 49–58.

[7] Azubuike, C. C., Chikere, C. B., and Okpokwasili, G. C. 2016. Bioremediation techniques–classification based on site of application: principles, advantages, limitations and prospects. *World Journal of Microbiology Biotechnol*ogy, 32:180

[8] Eludoyin O.S. and Oghenetega O.J. 2016. Temporal analysis of bioremediation of crude oil contaminated soil using remediation by enhanced natural attenuation (RENA) technique in Mbodo community, Port Harcourt, Nigeria. *Tropical Agriculture. (Trinidad)*, 93(1)

[9] Ebuehi, O.A.T., Abibo, I. B., Shekwolo, P.D., Sigismund, K. I., Adoki, A. and Okoro I.C. 2005. Remediation of Crude Oil Contaminated Soil. *Journal of Applied Science and Environmental Management*, 9 (1): 103-106.

[10] Onifade, A.K., Abubakar, F.A. and Ekudayo, F.O. 2007. Bioremediation of crude oil polluted soil in the Niger Delta Area of Nigeria using Enhanced Natural Attenuation. *Research Journal of Applied Sciences*, 2: 498-504.

[11] Thapa, B., Ajay, K.C. and Anish G. 2009. A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Journal of Applied Science Environmental Management*, 13(3): 99-103.

[12] Mmom, P.C. and Deekor, T. 2010. Assessing the effectiveness of land farming in the remediation of hydrocarbon polluted soils in the Niger Delta, Nigeria. *Research Journal of Applied Sciences, Engineering and Technology*, 2(7): 654-660.

[13] Odukoya, J. and Ronald, L. 2015. Remediation by enhanced Natural attenuation (RENA): A beneficial strategy for Polycyclic Aromatic Hydrocarbon degradation and Agrifood production. *Proceedings of the World Congress on New Technologies, Barcelona, Spain*. Paper No.183.

[14] Wegwu, M. O., Uwakwe, A. A. and Anabi, M.A. 2010. Efficacy of enhanced natural attenuation (land farming) technique in the remediation of crude oil-polluted agricultural land. *Archives of Applied Science Research*, 2 (2):431-442.

[15] Dickson, U.J. and Udoessien, E. I. 2012. Physicochemical studies of Nigeria's crude oil blend. *Petroleum and Coal*, 54(3): 243-252

[16] Ayotamuno, M.J., Kogbara, R. B., Ogaji, S.O.T. and Probert, S.D. 2006. Bioremediation of a crude-oil polluted agricultural soil at Port Harcourt, Nigeria. *Applied Energy*, 83(11): 1249-1257.

[17] Walkley, A. and Black, I.A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-38.

[18] Amadi, A., Abbey, S.D. and Nma, A. 1996. Chronic effects of oil spill on soil properties and microflora of rainforest ecosystem in

Nigeria. *Water, Air, and Soil Pollution*, 86 (1): 1-11.

[19] Macci, C., Peruzzi, E., Doni, S., Poggio, G. and Masciandaro, G. 2016. The phytoremediation of an organic and inorganic polluted soil: A real scale experience, *International Journal of Phytoremediation*, 18:4, 378-386, DOI: 10.1080/15226514.2015.1109595

[20] Lorestani, B. 2014. Bioremediation (natural attenuation) of hydrocarbon contaminated soil. *Journal of Biodiversity and Environmental Science*, 5(6): 416-424.

[21] Barua, D., Buragohain, J. and Sarma, S.K. 2011. Certain physico-chemical changes in the soil brought about by contamination of crude oil in two oil fields of Assam, NE India *European Journal of Experimental Biology*, 1 (3):154-161.

[22] Boufadel, M C., Suidan, M.T. and Venosa, A. D. 2006. Tracer studies in laboratory beach simulating tidal influences. *Journal of Environmental Engineering*, 132 (6): 616.

[23] Inda Junior, AV, Torrent J, Barrón V, Bayer C, Fink, JR. 2013. Iron oxides dynamics in a subtropical Brazilian Paleudult under long-term no-tillage management. *Scientia Agricola*, 70: 48-54.

[24] Onojake M.C. and Okonkwo, V. 2011. Trace metals associated with oil spillage: a case study. *Journal of Chemical and Pharmaceutical Research*, 3 (6):742-751.

[25] Joel, A., Ogbonna, F. and Chinomso, A. 2009. Physicochemical Characteristics and Microbial Quality of an Oil Polluted Site in Gokana, Rivers State. *Journal of Chemical and Pharmaceutical Research*, 3(6): 742-751.

[26] Ipeaiyeda, A.R., Nwauzor, G.O., Akporido, S.O. 2015. Biodegradation of Polycyclic Aromatic Hydrocarbons in Agricultural Soil Contaminated with Crude Oil from Nigeria Refinery using *Pleurotussajor-caju*. *Journal of Bioremediation Biodegradation*, 6: 301.

[27] Xu, G. L., Liu, H., Li, M. J., Li, Z. M., Peng, Z. H., Zuo, L. M., He, X., Liu, W. W. and Cai, L.G. 2016. In situ bioremediation of crude oil contaminated site: A case study in Jianghan oil field, China. *Petroleum Science and Technology*, 34(1): 63-70.

[28] Abdel-Shafy, H. I. and Mansour, M. S. M. 2016. A review on polycyclic aromatic hydrocarbons: Source, environmental impact, effect on human health and remediation. *Egyptian Journal of Petroleum*, 25: 107-123.

[29] IARC (International Agency for Research on Cancer). 2002. Naphthalene. *IARC* Monogaraphs on the evaluation of the carcinogenic risk of chemicals to humans 82:367-435.

[30] US EPA (Environmental Protection Agency). 2008. Polycyclic aromatic hydrocarbons (PAHs) — EPA fact sheet. Washington (DC): National Center for Environmental Assessment, Office of Research and Development.

[31] Onyenjoro, C.H. 2011. Modelling the rate of degradation in crude oil polluted sites. Unpublished M. Engr. Dissertation, *Department of Civil Engineering Faculty of Engineering University of Nigeria Nsukka, Nigeria*.