IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402,p- ISSN: 2319-2399.Volume 16, Issue 1 Ser. I (January 2022), PP 50-57
www.iosrjournals.org

Pre-Bioremediation Assessment of Crude Oil Contaminated Soil in Kokori and Environs, Niger Delta Region, Nigeria.

Birma, G. J.[1,2*], Tawari–Fufeyin, P[2]., Issa, B. R[1, 2] and Ogunkeyede, A. O[2].
[1]*Industrial Safety and Environmental Technology (ISET) Department, Petroleum Training Institute, P.M.B 20 Effurun Delta state Nigeria.*
[2]*Department of Environmental Management and Toxicology (EMT), Federal University of Petroleum Resources, P.M.B 1221 Effurun, Delta state Nigeria.*
Corresponding Author: birma_jg@pti.edu.ng, +2348059507478

Abstract
Pollution of the soil environment by petroleum hydrocarbon has been recognized as one of the most serious problems in the world, resulting to damage of flora and fauna. This damage is not limited to potent immune-toxicity and carcinogenic nature of the hydrocarbon, but its ability to reduce soil aeration leading to low oxygen availability to living organisms as well as obstruction in soil nutrients uptake by the flora community. The pre bioremediation assessment study of crude oil spilled soil was conducted in kokori area with sole purpose of determining the extent of hydrocarbon pollution, estimation of microbial population as well as analysis of physical and chemical properties of the soil using standard analytical procedures. The results showed the soil pH between 4.1 to 5.7 and the petroleum hydrocarbon concentration ranged from 81.17 mg/kg to 736.86mg/kg. The total bacterial count reduced from 8.5 x 10^4, cfu/g (control site) to 6.2 x 10^4, cfu/g in one of the sampling points. All samples from the spilled areas also recorded growth of the hydrocarbon utilizing bacteria which indicates the presence of crude the serves as the only sources of carbon and energy to them.
Key Words: *Crude oil, soil, kokori, Niger Delta, Environment*

Date of Submission: 08-01-2022 Date of Acceptance: 22-01-2022

I. Introduction

Environmental pollution with petroleum Hydrocarbons has been recognized as one of the most important and serious current problems around the world (*Veni et al.,2020*; *Ashikodi and Abu, 2017*). This pollutant is introduced into the environment majorly through anthropogenic and natural sources. People working in garage, fuel dooms etc. are always exposed to oily sludge which are potent immune-toxicants and carcinogens. Accidental leakages from petroleum carrying tankers, ships, pipelines as well as deliberate vandalization of pipelines lead to oily layers over the surfaces of water and land, posing great threat to the existing flora and fauna. According to *Kiraye et al.,* 2016 and supported by *Vasilyeva et al., 2019*, Petroleum Hydrocarbons (PHC) is progressively becoming both aquatic and terrestrial contaminants of great worry within the environment as they bio-accumulate in the food chain and can be delivered to other high trophic levels of the food chain.

Numerous potential hydrocarbon microbial degraders have been reported, and bacterial population constitute the most abundant group, which has been well researched for hydrocarbon degradation. Stancu (2018) have identified *Bacillus, Klebsiella, Lysinibacillus, Pseudomonas, Rhodococcus, Shewanella, Serratia, and Vibrio* as generea that are able to survive in the presence of different toxic petroleum hydrocarbons.

The purpose of this research work is to conduct preliminary investigation of a crude oil spilled site in Kokori general area in Delta State, Nigeria in order to ascertain the level contamination of the soil by crude oil. Furthermore, the study estimates microbial population (especially biodegraders) as well as physical and chemical parameter of the soil. The future research work maybe possible isolating the indigenous microorganisms for employment in bioremediation and clean up processes.

II. Materials and Methods

2.1 Study area and soil sample collection

The study area Kokori and Environs, typically of a Niger Delta region is the home of the petroleum industry in Nigeria with network distribution of onshore and offshore oil field operations. It is estimated that Nigeria earns over 90 percent of its foreign exchange and over 80 percent of government revenues from the oil industry (Okoko and Nna, 1998). The meteorology of the area according to study by Gobo, 1998 reveals that the relative humidity values on daily bases ranges from 55.5% to 96% in dry to rainy seasons respectively. The average atmospheric temperature records between 25.50^{C} during rainy season and 30.0^{C} in the dry season. Rainfall in the area averages 2500mm annually. The rainfall pattern shows two distinctive seasons: the rainy season (April to October) and a relatively short dry season (November to March). The vegetation of the Kokori general area is characterized by saline mangrove forest; fresh water swamp forest as well as tropical rain forest. The area is the largest wetland in West Africa and one of the largest mangrove forests in the world (Darafeka, 2003).

Table 2.1: Coordinates of soil sampling points

S/N	Name of Area	Coordinates
1	Omavure	5.550325N
		6.056426E
2	Idjerhe	5.592652N
		6.066729E
3	Agba	5.607598N
		6.066735E
4	Erheoke	5.652052N
		6.070360E
5	Control	5.650500N
		6.089307E

Samples of crude oil polluted soil and unpolluted soil (control) were collected at Kokori Community areas from five (5) geo-referenced sampling points (Omavure, Idjerhe, Agba, Erheoke and Control) as shown in Table 2.1 above and figure 2.1below (Birma *et al.,* 2018). Soil samples at the depth of 0-30cm will be collected using pre-cleaned soil auger and glass containers and these will be placed on Ice in cooler boxes and transported to experimental Laboratory. The contaminated as well as uncontaminated soil samples were used to estimates the bacterial population of total heterotrophic bacterial count, hydrocarbon bio-degraders as well as physical and chemical characterization.

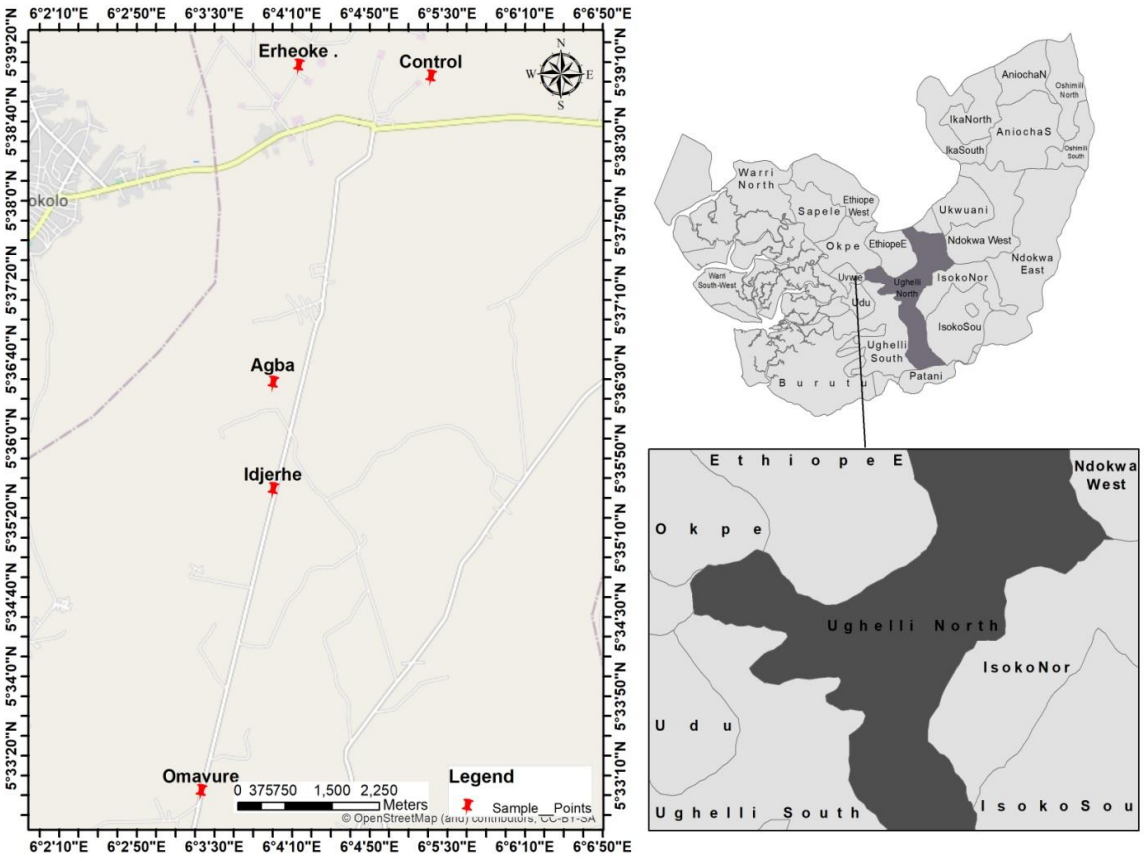


Figure 2.1: Map of the study area showing soil sampling points

2.2 Preparation and Characterization of Soil samples

The contaminated and uncontaminated soil samples were air dried for a period of two week *(Kazemzadeh, et al., 2020)*. The dried soil was crushed and sieved with 2mm mesh sieve (*Cubitto and Gentili et al., 2015; Hamza et al., 2014). Some p*ortions of the sieved soil were used to determine the some baseline physical, chemical and biological parameters that were carefully selected.

2.3 Extraction and determination of Petroleum hydrocarbon

The extraction of Total Petroleum hydrocarbons from soil samples will be done using *n*-hexane following a method described by EPA 3510 and EPA 3540C respectively. This extraction will be applied to the samples immediately after collection and at the end of each incubation periods (Vasilyeva *et al., 2019).*

Quantitative determination of the Total Petroleum Hydrocarbons (TPH) was done using Gas chromatography (GC) instrument method for as described by (Agarry *et al., 2013*).

2.4. Determination of the microbial and physico-chemical parameters

The soil pH of the samples was determined using ASTM D 4972, 2006, which employ the applications of pH probes. The moisture content and the soil texture were measured using gravimetric and sieve analysis methods as described by ASTM D 4959 and ASTM D6913 respectively. Heterotrophic bacterial count was determined using pour plate standard method procedure SM9215B, while hydrocarbon utilising bacteria was determined using mineral salt medium as described by *(*Laurelta *et al, 2020)*. Sulphate, Phosphate and total Nitrogen were estimated using the standard methods SM4500- SO_4^{2-}, SM4500-NO_3^- and SM 4500-PO_4^{2-} respectively as provided in American Public Health Association (APHA, 2018).

III. Result and Discussion

The overall results of analysis of the soil samples from study area are presented in table 2.0 below. The result showed that soil within the study is acidic and clay in nature which is characteristic of soil properties from Niger Delta region as documented by (Puyate and Rim-Rukeh, 2008). The result of the soil texture at the various sampling points uses, showed no difference in the composite samples from all points. This indicates the

homogeneity of soil-forming processes and the similarity of parent materials as described by (Soo Ying Ho *et al*, 2019)

Table 3.1: Result of soil sample analysis

S/N	PARAMETER	Omavure	Adjerhe	Agba	Erheoke	Control
1	Ph	4.5	4.10	4.30	5.70	5.40
2	Total Nitrogen, mg/kg	10.0	390.0	540.0	370.0	220.0
3	Phosphate, mg/kg	1.04	1.82	2.52	1.04	1.82
4	Total Petroleum Hydrocarbon (TPH), mg/kg	343.79	116.00	81.17	736.86	11.36
5	Total Bacterial Count (TBC), cfu/g	$8.2x10^4$	$6.30 x10^4$	$6.50 x10^4$	$7.50 x10^4$	$8.50 x10^4$
6	Hydrocarbon Degrading Bacteria (HDB), cfu/g	$3.7x10^2$	$3.0 x10^2$	$2.0 x10^2$	$5.2 x10^2$	NIL
7	Moisture content, %	43	36	33	42	38
8	Sulphate, mg/kg	3.9	3.2	2.8	4.2	4.1
9	Total Organic Carbon, mg/kg	61.82	17.44	16.9	72.9	20.6
10	PAH, mg/kg	76.2	35.8	25.32	143.89	6.34
11	Clay %	78	80	73	79	82
	Silt %	15	15	16	14	11
	Sand %	7	5	11	7	7

The results of pH determination (see figure 2) of the soil samples indicate that the soil is acidic in the nature as the pH values ranges from 4.10 to 5.70 with a value of 4.65 within the crude oil spilled area. The significance of soil pH is not only important in catalysing reactions between water and nutrients, but its great influence in determining nutrients availability for plants uptake from the soil. According to Imran *et al*, 2010 primary nutrients such phosphate, Nitrogen and Potassium as well as secondary nutrients like sulphur, calcium etc are better utilized within soil pH range of 5.5 to 7.9.

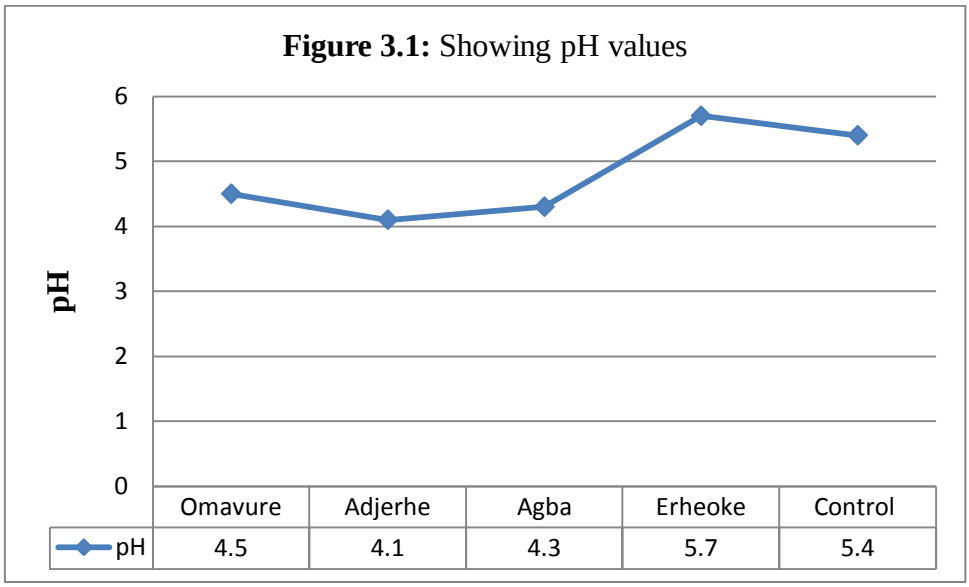
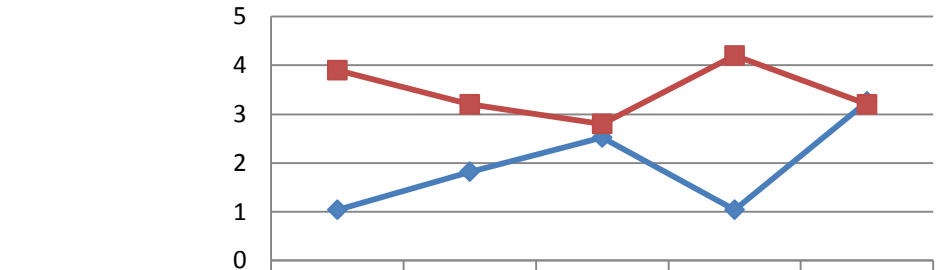


Figure 3.1: Showing pH values

The average concentration values of sulphate and phosphate is presented in figure3 below. The result values for phosphate ranges from 1.04 to 3.26 mg/kg, while that one of sulphate has 4.2 mg/kg and 2.8mg/kg for maximum and minimum respectively. The main average concentration values for phosphate and sulphate within the crude oil polluted site are 1.61 mg/kg and 3.53 mg/kg respectively. The result of the main average for phosphate (1,61 mg/kg) is significantly different from the concentration value (3.26 mg/kg) of the control site. While the sulphate concentrations of 3.53 mg/kg for main average and 3.2 mg/kg for control are similar.

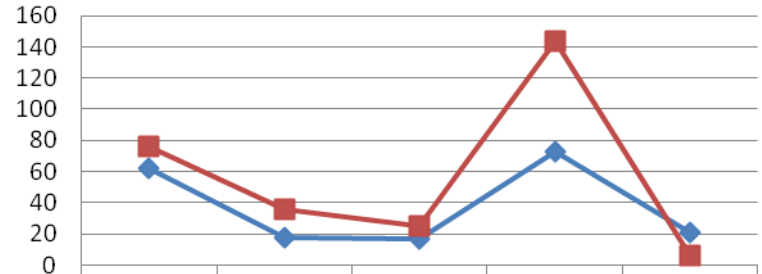
Figure 3.2: Showing concentration of Sulphate and Phosphate



	Omavure	Adjerhe	Agba	Erheoke	Control
Phosphate, mg/kg	1.04	1.82	2.52	1.04	3.26
Sulphate, mg/kg	3.9	3.2	2.8	4.2	3.2

The organic content of the soil samples basically serves as the foodstuff for microbial population in the soil. It may contain some major components of micro as well as macronutrients available to plants. The concentrations of the total organic carbon (see figure 3.3) which may serve as carbon and energy sources to the microbes varies from 16.9 mg/kg to 72.9 mg/kg with an average value of 42.27 mg/kg. The result showed that total organic content is enhanced by the crude oil pollutions within the impacted areas when compared to the concentration value (20.6 mg/kg) from the control site. The concentration values for the poly aromatic hydrocarbon vary from 25.32 mg/kg to 143.89 mg/kg. This is as a result of crude oil spill within the study.

Figure 3.3: Concentration Values of Total Organic carbon, mg/kg and PAH, mg/kg



	Omavure	Adjerhe	Agba	Erheoke	Control
Total Organic Carbon, mg/kg	61.82	17.44	16.9	72.9	20.6
PAH, mg/kg	76.2	35.8	25.32	143.89	6.34

The total Nitrogen concentration is presented in figure 3.4 below. The concentration values vary from 10 mg/kg to 370 mg/kg of soil. The average Nitrogen concentration from the spilled and control area were 327.5 mg/kg and 220mg/kg respectively. Adequate availability of nitrate and other micro and macronutrients like sulphate, phosphate are essential for increased activity of microorganisms since nitrogen, sulphur, and carbon are essential elements for cellular metabolism in microbial life. This cellular activities includes synthesis of new cells and generation of energy that sustained bacterial growth in any soil environment (Puyate and Rim-Rukeh, 2008)

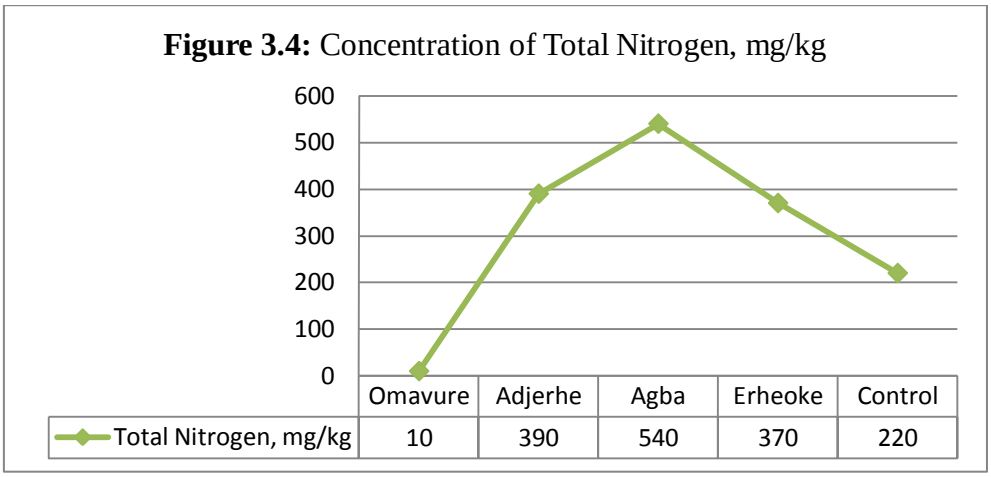


Figure 3.4: Concentration of Total Nitrogen, mg/kg

Generally, changes in soil physico-chemical properties investigated within the study areas may be caused by variation in topographical factors, climatic conditions, tillage practices, nature of the base rocks and soil forming factors (Mangosogo *et al*, 2019).

The results of Total Bacterial Count (See figure 3.5) had the highest (8.2 x 10^4 , cfu/g) and lowest (6.3 x 10^4, cfu/g values from Omavure and Adjerhe respectively within the crude oil contaminated sites. However, the value of total bacterial load from control site recorded the overall highest value (8.5 x 10^4, cfu/g). This decline in microbial population is as a result toxic impact of crude oil on flora and fauna community resulting to damages of their food resources and habitats as well as its ability to reduce oxygen aeration and nutrients availability to living organism within the soil environment (Tewari and Sirvaiya, 2015).

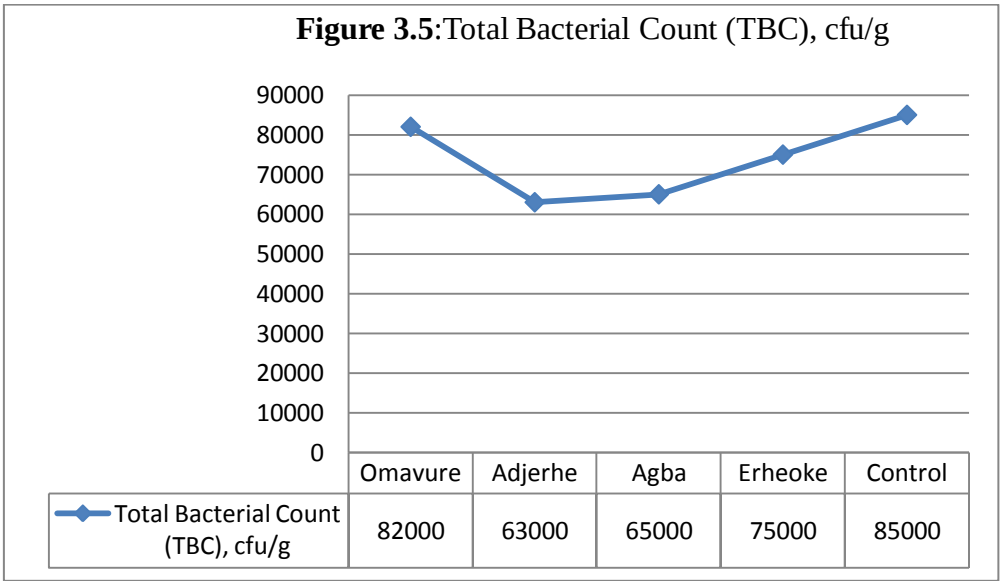
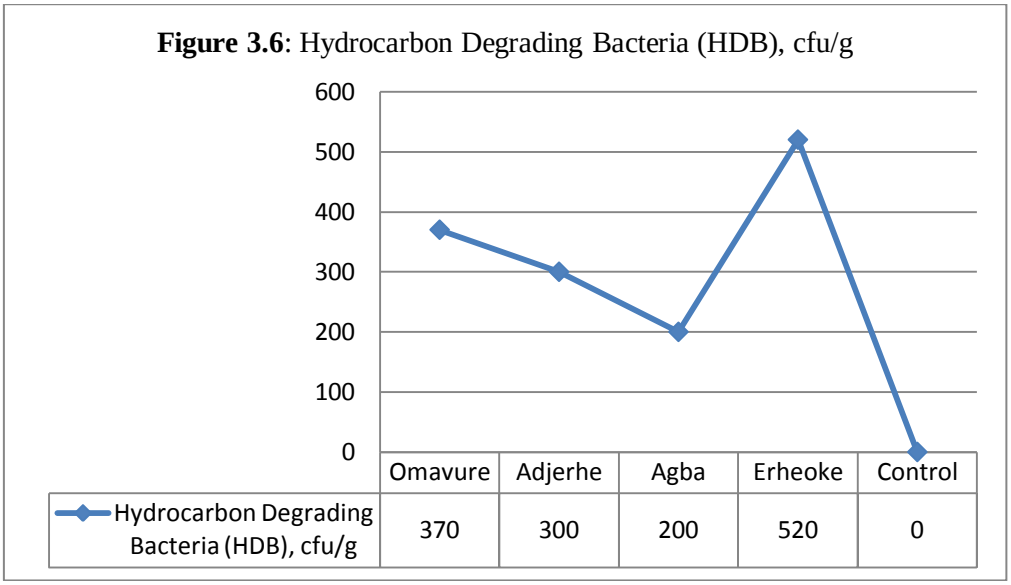


Figure 3.5:Total Bacterial Count (TBC), cfu/g

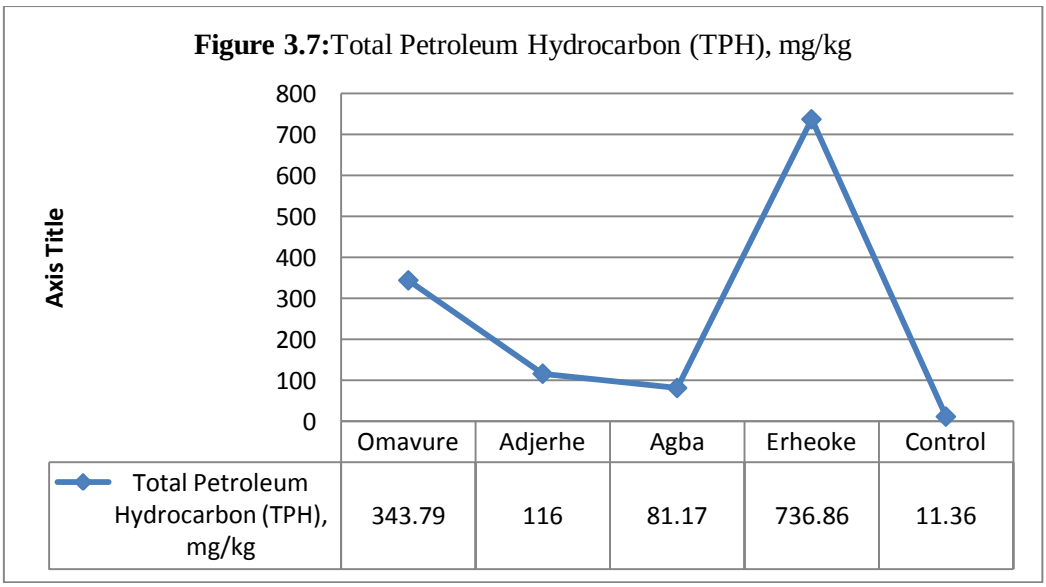
The Hydrocarbon Degrading Bacteria (HDB) is presented in figure 200 cfu/g to 520 cfu/g from the crude oil contaminated areas. However the control site without crude oil contamination showed no growth. The Hydrocarbon utilizing bacteria use the crude as the only sources of carbon and energy, as such cannot thrive in the absence of petroleum or petroleum products. The growth of Hydrocarbon bacteria observed also gives reason for possibility of isolating these indigenous species for bioremediation processes.

Figure 3.6: Hydrocarbon Degrading Bacteria (HDB), cfu/g



The result of the Total Petroleum Hydrocarbon (TPH) is presented in figure 3.7 below. The result recorded substantial concentration values (ranged from 81.17 mg/kg to 736.86mg/kg) of TPH which indicated crude oil contaminations for all soil samples from the contaminated area. These concentrations were sufficient to support the growth of HUB from all sampling point. The small concentration (11.36 mg/kg) from the control site was not enough to support and/or sustained HUD population.

Figure 3.7: Total Petroleum Hydrocarbon (TPH), mg/kg



IV. Conclusion

The study revealed that nutrients are present in the soil samples collected based on the result of the physico-chemical analysis. This means that the soil can support and sustain the microbial growths, which is essential for fixing some plants nutritional requirement and support their growth. However, the presence of crude oil hydrocarbon in the soil leads to the reduction of microbial population. The presence of crude oil within the soil environment consequently will lead to reduced aeration causing reduced oxygen availability as well as obstruction of nutrients uptake by the plants for their normal growth. The result also showed natural selection and development of the hydrocarbon utilizing bacteria as seen from the results of samples from the contaminated sites. The crude oil impacted soil may drastically fail to support plant growth and as well as complete destruction and distortion of ecosystem within the contaminated area.

It is recommended that bioremediation exercise should be conducted in order to regain and reclaim the fertility of the soil and its full potential for plants growth and bacterial activities. The native bacteria that grow within the crude oil spilled environment can be harvested and utilized for the bioremediation process of the polluted sited, because of developed potential to survive and thrive within the environment.

Reference

[1]. Agarry, S.E., Aremu, M.O. and Aworanti ,O.A. (2013). Kinetic modelling and half-life study on enhanced soil bioremediation of bonny light crude oil amended with animal-derived organic waste . J Pet Environ Biotechnology. 4:137

[2]. Almansoory, A.F., Hasan, H. A., Idris, M., Abdullah, S. R. S. and Anuar, N. (2015). Potential application of a biosurfactant in phytoremediation technology for treatment of gasoline-contaminated soil. Ecol Eng. 84:113–120. doi:10.1016/j.ecoleng.2015.08.001.Availableat: https://www.ajol.info/index.php/jasem

[3]. American Public Health Association, APHA (2018). Standard Methods for the Examination of Water and Waste Water 23rd Edition

[4]. American society for Testing and Materials, ASTM (2006) Standard methods for testing of Soil and rock. Vol. 10.1

[5]. American society for Testing and Materials, ASTM (2018) Standard methods for testing of petroleum and Petroleum products. Vol. 10.1

[6]. Association of Official Analytical Chemists, AOAC (1999). Standard method for the analysis of fertilizers, foods, feeds, pesticides, drugs, cos-metics, hazardous substances, and other materials related to agriculture, health and welfare, and the environment.

[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 J Microbiol Biotechnol (2016) **32**:180

[8]. Birma, G. J., Ukpebo, E. E. and Issa, B. R. (2018). Assessment of Environmental Noise Pollution In Warri And Effurun Metropolitan Cities. Current World Environment. 13(3). Available from: http://www.cwejournal.org?p=1093/

[9]. Darefeka, M. D. (2003). Water quality standards and guidelines in Nigeria, Paper presented at the 2nd Conference on Water, Port Harcourt, Rivers State.

[10]. Hamzah, A., Salleh, S. N, M. and Sarmani, S. (2014). Enhancing Biodegradation of Crude Oil in Soil Using Fertilizer and Empty Fruit Bunch of Oil Palm. Sains Malaysiana , **43**(9) :pp1327–1332

[11]. Imran, M., Khan, A., Hassan, A., Kanwal, F., Liviu, M., Amir, M. and Iqbal, M.A. (2010), Evaluation of physico-chemical characteristics of soil samples collected from Harrapa-Sahiwal (Pakistan). Asian Journal of Chemistry, **22**(6): 4823-4830.

[12]. J. Appl. Sci. Environ. Manage. Vol. 12**(2),** 135 - 141

[13]. Kiraye, M., John, W. and Gabriel, K .(2016). Bioremediation Rate of Total Petroleum Hydrocarbons from Contaminated Water by Pseudomonas aeruginosa Case Study: Lake Albert, Uganda. Journal of Bioremediation and Biodegradation 7: 335. DOI: 10.4172/2155-6199.1000335

[14]. Okoko, K. A. B. and Nna, J. N. (1998). Emerging trends and community perception in the Nigerian oil industry, Nigerian Journal of Oil and Politics, 1(7), 44-55.

[15]. Puyate Y.T. and Rim-Rukeh, A. (2008). Some physico-chemical and biological characteristics of soil and water samples of part of the Niger Delta area, Nigeria

[16]. Soo Ying Ho , Mohd Effendi Bin Wasli , and Mugunthan Perumal, (2019).Evaluation of Physicochemical Properties of Sandy-Textured Soils under Smallholder Agricultural Land Use Practices in Sarawak, East Malaysia. Applied and Environmental Soil Science. Volume 2019, Article ID 7685451, 14pp. DOI: https://doi.org/10.1155/2019/7685451

[17]. Stancu, M. M. (2018). Bacterial Degradation of Petroleum and Petroleum Products. J Mol Microbiol. 2 (1: 2). Available at: http://www.imedpub.com/journal-molecular-microbiology/

[18]. Sunita J. Varjani (2017) Microbial degradation of petroleum hydrocarbons: Review. Bioresource Technology. 223; 277–286. Available at: http://dx.doi.org/10.1016/j.biortech.2016.10.037

[19]. Tewari, S and Sirvaiya, A. (2015). Oil Spill Remediation and its Regulation. International Journal of Research In Science & Engineering.1(6) . pp8. Available at: https://www.researchgate.net/publication/283644184

[20]. Tudararo-Aherobo, L., Sylvester, O., Tega, A. & Avwerosuoghene, S. (2020). Biotreatability of Crude Oil Polluted Aquatic Environment Using Indigenous Hydrocarbon Utilizing Bacteria. Asian Journal of Environment & Ecology.**12**(4): 52-62

[21]. Varjani, S. .J, Rana, D. P., Jain, A. K., Bateja, S. & Upasani, V. N. (2015). Synergistic ex-situ biodegradation of crude oil by halotolerant bacterial consortium of indigenous strains isolated from on shore sites of Gujarat, India Int. Biodeterior. Biodegrad.103 116-124

[22]. Vasconcelos ,R.N., Lima, A.T.C., Lentini, C. A. D., Miranda., G.V., Mendonça, L. F., Silva, M. A., Cambuí ,E. C. B., Lopes, J. M. & Porsani, M.J.(2020). Oil Spill Detection and Mapping: A 50-Year Bibliometric Analysis. Remote Sensing. 2020; 12(21):3647. https://doi.org/10.3390/rs12213647

[23]. Vasilyeva, G., Kondrashina, V., Strijakova, E., et al., (2019). Adsorptive bioremediation of soil highly contaminated with crude oil, Science of the Total Environment. Available at: https://doi.org/10.1016/j.scitotenv.2019.135739

[24]. Veni, P., · Satish, C. P., Diksha, S., · Veena, P. & Mukesh, S.(2020). Bioremediation: an emerging effective approach towards environment restoration. Society for Environmental Sustainability. Available at: https://doi.org/10.1007/s42398-020-00099-w

[25]. Yuniati, M. D. (2018). Bioremediation of petroleum-contaminated soil: A Review. IOP Conf. Series: Earth and Environmental Science 118: 012063. doi :10.1088/1755-1315/118/1/01206

Birma, G. J, et. al. "Pre-Bioremediation Assessment of Crude Oil Contaminated Soil in Kokori And Environs, Niger Delta Region, Nigeria." *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 16(01), (2022): pp 50-57.